Judith S. Weis

Physiological, Developmental and Behavioral Effects of Marine Pollution

Springer

Physiological, Developmental and Behavioral Effects of Marine Pollution

Judith S. Weis

Physiological, Developmental and Behavioral Effects of Marine Pollution

Springer

Judith S. Weis
Department of Biological Sciences
Rutgers University
Newark, NJ, USA

ISBN 978-94-007-6948-9 ISBN 978-94-007-6949-6 (eBook)
DOI 10.1007/978-94-007-6949-6
Springer Dordrecht Heidelberg New York London

Library of Congress Control Number: 2013941886

© Springer Science+Business Media Dordrecht 2014
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

When I first started out working in this field, I participated in a series of conferences in the 1970s and 1980s, organized by John and Winona Vernberg of the University of South Carolina, and Anthony Calabrese and Fred Thurberg of the NOAA laboratory in Milford CT, in which marine biologists interested in organismal biology started examining responses to pollutants. These were small meetings of around 100 people, and were among the most stimulating and enjoyable meetings I have participated in. This was an exciting beginning of a new field of study. The participants were physiologists and other biologists who had not been trained in "aquatic toxicology," as that field was still in the early stages of development. These meetings resulted in a series of peer-reviewed volumes with titles that were variants on "Physiological Responses of Marine Organisms to Pollution," but each volume had a somewhat different title, thus using up many possible titles I might have used for this book. At around the same time, another group of people, as yet unknown to me, were establishing the field of "aquatic toxicology" with a goal of developing "standard toxicity tests." I first came upon this approach when I was speaking with some EPA people about the interesting variation we had seen in killifish embryos exposed to the same concentration of mercury – some females produced very sensitive embryos and others produced very tolerant ones. I asked if they might be interested in funding further research into this intriguing observation. The response was "Could you turn this into a toxicity test?" I had no interest in toxicity tests; I merely wanted to follow up an interesting observation and learn what was going on. As it turned out, I pursued the research without EPA support. I also learned that much of the work going on in the field, unlike the research of the people who came to the "Vernberg meetings" focused on lethality as an endpoint – the "kill 'em and count 'em" approach. These projects calculated the LC_{50} for different chemicals, numbers that were used in the development of regulations. Even today, research papers are still coming out with this kind of data, using a new species or different conditions. A paper entitled "Effects of X {chemical} on Y (species)" might very well turn out to be how much of chemical X was needed to kill 50 % of species Y. I find this uninteresting, and think it unfortunate for two reasons: (1) Scientists are intelligent creative people who should be developing new hypotheses and expanding the intellectual range of the field and should not be wasting their time doing this sort of routine work – the field is in need of progress and advancing along more scientifically sophisticated routes. (2) Regulatory agencies should not be relying on such crude measures for setting criteria and standards. The science has advanced far beyond this, and we know a lot about subtle sublethal responses as well as delayed responses. Setting numbers on the basis of dividing 96 h LC_{50}s by some arbitrary number is an antiquated approach. If this approach to standard setting is no longer being used, why are people still doing this kind of work?

Another aspect of the field is the rapid development over the past few decades of biochemical and molecular approaches. The search for new biochemical "biomarkers" of exposure or response to contaminants is a major part of the field. This reductionist approach leads to greater insight and understanding of the mechanisms by which chemicals produce effects on organisms. For the past 30 or so years there has been a series of relatively small meetings, comparable to the early "Vernberg meetings," called "Pollution Responses in Marine Organisms" (PRIMO). The papers presented at these meetings are almost exclusively biochemical and molecular. Even newer approaches are genomic and other types of "omic" approaches. However, the connection between these biochemical responses and an effect at the organism level is often difficult to draw. How does it affect the life of the animal that it is producing more or less of a certain enzyme? The study of physiological, developmental, and behavioral effects that are the focus of this book are whole animal responses that are easily related to effects at the population level, and their ecological significance is more obvious. While these kinds of studies have been somewhat overshadowed by the biochemical/molecular approaches in recent years, it is my earnest hope that they will remain active and essential components of the field, as they are the best way to link to effects on the ecosystem. This book does not cover biochemical, molecular and 'omic studies, including immunotoxicology and genotoxicology. For the topics covered there is a very extensive literature, so the book is not exhaustive, and of necessity many studies have not been included.

The marine environment is under assault from overfishing, habitat loss and pollution from increasing types of sources. New kinds of pollutants ("contaminants of emerging concern") include both new pollutants and old pollutants that no one ever paid attention to before. These include pharmaceuticals which are designed to have effects on the body at very low concentrations – so they can have effects on marine life at low concentrations also. The unsightly volumes of marine debris, often persistent plastic, washing up on beaches and collecting in Great Garbage Patches in the Atlantic and Pacific Oceans is something that most people have heard about. New awareness of the damaging effects of loud noise on marine animals, especially mammals, is a great concern as it may relate to cetacean beaching incidents. There have been a huge number of papers coming out in recent years on effects of ocean acidification. While many focus on effects on shell formation/calcification, since it is the most obvious problem caused by lower pH, people are also investigating and uncovering effects on physiology and behavior as well. Fortunately for this field, the toxicity testing folks have not gotten involved, and I am happy to report that I have not come across any publications that determine how low the pH has to be in order to kill half of the test animals. Perhaps the most widespread and serious type of pollution worldwide is eutrophication resulting from excess nutrients, which stimulates algal blooms and results in hypoxia. On a global scale, eutrophic/hypoxic areas are increasing, and there is considerable research into the sublethal effects of low D.O. on marine organisms. On the other hand, there is some “good news” in that many persistent organic pollutants have been banned and are no longer manufactured in many countries (even though as legacy pollutants they still persist in sediments, accumulate in marine life, and exert effects). Also, the frequency of oil spills has gone down in the past few decades. In addition to this reduction of inputs of some of the historical pollutants, efforts have begun to physically remove highly contaminated sediments from some of the estuarine toxic hot-spots in the U.S. under the auspices of the Superfund Program. After decades of delay, sediments highly contaminated with dioxins, PCBs and mercury are finally being removed from the Passaic River in New Jersey and other notorious sites through Superfund remediation programs.

Acknowledgements

First and foremost, I would like to thank my husband, Dr. Peddrick (Pete) Weis, for handling and preparing the figures in this book and for being a research partner for many years of research into effects of pollutants on marine organisms. I am thankful to John and Winona Vernbergs, Anthony Calabrese and Fred Thurberg for organizing those early conferences that got me started in the field. I also would like to thank my editor, Alexandrine Cheronet of Springer, for her encouragement and assistance during the preparation of this volume. Graduate students and post-docs who worked in my lab on pollution-related research have contributed a great deal. These include Margarete Heber, Howard Solomon, Swati Toppin, Mark Renna, Patrick Callahan, Abu Khan, Mark Kraus, Catherine Davis, Tong Zhou, William Romeo, David Harpell, Graeme Smith, Jennifer Samson, Maryanne Carletta, Lisamarie Windham, Lauren Bergey, Jessica Reichmuth, and Allison Candelmo.

I would like to the acknowledge the scientists who got me interested in marine life and pollution – Evelyn Shaw, who took me on as an undergraduate summer helper at Woods Hole Oceanographic Institution to study fish schooling behavior; Eugene Odum, Howard Sanders and Larry Slobodkin, who taught the Marine Ecology course at the Marine Biological Lab at Woods Hole, and who stimulated and broadened my interest in the subject; and Alfred Perlmutter of New York University, who perked my interest in pollution and other environmental issues. Finally I would like to express my thanks to *Fundulus heteroclitus, Uca pugilator*, *Uca pugnax*, and *Palaemonetes pugio*, and also to *Callinectes sapidus* and *Pomatomus saltatrix* for being such interesting subjects and sources of information. Investigating how they survive in and cope with the contaminated estuaries of northern New Jersey has been a long-standing interest and challenge. I have enjoyed learning how they are affected by their stressful environment. I thank them for allowing us to learn about the similarities and differences of their fascinating responses and adaptations.

Contents

Part I Physiology

Part II Reproduction and Development

Part III Behavior

Part IV Dealing with Pollutants

Part I
Physiology

Chapter 1
Introduction to Marine Pollution

Abstract The ocean plays a key role in cycles of carbon, nitrogen, phosphorus and a variety of other important chemicals. Ocean chemistry has been changing due to human activities, both regionally in coastal waters and in the open ocean. Some of the greatest impacts are on carbon, nitrogen, and dissolved oxygen, which affect biological productivity. The rate of primary production is determined primarily by light and nutrients. Decades of pollution of marine waters, along with coastal habitat destruction, overfishing and bottom trawling have had devastating impacts on biodiversity and habitats. The increasing demand for seafood worldwide has depleted fish populations and devastated the economic well-being of coastal communities. At the same time, climate change is altering the oceans in major ways that we are only beginning to understand.

Land-based sources pollute estuaries and coastal waters with nutrients, sediments, pathogens as well as many thousands of toxic chemicals, including metals, pesticides, industrial products, pharmaceuticals and more. Following the industrial revolution, increasing amounts of materials have been discharged into the environment from chemical industries, sewage treatment plants, and agriculture, eventually reaching marine ecosystems. Highly visible events such as the *Exxon Valdez*, and the Gulf of Mexico "gusher" have raised public awareness of marine pollution in recent decades. There is growing scientific evidence demonstrating serious, sometimes disastrous, impacts of pollution in the marine environment. Pollutants of major concern are those that are widespread and persistent in the environment, accumulate in biota, and induce effects at low concentrations. Toxic chemicals are varied and often difficult to detect. In recent years, attention is being devoted to new or newly recognized threats to the environment – contaminants of emerging concern (CEC), ocean acidification, and noise pollution.

Keywords Metal • Pesticide • Acidification • Eutrophication • Litter • Metal • Nitrogen • Noise • Nutrient • Sediment

J.S. Weis, *Physiological, Developmental and Behavioral Effects of Marine Pollution*,
DOI 10.1007/978-94-007-6949-6_1,

1.1 Sources and Fate in the Environment

Sources of contaminants in the marine environment are mostly based on land. While many pollutants come from industrial or residential areas, others come from agricultural areas. Factories and sewage treatment plants discharge into receiving waters through a pipe – this is referred to as a "point source" and it can be monitored and regulated by environmental protection agencies. Since passage of the Clean Water Act in the United States in 1972, much progress has been made in controlling pollution from point sources. However, the historic use of some chemicals no longer manufactured in the United States (e.g., DDT, PCBs) has left a legacy of contamination. Sediments remain contaminated with these persistent chemicals, which continue to affect marine life long after their inputs have ceased. In recent decades attention has moved from end-of-pipe discharges to diffuse pathways of runoff and atmospheric deposition. Sources of contaminants that wash into the water during rainfall are diffuse and enter water bodies in many places, as do pollutants from the atmosphere that come down in precipitation. This diffuse pollution is referred to as "non-point source," and is not so easily regulated. Non-point sources, such as farms, roadways, and urban or suburban landscapes remain largely uncontrolled, and are major sources of continuing pollution inputs. The few sources that are not land-based include oil spills from tankers and drilling platforms, leaching of antifouling paints and discharge of wastes from vessels. Point sources of pollution from industrial discharges and oil spills are highly destructive to local areas where they occur, but lower concentrations of these chemicals are widespread in the global oceans. Elevated levels of persistent organic pollutants and methylmercury are widespread and of concern since these chemicals build up in food chains and pose a threat to humans from eating contaminated fish and other seafood.

1.1.1 Metals

Metals released from mining and industrial processes are among the major contaminants of concern in coastal environments. Many studies have shown their accumulation in sediments and coastal organisms. Mercury, cadmium, copper, zinc, and silver are major contaminants from industrial processes including power plants. Since mercury is present in coal, when it is burned the mercury enters the atmosphere, where it can be transported long distances before being deposited far from its source. While some metals (copper and zinc) are essential for life at low concentrations, other metals play no normal biological role. While most metal contaminants originate from land-based industrial sources, metals also are used in anti-fouling paints for ships. Since fouling organisms can accumulate on ship bottoms (reducing streamlining, thus increasing fuel consumption), antifoulant coatings have been developed. For thousands of years ship hulls have been treated with various substances to reduce fouling. Paints containing copper have been used for many years. Starting in the 1940s organotin compounds were developed for this purpose and one of the most effective and long-lasting is tributyltin, which is one of the most toxic to other non-target organisms.

In aquatic environments, copper exists in particulate, colloidal and soluble states, predominantly as metallic (Cu^0) and cupric copper (Cu^{2+}). It forms complexes with both inorganic and organic ligands. The toxicity of copper is directly associated with the free ion, as is the toxicity of Cd, so measurements of total Cu or total Cd in the water overestimate the amount the is bioavailable (Sunda et al. 1978; Sunda and Lewis 1978).

Mercury is a highly toxic element that is found both naturally and as a contaminant. Although its potential for toxicity in highly contaminated areas such as Minamata Bay, Japan, in the 1950s and 1960s, is well documented, mercury can also be a threat to the health of people and wildlife in environments that are not obviously polluted. The risk is determined by the form of mercury present and the geochemical and biological factors that influence how it moves and changes form in the environment. Mercury can exist in three oxidation states in natural waters: Hg^0, Hg^{1+} and Hg^{2+}. The distribution of the forms depends on the pH, redox potential, and availability of anions to form complexes with the mercury. In the environment, inorganic mercury can be transformed into organic mercury compounds. Methylmercury (meHg) is a highly toxic form, and inorganic Hg can be converted to meHg by bacteria in marine sediments (Fig. 1.1). Bacteria capable of methylating Hg^{2+} have been isolated from sediment, water, soil and fish tissue. However, little is known about the physiology and the mechanisms controlling methylation. MeHg, in addition to being far more toxic than inorganic forms of the metal, also is biomagnified up the food chain, so tissue concentrations increase as it passes up the food chain. People are exposed to meHg primarily by eating fish that are high on aquatic food chains.

The other organometal of concern is tributyltin, which was formerly used in antifouling paints for vessels, but unlike Hg, tributyltin (TBT) breaks down in the environment, losing its butyl groups over time, reducing its toxicity as it eventually becomes inorganic tin, which is not toxic. However, the breakdown is not as rapid as initially thought, so effects can persist for some years.

Metals tend to bind to sediments, from which they are available to varying degrees to marine organisms, particularly benthic ones, from which the metals can be moved up the food chain. Bioavailability of sediment-bound metals is a critical issue for their toxicity.

Acid volatile sulfide (AVS) has been used to predict the toxicity in sediments of divalent metals, including copper (Cu), cadmium (Cd), nickel (Ni), lead (Pb) and zinc (Zn) (Ankley et al. 1996; Berry et al. 1996). The rationale is that the AVS in sediment reacts with the simultaneously extracted metal (SEM), the reactive metal

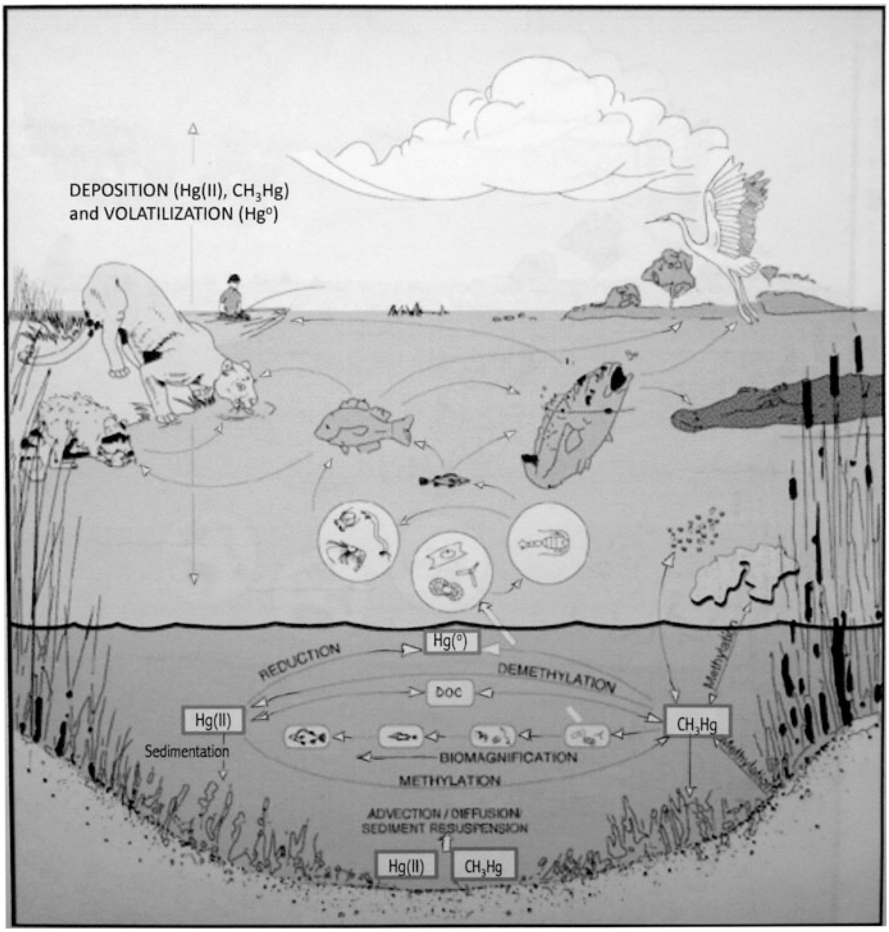


Fig. 1.1 Mercury cycle (From USGS)

fraction that is measured in the cold acid extract. This reaction forms an insoluble metal sulfide that is relatively non-available for uptake. Estuarine sediments tend to have high levels of sulfide, and thus relatively low bioavailability of sediment-bound metals. Ironically, elevating the oxygen in overlying water increases the redox potential in the sediment while decreasing AVS, thus increasing metal availability in the sediment's pore water. Thus, increased oxygen from water quality improvements can increase the mobility of trace metals and may cause sediment-bound metals to leach into the overlying surface water. In contrast, prolonged hypoxia promotes the release of iron and manganese from contaminated estuarine sediments (Banks et al. 2012).

1.1.2 Organics

Oil

Petroleum hydrocarbons (including both linear alkanes and polyaromatic hydrocarbons, PAHs) in the marine environment have been a long-standing problem. There is great public concern about oil spills and the resultant shoreline fouling and mortality of large numbers of marine birds and mammals. Major oil disasters in recent years include the *Exxon Valdez* in Alaska, and the blow out of the mile-deep BP well *Deepwater Horizon* in the Gulf of Mexico.

Many of the major spills had long-term consequences, associated mainly with estuaries and marshes, due to the persistence of oil or petroleum fractions in these low-energy environments. The bioavailability of residual oil to benthic infauna is influenced by several factors, such as water solubility, weathering rate and sediment grain size. These effects may last for decades on processes including behavior, development, genetics, growth, feeding, and reproduction. Long-term effects have been studied after spills, and they vary depending on the nature of the oil, the temperature, and the nature of the area of the spill. After a spill, most of the oil undergoes a weathering process. However, oil in marshes or sandy beaches can sink down to depths where it persists for decades in the absence of oxygen.

The number of spills from tanker ships has decreased over the past three decades. There were about three times as many spills in the 1970s as in the 1990s. However, the number of spills does not consider the volume of oil; the frequency of large spills has decreased as well as the frequency of small ones.

The *Exxon Valdez* Oil Spill

The *Exxon Valdez* oil spill occurred in Prince William Sound, Alaska, on March 24, 1989, when *Exxon Valdez*, an oil tanker struck Prince William Sound's Bligh Reef and spilled 260,000–750,000 barrels (41,000–119,000 m^3) of crude oil. It is considered to be one of the most devastating human-caused environmental disasters. Within 6 h of the grounding, the *Exxon Valdez* spilled approximately 10.9 million gallons of its 53 million gallon cargo of Prudhoe Bay crude oil. The *Valdez* spill was the largest ever in U.S. waters until the 2010 *Deepwater Horizon* oil spill, in terms of volume released. However, the remote location, which could be reached only by helicopter, plane, and boat, made government and industry response efforts very difficult. The oil eventually covered 1,300 miles (2,100 km) of coastline, and 11,000 square miles (28,000 km^2) of ocean. After the spill, the subsurface oil persisted, and chronic exposures continued to affect biota for over a decade. The region is a habitat for salmon, sea otters, seals and seabirds, many of which were obvious victims of the spill, which involved 1,500 miles of oiled shoreline, several hundred thousand dead birds and marine mammals. Three years after the spill, most of the remaining oil was sequestered in places where degradation was inhibited, such as intertidal subsurface sediments or under mussel beds. Heavily oiled coarse sediments protected oil reservoirs beneath the surface, preventing it from weathering in intertidal sites. These sites often contained fish eggs and other vulnerable biota (Peterson et al. 2003).

Various reasons for the spill include the following: Exxon Shipping Company failed to supervise and provide a rested and sufficient crew. The third mate failed to properly maneuver the vessel, possibly due to fatigue or excessive workload. (The 1989 tanker crew was half the size of the 1977 crew, worked 12–14 h shifts, plus overtime.) *Exxon Valdez* was sailing outside the normal sea lane to avoid small icebergs thought to be in the area. Exxon failed to properly maintain the Collision Avoidance System (RAYCAS) radar, which should have indicated to the third mate an impending collision with the Bligh Reef. The captain was asleep when the ship crashed, having had too much to drink. At the helm, the third mate did not look at the radar, because it was not turned on, having been broken and disabled for over a year. Coast Guard tanker inspections in Valdez were not done, and the number of staff was reduced (National Transportation Safety Board 1990).

Lack of available equipment and personnel hampered the spill cleanup, which was delayed during a few days of relatively calm weather because of confusion over which entity (Exxon, the EPA, the State of Alaska) was in charge. Many cleanup techniques were tried with only moderate success. One trial burning was conducted during the early stages of the spill to burn the oil, in a region of the spill isolated from the rest by another explosion. The test reduced 113,400 l of oil to 1,134 l of removable residue, but because of unfavorable weather no additional burning was attempted. The dispersant Corexit® 9580 was tried as part of the cleanup. Corexit has been found to be effective but toxic to wildlife. The primary means of open water oil recovery was with skimmers, but the skimmers were not readily available during the first 24 h following the spill. In general, most skimmers became less effective once the oil had spread, emulsified and mixed with debris. Thick oil tended to clog the equipment. Sorbents were used to recover oil in cases where mechanical means were less practical. The drawback to sorbents was that they were labor intensive and generated additional solid waste. In 1989, hoses spraying seawater were used to flush oil from shorelines. The released oil was then trapped with offshore boom, and removed using skimmers, vacuum trucks (useful for thick layers of oil) and boom (e.g., sorbents). Because there were rocky coves where the oil collected, the decision was made to displace it with high-pressure hot water. However, this also displaced and destroyed the microbial and meiofaunal populations on the shoreline; many of these organisms are important ecologically and/or capable of biodegradation of oil. At the time, both scientific advice and public pressure was to clean everything, but since then greater understanding of bioremediation processes has developed. The general opinion is that the high pressure hot water treatment did more harm than good. Beach applications of dispersants were also tried in several locations. Corexit® 7664 was applied on Ingot Island, followed by a warm water wash. No significant change in oil cover or the physical state of the oil was observed after the treatment, but some ecological impacts were found in the treated areas. It appeared that the effects were due more to the intensive washing than to the use of dispersant, and were evident in intertidal epibenthic macrobiota. Despite the extensive cleanup attempts, less than 10 % of the oil was recovered. An important observation that resulted from the *Exxon Valdez* oil spill was that natural cleaning processes (bioremediation), on both sheltered and exposed beaches, could be very effective at degrading oil. It took longer for some areas of shoreline to recover from invasive cleaning methods (hot water flushing) than from the oil itself. NOAA determined that as of early 2007 more than 26,000 gallons (98 m^3) of oil remained in the sandy soil of the contaminated shoreline, declining at a rate of less than 4 % per year (Cleveland et al. 2010).

The Gulf Oil Spill and Dispersants

On April 20, 2010, the Macondo well blowout occurred approximately 5,000 ft below the surface of the Gulf of Mexico, causing the BP-Transocean drilling platform *Deepwater Horizon* to explode, killing 11 workers and injuring others. About five million barrels of crude oil were released into the sea; on average, 60,000 barrels a day (about 11,350 t of gas and oil per day) before the gusher was capped on July 15. Over 630 miles of Gulf Coast shoreline were oiled, mostly in Louisiana. There were over 400 controlled burns, which killed hundreds of sea turtles and unknown numbers of dolphins. To protect marshes from incoming oil, booms were set around islands and shorelines, and two million gallons of the dispersant Corexit® were applied on and beneath the surface of the sea to break up the oil. After extensive use, oil was no longer visible on the surface of the water, and some claimed it was "gone" and degraded by microbes. The use of the dispersants was highly controversial. By enhancing the amount of oil that physically mixes into the water column, dispersants reduce the amount of oil that reaches shoreline habitats, but dispersants are controversial because of the toxicity of dispersed mixtures. Also, once oil is dispersed in deep water, it cannot be recovered. Oil, when combined with dispersants in the water is usually more toxic than either the oil or the dispersant alone. Most studies found that the combination of oil and dispersant increased toxic effects. Two dispersants, Corexit® 9500 and 9527A, were used, which are complex mixtures of chemicals that have surfactant (wetting) properties, which allows them to act as emulsifiers (USEPA 2010). Although these two are EPA-approved, they are more toxic and less effective than other approved dispersants (Scarlett et al. 2005).

EPA performed short-term tests on mysid shrimp and inland silverside fish stated that JD-2000® and Corexit® 9500 were generally less toxic to small fish and JD-2000 and SAF-RON GOLD® were least toxic to mysid shrimp. However, on May 20, EPA gave BP 24 h to find a less toxic alternative, which BP ignored. On May 25, the EPA gave BP a directive to reduce dispersant use, but the Coast Guard granted exemptions, allowing continued use of Corexit®. The goal of the EPA was to decrease toxicity, while that of the Coast Guard was to protect the coastline from being oiled.

The blowout occurred in deep water, where a turbulent discharge of hot pressurized oil and gas mixed with seawater and dispersed by itself into droplets and gas hydrates without the use of chemicals. This mixture did not rise to float on the surface but stayed in a subsurface plume. Amid reports of the oil being nearly gone, a plume of hydrocarbons about 22 miles long in deep water over 3,000 ft below the surface was discovered. Camilli et al. (2010) found a continuous plume of oil, which persisted for months without substantial biodegradation. Samples collected from the plume had monoaromatic petroleum hydrocarbon concentrations $>50\ u\text{g l}^{-1}$. Dissolved oxygen concentrations suggest that microbial respiration in the plume was not more than 1 uM oxygen per day. The high pressures and low temperatures made the mixture of oil, dispersants, sea water and methane neutrally buoyant.

Subsequently, Kujawinski et al. (2011) reported that a major component of the dispersant was contained in the plume in the deep ocean and had still not degraded after 3 months. They measured one of Corexit® 9500A's main ingredients in May and June and again in September, 2 months after the well was capped. The results raise questions about what impact the oil and dispersant might have had on the environment. The toxicity of this mixture on deep sea corals and cold seep communities is unknown, as are the impacts on planktonic filter feeders and fish eggs/larvae in the water column. Eventually intense microbial activity degraded the oil (Kessler et al. 2011).

The *Deepwater Horizon* blowout was unprecedented because of the use of dispersants at the wellhead, retention of oil as finely dispersed droplets and emulsions and deepwater retention of plumes of natural gas that underwent rapid microbial degradation. Subsurface effects of oil had not been seen before. According to the government's "oil budget," released by NOAA in November 2010, one fourth of the oil evaporated or dissolved into the water, and 13 % was blown into fine droplets as it rushed from the broken pipe. Corexit® 9500 sprayed at the wellhead dispersed another 16 % into fine droplets, which joined the plume. Natural oil-degrading Proteobacteria then worked on the plumes (Hazen et al. 2010). Rapid degradation was seen at 5 °C. Thus, intrinsic bioremediation of the plume took place in the deep water, as a result of the geography of the Gulf of Mexico, which is fairly enclosed. When the hydrocarbons were released from the well, bacteria bloomed, and then swirled around and often came back repeatedly over the leaking well. Water with a bacterial community got a second input of hydrocarbons and the organisms attacked and degraded the new oil (Valentine et al. 2012). In addition to the oil that was degraded, the Unified Command, led by the U.S. Coast Guard, physically removed about a third of it, and burning at the surface removed another 5 %. However, the budget was criticized as incomplete. Samantha Joye of the University of Georgia said her data showed that oil and gas at depth remained much longer than the oil budget suggested. There was also the "residual" oil unaccounted for, which is still out there, on or under beaches, in marshes, sunk to the bottom, or floating as tarballs.

If dispersants had not been used, the surface oil would have been weathered (tar balls) by the time it reached the coast. This would have created a public relations nightmare on beaches and affected the socio-economic activities of residents and tourists. The dispersed oil below the ocean surface appears to have killed benthic animals in intertidal and shallow subtidal regions on and near sandy beaches. In the wetlands only the fringe-edge marsh plants were damaged by the toxic

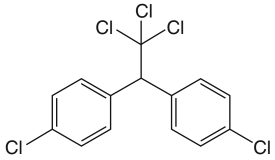


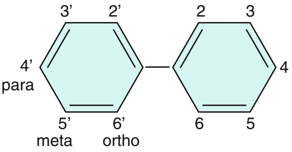
Fig. 1.2 DDT molecule

oil/dispersants in the surface water, since the plants appear to have absorbed these chemicals which caused the death of shoots. At the time this book is being written, in 2011 and 2012, there have been relatively few published reports on effects, as many scientists are not permitted to publish their findings yet, and it is too early to say anything about long-term effects on the Gulf ecosystems.

Pesticides

Halogenated hydrocarbons (mostly DDT-related pesticides, PCBs, and dioxins) have been studied intensively for decades. DDT (dichlorodiphenyltrichloroethane) (Fig. 1.2), the most powerful pesticide the world had ever known, could kill hundreds of different kinds of insects.

Synthesized in 1874, its insecticidal ability was identified in 1939 by the chemist Paul Hermann Müller. It was used in World War II, clearing South Pacific islands of malaria-causing insects, and was used as a de-lousing powder in Europe. When it became available for civilian use, few people expressed concern about this miracle compound. In 1948, Müller won the Nobel Prize for Physiology and Medicine. Related pesticides included aldrin, dieldrin, chlordane, heptachlor, and toxaphene, which caused fish kills when applied near the water. These chemicals are lipophilic and highly persistent. Rachel Carson's writing about the dangers of DDT was stimulated by bird kills that occurred as the result of DDT sprayings. Having already collected a large amount of research on the subject, she decided to write a book. *Silent Spring* described how DDT entered the food chain and accumulated in the fatty tissues of animals, including humans, and caused cancer and genetic damage. A single application on a crop, she wrote, killed insects for weeks and months (not only the targeted insects but many others) and remained toxic even after dilution by rainwater. She concluded that DDT and related pesticides had harmed birds and other animals and had contaminated the world food supply. The book alarmed readers and triggered an indignant response from the chemical industry. "If man were to faithfully follow the teachings of Miss Carson," complained an executive of the American Cyanamid Company, "we would return to the Dark Ages, and the insects and diseases and vermin would once again inherit the earth." Anticipating such a reaction, Carson had written *Silent Spring* with numerous scientific citations and a list of expert scientists who had approved it. Many eminent scientists supported it, and President Kennedy's Science Advisory Committee vindicated the book. As a result, DDT came under much closer government scrutiny and was eventually banned. Most other chlorinated hydrocarbons were also phased out



Structure of Polychlorinated Biphenyl (PCB) Molecule

Fig. 1.3 PCB molecule, showing positions where Cl could be attached

in subsequent decades. An important legacy of *Silent Spring* was a new public awareness that nature was vulnerable to human activities. The growth of the environmental movement was partly a response to this new awareness. Most uses of DDT and other chlorinated hydrocarbons were banned in the1970s. In the United States, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) requires that adverse ecological effects be balanced against the economic costs of regulating pesticide use and benefits the pesticide provides.

The diversity of pesticides has increased greatly. Hundreds of chemicals are now in use, and they generally occur in mixtures, whose toxicity may be additive or synergistic or antagonistic. Newer chemicals are less persistent than the “legacy pesticides” and do not generally cause kills. However, they can produce sublethal effects such as endocrine disruption, altered development and behavior, reduced growth, and other effects that are the major focus of this book. “Second generation” pesticides such as organophosphates and carbamates are much less persistent in the environment. Nevertheless, if spraying coincides with the time of reproduction and early life stages of susceptible organisms, they can also have deleterious effects. “Third generation” pesticides such as insect growth inhibitors, chitin synthesis inhibitors, and juvenile hormone mimics such as methoprene are more narrowly focused on insect biology, but tend to have severe effects on crustaceans, especially early life stages.

Industrial Chemicals

PCBs

PCBs (polychlorinated biphenyls) are also chlorinated hydrocarbons, and were manufactured from 1929 until they were banned in the U.S. in 1979 (Fig. 1.3). They include over 200 congeners with differing numbers of chlorine on different locations on the biphenyl structure, have a range of toxicity, and vary in consistency from thin, light-colored liquids to yellow or black waxy solids. In terms of toxicity, there are two distinct categories – coplanar (or non-*ortho*-substituted) and non-coplanar (or *ortho*-substituted) congeners. Coplanar PCBs have a fairly rigid structure, with the two phenyl rings in the same plane, which gives the molecule a structure similar to polychlorinated dibenzo-p-dioxins (PCDDs) (see below), and allows them to act in the same way as these molecules. Non-coplanar PCBs, with chlorine atoms at the *ortho* positions, are not part of the dioxin group. Nevertheless they have some neurotoxic and immunotoxic effects, but at levels higher than normally associated with dioxins. Due to their non-flammability, chemical stability, high boiling point, and electrical insulating properties, PCBs were used in hundreds of industrial and commercial applications including electrical, heat transfer, and hydraulic equipment; as plasticizers in paints and rubber products; in pigments, dyes, and carbonless copy paper; and many other industrial applications. PCBs entered the environment during their manufacture and use in the United States. They can still be released into the environment from poorly maintained hazardous waste sites that contain PCBs; illegal or improper dumping of PCB wastes; leaks or releases from electrical transformers containing PCBs; and disposal of PCB-containing products into landfills not designed to handle hazardous waste. PCBs may also be released into the environment by the burning of some wastes in municipal and industrial incinerators.

Because of their persistence in the environment and low water solubility, chlorinated hydrocarbons tend to accumulate in sediments and in tissues. Chlorinated hydrocarbons are highly persistent and remain in the environment (especially in sediments) for many decades, so they continue to be found long after they have been banned. Since PCBs are particularly resistant to microbial degradation, they may remain for long periods of time cycling between air, water, and soil. PCBs can be carried long distances and have been found in snow and sea water in areas far away from where they were released. As a consequence, PCBs are found all over the world. In general, the lighter the form of PCB (i.e., fewer chlorine atoms per molecule), the further it can be transported. PCBs accumulate in aquatic biota, including plankton and fish. Like chlorinated pesticides and methylmercury, PCBs biomagnify. Thus, larger fishes higher on the food chain are likely to have higher concentrations than smaller fishes (Fig. 1.4).

As a result, people who ingest fish may be exposed high concentrations. The highest environmental concentrations of PCB are usually found in soil and sediment, with much lower levels found in air and water. Fortunately, PCB levels have been declining in the past few decades and have been the subject of a number of federal and state regulations and clean-up actions in the U.S.

Dioxins

Dioxins and furans are some of the most toxic chemicals known. Dioxin is a general term that describes a group of hundreds of chemicals that are highly persistent in the environment. The most toxic compound is 2,3,7,8-tetrachlorodibenzo-p-dioxin or TCDD (Fig. 1.5). Polychlorinated dibenzofurans are similar to dibenzodioxins, but with a single oxygen connecting the benzene rings instead of two.

The toxicity of other dioxins and chemicals such as dioxin-like PCBs are measured in relation to TCDD. Dioxins and furans are formed as unintentional by-products of many industrial processes that use chlorine, such as chemical and pesticide manufacturing, pulp and paper mills that use chlorine bleach, the production of polyvinyl chloride (PVC) plastics, the production of chlorinated

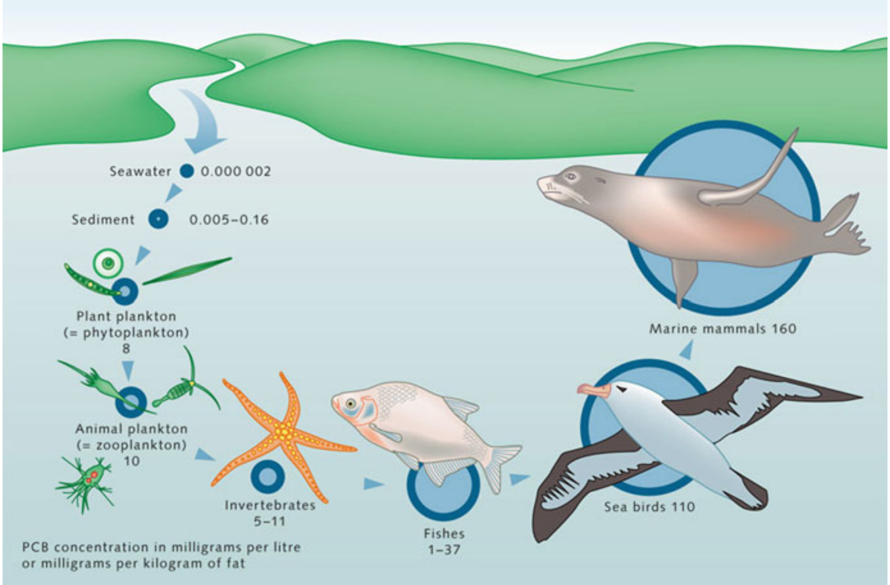
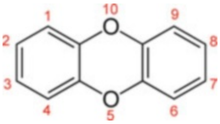


Fig. 1.4 Biomagnification of PCBs (From oceansoffun.org)

Fig. 1.5 Dioxin molecule, showing positions where Cl could be attached



chemicals, and incineration of waste including plastics,. Dioxin, a contaminant of Agent Orange, was found at Love Canal in Niagara Falls, NY and was the basis for evacuations at Times Beach, MO and Seveso, Italy. The industrial accident in Seveso led to many cases of Acquired Dioxin-Induced Skin Haematoma, or ADISH, in which the skin acquires disfiguring lumps. Perhaps the most famous case of ADISH was that of Viktor Yushchenko, who was poisoned while running for the presidency of Ukraine. The disfiguration led to the diagnosis. He went on to win the election, but had to undergo many surgical procedures to preserve his life. Like PCBs, dioxins are persistent in the environment and biomagnify in food chains.

1.1.3 Contaminants of Emerging Concern (CECs)

"Emerging contaminants" have been defined as any synthetic or naturally occurring chemical or microorganism that is not commonly monitored in the environment but has the potential to enter the environment and cause known or suspected adverse ecological or human health effects. In some cases, release of these contaminants to the environment has occurred for a long time, but the chemicals have not previously been regarded as contaminants and are widespread in the environment. They are derived from municipal, agricultural, and industrial wastewater sources. Some examples are: alkylphenols, used as detergents and known to disrupt the reproductive system; pharmaceuticals, and triclosan (trichlorohydroxydiphenyl ether), an antibacterial agent found in many personal care products and which has been identified as posing risks to humans and the environment.

Microplastics are both abundant and widespread in the oceans, found in highest concentrations along coastlines and within mid-ocean gyres. Ingestion of microplastics has been demonstrated in a range of marine organisms, a process which may facilitate the transfer of chemical additives or hydrophobic waterborne pollutants to biota.

Halogenated Organics

Polybrominated diphenylethers (PBDEs) are used as flame retardants on many consumer products. PBDEs are chemically similar to chlorinated hydrocarbons, with bromine instead of chlorine, and cause long-term adverse effects in humans and wildlife. They move from consumer products to the outdoor environment, and have been found in tissues of marine mammals in the Arctic, very far from sites of use. Fluorinated compounds are also of concern. Perfluorinated compounds (PFCs) are a family of manmade chemicals that are used to make products that resist heat, oil, stains, grease and water. Common uses include nonstick cookware, stain-resistant carpets and fabrics, coatings on some food packaging (e.g., microwave popcorn bags and fast food wrappers), and in fire-fighting foam. These chemicals, such as perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), are persistent and ubiquitous in the environment. PFCs are also likely to be toxic and have bioaccumulative properties. All of the chemicals listed above are, like DDT, PCBs and dioxin, halogenated – that is, include F, Cl or Br in their structure, which makes them resistant to microbial degradation.

Pharmaceuticals and Personal Care Products (PPCPs)

These contaminants are being discovered in our waters, as well as in fish tissue, at very low concentrations. Pharmaceuticals are prescription and over-the-counter therapeutic and veterinary drugs, including antibiotics, birth control pills, tranquilizers, etc., while personal care products include soaps, fragrances, sunscreen, and cosmetics. It is likely they have been present in the environment for as long as they have been in use. Many PPCPs remain in the environment because as they degrade, more are added, and their use is increasing. Because of increasing concentrations, effects in the environment are being noticed. When endocrine disruption was first being studied in aquatic biota in the early 1990s, people looked to the “usual suspects," the chlorinated hydrocarbons (which do have endocrine effects). Later it was noticed that estrogens themselves were in the water, coming from sewage treatment plants without being broken down, and it was realized that estrogens from birth control pills were playing a major role. Glucocorticoids are also found in the environment at concentrations higher than those of estrogens, which may be high enough to affect aquatic life. Even caffeine (found in many food and beverage products as well as some pharmaceuticals) has been found in coastal waters.

Nanoparticles

Nanomaterials or nanoparticles (<100 nm) are being used in many applications, including cosmetics, electronics, drug delivery, manufacturing, paints, and more. They may be composed of carbon (nanotubes, fullerenes), transition metals (gold, platinum, silver), metal oxides (titanium dioxide, zinc oxide), polystyrene, and silica, and are being manufactured in increasing amounts. Greater use of such products has led to their release into the environment in runoff and sewage effluent, and their accumulation in coastal environments. They have come under scrutiny as potential pollutants. For example, it was found that the nanoparticulate form of titanium dioxide (TiO2) exposed to ultraviolet radiation can be toxic to marine organisms (Miller et al. 2012).

While metal nanoparticles (NPs) may have fates similar to other forms of the same metal, metals in NPs may be tightly bound to the core material and not readily dissociate (Griffitt et al. 2008). However, Cleveland et al. (2012) studied fate of three nanosilver consumer products, two AgNP standards, and ionic silver (Ag^+) in estuarine mesocosms. The consumer product released significant amounts of Ag (>80 %) over 60 days, which moved from the water column into estuarine biota, including clams, grass shrimp, mud snails, cordgrass, biofilms, intertidal sediment, and sand. Ag was adsorbed from the water into the biofilms, sediment, and sand, then from the sand to the clams. Significant amounts were taken up by the organisms through trophic transfer.

Research is underway to develop analytical methods to measure these unregulated chemicals in water, sediment, and waste down to trace levels, determine their environmental occurrence, sources and pathways of release to the environment, their transport and fate, and potential ecological effects. There is a need to develop standardized analytical techniques and reliable extraction procedures, understand the role of wastewater treatment systems on the environmental fate of these contaminants, and characterize the mechanisms responsible for their transport and fate in the environment.

Noise Pollution

Though not fitting the definition of a "chemical," noise pollution in the ocean is another stress of emerging concern. For millions of years, the oceans have been filled with sounds from natural sources such as the clicks of whales and the snapping of shrimp. Many marine species have specialized hearing abilities, communication skills and echolocation abilities. However, humans generate a lot of sound. An increase in motorboats, primarily commercial shipping traffic, exploration and extraction of oil and minerals, air guns used for seismic exploration, sonar and even jet skis contribute to the increased level of underwater noise. Sound travels four times faster in water (1,230 m/s) than in air (340 m/s) so it travels farther under water. High intensity sound in the oceans can travel for thousands of miles. Since water is denser than air sound waves travel though water at higher energy levels and are hence louder. New sources of marine sound pollution have been added. One source having immediate and obvious negative effects has been the development and testing of "Low-frequency Active (LFA) Sonar" that has a potential worldwide deployment by the U.S. Navy. Several tests have resulted in large losses in marine life.

1.1.4 Nutrients and Sewage

Nutrient enrichment due to excessive amounts of nitrogen (N) is the primary cause of impaired coastal waters worldwide, while excessive phosphorus (P) tends to be associated with enrichment in fresh waters. Nitrogen occurs in several oxidized forms, collectively termed NO_x. N is an essential nutrient and a fertilizer that contributes to agricultural productivity but also a pollutant. It is a benefit or a hazard, depending on its form, location, and quantity. Human activities, primarily increased use of commercial fertilizers, have increased N inputs by ten-fold in many parts of the world. Another major source is burning of fossil fuels, which emit NO_x into the atmosphere, creating acid rain and air pollution as well as water pollution. Coastal oceans receive enormous inputs of nitrogen and phosphorus from sewage treatment plants, runoff of fertilizers and atmospheric deposition. Sewage, even after treatment, contains high levels of nutrients. Excess N flows from agricultural fields, suburban lawns, and stockyards, generally as ammonia and nitrate, entering freshwater and going down to estuaries via streams and rivers, altering water chemistry and ecological communities. It also is released from septic tanks and reaches coastal waters via groundwater, and comes down from the atmosphere in precipitation. These nutrients cause algal blooms, followed by hypoxia (low oxygen) in deeper waters (Fig. 1.6). The global rise in eutrophic and hypoxic events is due to increases in intensive agriculture, industrial activities, and population, which have increased N and P flows in the environment. There are variations in the importance of each source among regions. For example, in the U.S. and Europe, agricultural sources (animal manure and fertilizers) are generally the primary contributors, while sewage and industrial discharges (which usually receive treatment prior to discharge) are a secondary source. Atmospheric sources are also a significant contributor of N in coastal areas. N from fossil fuel combustion and volatilization from fertilizers and manure is released into the atmosphere and deposited on land

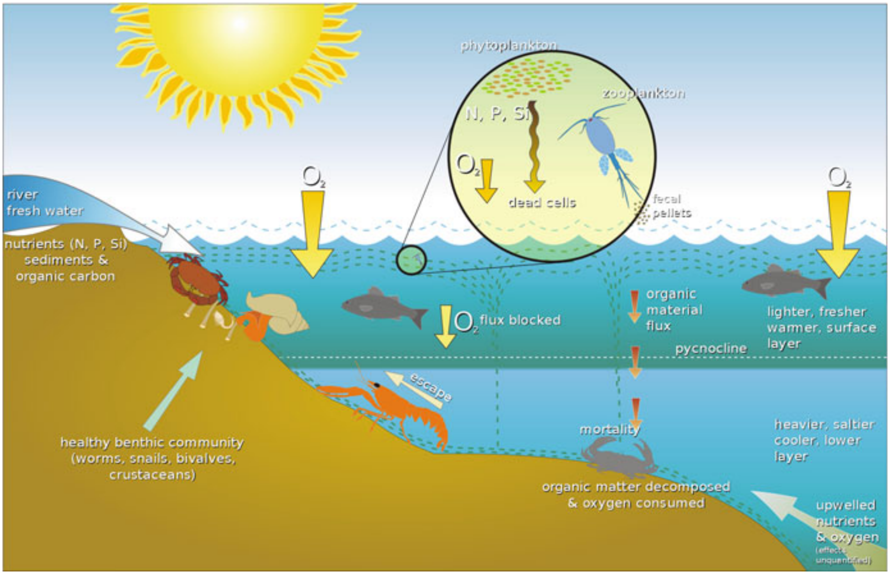


Fig. 1.6 Eutrophication diagram (Taken from Wikimedia (original source US EPA))

and water by wind and precipitation. In Chesapeake Bay, atmospheric sources account for a third of all controllable N that enters the bay. However, in Latin America, Asia, and Africa, wastewater from sewage and industry are often untreated and may be the primary contributors to eutrophication.

Nutrient enrichment of marine waters promotes excessive growth of algae, both attached multicellular forms (e.g., sea lettuce) and microscopic phytoplankton. Small increases in algal biomass can increase production in food webs sustaining fish and shellfish. However, over-stimulation of algal growth can severely degrade water quality and threaten human health and living resources. Some phytoplankton species, generally dinoflagellates, produce toxins that can impair respiratory, nervous, digestive and reproductive system function, and even cause death of fish, shellfish, seabirds, mammals, and humans. These harmful algal blooms (HABs) can cause fish kills, human illness through shellfish poisoning, and death of marine mammals and shore birds. HABs may be called "red tides" or "brown tides" because of water discoloration when these blooms occur. Their economic impacts can be severe, as shellfish harvest and fishing are closed. HABs have been increasing worldwide, and strong correlations have been shown between N input and phytoplankton production. There are examples in regions around the world such as Chesapeake Bay, the Inland Sea of Japan, the Black Sea, and Chinese coastal waters, where increases in nutrient loading have been linked with the development of large blooms, which can lead to toxic effects on ecosystems, fisheries resources, and human health (Anderson et al. 2002). Not only has the frequency of HABs been increasing, new toxin-producing species have been found to cause serious problems.

With species that do not produce toxins, algal blooms eventually die and sink and are degraded by bacteria, whose metabolism consumes oxygen. If the aeration of water by mixing is less than bacterial metabolism, the bottom waters will become hypoxic (low oxygen) or anoxic (devoid of oxygen), creating stressful or lethal conditions. This is a major problem in many estuaries, especially in late summer and early fall, and is termed eutrophication. Hypoxia has been increasing globally; increasing numbers of "dead zones" have been reported a result of fertilizer runoff and nitrogen deposition from fossil fuel burning. Eutrophication is especially harmful to coral reefs, where the nutrients cause benthic algae to proliferate and cover and smother the corals, eventually leading to the replacement of the coral community with an algal community, especially when grazers (e.g., parrot fish) are not plentiful (Bell 1992). Of the 415 areas around the world identified as experiencing some form of eutrophication, 169 are hypoxic and only 13 are classified as "in recovery." Seasonal occurrences of dead zones with no oxygen have expanded in the Gulf of Mexico (where the dead zone has approached the size of New Jersey) and many other regions worldwide. While trends show increases worldwide, some localized areas are improving (Diaz and Rosenberg 2008). Efforts to reduce the flow of fertilizers, animal waste and other pollutants into the Chesapeake Bay appear to be helping the bay's health. The size of mid- to late-summer "dead zones," in deep channels of the bay has been declining.

Sewage and nonpoint runoff also discharge microbes into the water. Microorganisms such as hepatitis A virus, and pathogenic bacteria (e.g., *Salmonella, Listeria monocytogenes, Vibrio cholerae* and *Vibrio parahaemolyticus*) have been reported in coastal waters. Coliform bacteria are a commonly used bacterial indicator of water pollution, although not an actual cause of disease. Microbiological contamination can occur in marine biota when sewage from humans or animals is discharged to coastal waters or arrives in river flow. High levels of pathogens may result from inadequately treated sewage, which may be from a sewage plant without secondary treatment. Older cities with aging systems may have leaky pipes, pumps, or valves, which can cause sanitary sewer overflows. Some older cities also have combined sewers (sanitary and storm sewers) which may discharge untreated sewage during rain storms when the volume of water exceeds the capacity of the system. Pathogens may be from animal feces, from poorly managed livestock operations or dense concentrations of wild animals. Bacteria and viruses from humans and animals, mainly attached to fine particulate matter, can affect bathing water quality and accumulate in filter feeding shellfish.

Microbial pollution by pathogens from sewage or animal waste is of concern for drinking water supplies, but is also a human health issue in coastal waters where swimmers may become ill after rain has washed bacterial pollution in from combined sewers or runoff from land. Infections and illness due to recreational water contact are generally mild and difficult to detect. Even when illness is more severe, it may still be difficult to attribute to water exposure. Epidemiological studies have shown gastrointestinal and respiratory infections associated with polluted recreational water. Bathing beaches may be closed by officials when they have excessive levels of bacterial pollution. Another concern is accumulation of pathogenic bacteria in edible shellfish. Since many estuaries are contaminated by municipal and agricultural wastes, bacteria and viruses can cause disease associated with shellfish consumption. Gastroenteritis and hepatitis A are the most important diseases transmitted to humans through shellfish, but cholera and typhoid fever were the first to be linked to consumption of contaminated shellfish. Viral outbreaks are also associated with eating contaminated shellfish. Initially, the analysis of outbreaks was based on epidemiological data but advances in molecular biology and the ability to detect low levels of enteric viruses in shellfish has provided more accurate assessment of shellfish as a path for disease transmission. Shellfish beds are closed when tests indicate elevated bacterial levels. Microbiological quality of coastal waters has been improving following better waste water treatment.

1.1.5 Carbon Dioxide, Climate Change and Ocean Acidification

The burning of fossil fuels emits carbon dioxide into the atmosphere, which results in the “greenhouse effect,” raising the earth’s temperature. Studies confirm that in the past century the oceans have warmed by about 1 °F to a depth of 200 ft, and the overwhelming scientific consensus is that increasing levels of human-caused greenhouse gases in the atmosphere are the principal cause. As surface water warms, vertical water movements (upwelling) which bring nutrients up to surface waters where most phytoplankton are found, are reduced and thermal stratification increases. There is a negative relationship between ocean temperatures in the tropics and subtropics and productivity of plankton, probably because of reduced upwelling and increased stratification. Much of the CO_2 is absorbed by the ocean where it is converted to carbonic acid, which releases hydrogen ions into the water, reducing its pH and making it more acidic. Since the industrial age began, the pH of the oceans has declined by 0.1 pH unit, which, because the pH scale is logarithmic, represents a 30 % increase in acidity. According to projections of the IPCC (Intergovernmental Panel on Climate Change), pH values will decrease another 0.2–0.3 units by 2100, thus doubling the current acidity.

The extent to which human activities have raised ocean acidity has been difficult to calculate because it varies naturally between seasons, from one year to the next, and between regions, and direct observations go back only 30 years. Combining computer modeling with observations, Friedrich and colleagues (2012) concluded that CO_2 emissions over the last 100–200 years have already raised ocean acidity far beyond the range of natural variations. The excess hydrogen ions reduce seawater concentrations of carbonate ions. They studied changes in the saturation level of aragonite (a form of calcium carbonate) typically used to measure of ocean acidification. As seawater becomes more acid, the saturation level of aragonite drops. Their models captured the current seasonal and annual variations of aragonite in coral reefs, where today's levels of aragonite saturation are already five times below the pre-industrial range. The saturation state, denoted by the Greek letter Ω, refers to the degree to which seawater is saturated with a carbonate mineral and is inversely proportional to the mineral's solubility. The saturation state is determined by the concentration of calcium and carbonate ions in relation to the solubility coefficient for the particular calcium carbonate mineral (aragonite, calcite). Aragonite saturation is very sensitive to acidity because it is more soluble. It is also the form most often used for mollusk shell formation, so this can affect the growth of these organisms. When the saturation state equals 1, there is an equal chance of dissolution or formation of calcium carbonate; when it is <1 dissolution is favored, and when it is >1 formation of calcium carbonate is favored. If the aragonite saturation state falls below 1 (undersaturation) already-formed shells will dissolve. If it falls below 1.5, some organisms are unable to build new shells. The saturation state is highest in shallow warm tropical waters and lowest in deep and cold high latitude waters (Feely et al. 2004). This suggests that effects of acidification will be more severe in cold high latitudes.

Acidification can interact with eutrophication. When nutrient-rich river water enters coastal waters, phytoplankton bloom. When the algae die, sink to the sea floor and decompose, carbon dioxide is released and oxygen depleted. The dissolved CO_2 reacts with water, forming carbonic acid. Ocean acidity also increases when excess carbon dioxide is absorbed from the air at the ocean's surface. The combination of these two sources of CO_2 increases acidity beyond what would be expected from the individual processes (Cai et al. 2011).

Rising temperatures due to increases in greenhouse gases are also warming the surface water of the oceans. Thermal expansion as well as increased meltwater and discharged ice from terrestrial glaciers and ice sheets are causing sea level to rise. Warmer ocean currents also can move migrating fish and invasive species to areas they previously didn't previously inhabit, and alter timing of reproduction or migration. Warm water holds less oxygen, and tends to amplify the threats of toxic pollution.

1.1.6 Litter, Marine Debris

Marine debris is any man-made object discarded, disposed of, or abandoned that enters the coastal or marine environment. It may enter directly from a ship, or indirectly when washed out to sea. Materials can be dumped, swept, or blown off vessels and platforms at sea. Sources of the debris are littering, dumping in rivers and streams, and industrial losses, e.g. spillage of materials during production, transportation, and processing. It is estimated that about 14 billion pounds (6.4×10^9 kg) of trash end up in the oceans every year. Plastics comprise a large proportion of the debris, and the variety and quantity of plastic items has increased dramatically, including domestic material (shopping bags, cups, bottles,

Fig. 1.7 Marine litter (From NOAA)

bottle caps, food wrappers, balloons) (Fig. 1.7), industrial products (strapping bands, plastic sheeting, hard hats, resin pellets), and lost or discarded fishing gear (nets, buoys, traps, lines).

Glass, metal, styrofoam, and rubber are used for a wide range of products. While they can be worn away – broken down into smaller and smaller fragments, they generally do not biodegrade entirely. As these materials are used commonly, they are common in marine debris. Derelict fishing gear includes nets, lines, crab/shrimp pots, and other recreational or commercial fishing equipment that has been lost, abandoned, or discarded in the marine environment. Modern gear is generally made of synthetic materials and metal, so lost gear can persist for a very long time.

Marine debris accumulates along shorelines and in coastal waters, estuaries, and oceans throughout the world. It can be blown by the wind, or follow the flow of ocean currents, often ending up in the middle of oceanic gyres where currents are weakest. The Great Pacific Garbage Patch is one such example; comprising a vast region of the North Pacific Ocean. Estimated to be double the size of Texas, the area contains over 3 million tons of plastic, mostly in very small pieces. Islands within gyres frequently have their coastlines covered by litter that washes ashore; prime examples being Midway and Hawaii, where plankton tows sometimes come up with more plastic pieces than plankton. The next biggest known marine garbage patch is the North Atlantic Garbage Patch, estimated to be some hundreds of km across. All estimates of the amount of litter are underestimates. Wind pushes the lightweight plastic particles below the surface, suggesting that research into how much plastic litter is in the ocean conducted by skimming the surface may vastly underestimate the true amount (Kukulka et al. 2012).

In addition to the visible litter that washes up on beaches, microscopic plastic debris from washing clothes is accumulating in the marine environment and could be entering the food chain. Researchers traced the “microplastic” back to synthetic clothes, which release up to 1,900 tiny fibers per garment every time they are washed (Browne et al. 2011). Earlier research showed plastic smaller than 1mm was being eaten by animals and getting into the food chain. In order to identify how widespread the presence of microplastic was on shorelines, the team took samples from 18 beaches around the globe, and found that samples contained pieces of microplastic. Polyester, acrylic and polyamides (nylon) were the major ones, and their concentration was greatest near large urban areas. They found exactly the same proportion of plastics in sewage, which led them to conclude that sewage was the source of the fibers.

1.2 Measuring Effects on Biota

Toxic effects, both lethal and sublethal, have been documented extensively in laboratory experiments. There have been fewer field studies of effects on populations of marine organisms. Early work on pollutant effects relied on tests that measured lethality. The LC_{50} – the concentration of a toxicant that produced 50 % mortality – was the benchmark. Regulations under FIFRA (the U.S. Federal Insecticide Fungicide and Rodenticide Act) for developing criteria for pesticides for the protection of aquatic life require standard endpoints, the LC_{50}, which is of little ecological relevance. Toxicity tests are required for a few species: rainbow trout, bluegill, and daphnids – one cold-water fish, one warm water fish and one crustacean – all freshwater. Unfortunately, even today, over half a century later, many studies still rely on this approach – acute toxicity tests – that are still considered most useful in a regulatory context. These tests do not consider sublethal toxicity or toxicity that is delayed in time, or differences in life history among species. Knowing sublethal effects of chronic lower dose exposures on physiology, behavior, development, etc., is essential for understanding ecological impacts of pollutants in nature and is the focus of this book.

Extensive research has shown that toxicants can disrupt the metabolic, regulatory, or disease defense systems, eventually compromising survival or reproduction. Sublethal effects can lead to understanding of mechanisms and also to understanding of ecological effects in the “real world.” Another insight is that early life stages – gametes, fertilization, embryonic and larval development – are most sensitive to stresses including contaminants. The hormonal control of reproduction can be affected by many contaminants, now called “endocrine disruptors.” Exposures during early life stages may cause effects that appear later, sometimes many years later. Thus, long-term delayed effects and indirect effects are important to evaluate. There has been a trend towards greater ecological realism in ecotoxicology, but advances have been mainly in freshwater ecosystems.

1.2.1 Hormesis

In toxicological investigations, whatever the endpoint, the degree of response typically goes up with the dose, a fundamental principle of toxicology. However, this is not always the case; there are cases in which low levels of a toxicant produce "positive" effects, while higher concentrations produce "negative" effects (e.g., increased growth vs. reduced growth). In these cases, the dose response curve is J-shaped, or inverted U- shaped. Some of the early reports of this phenomenon, termed "hormesis", were made with marine organisms. Laughlin et al. (1981) reported that crab zoeae (*Rhithropanopeus harrisii*) exposed to low concentrations of jet fuel oil had increased weight above that of controls. Stebbing (1981) similarly found increased growth in colonies of the hydroid *Campanularia flexuosa* in low concentrations of cadmium and copper. Many studies on different organisms with different toxicants have shown a similar pattern, which led Calabrese and Baldwin (1997) to conclude that hormesis is generalizable with respect to organism, endpoint, and chemical class, though this has not been universally agreed upon.

1.2.2 Mechanistic Approaches

A dominant approach of ecotoxicology focuses on discovering mechanisms of action at the biochemical level. There is considerable effort and an enormous literature devoted to development of biochemical biomarkers, which are measured in tissues and body fluids of organisms exposed to environmental chemicals. These responses presumably occur prior to effects at higher levels of organization and can be early warning signals. This reductionistic approach is useful for learning molecular mechanisms, but it cannot predict effects on whole organisms, populations, communities, or ecosystems. Many are biomarkers of exposure, rather than of effects, and do not necessarily link to impairment of growth, reproduction, energy utilization, etc. However, in some cases such connections are made. For example, Sanders et al. (1991) related changes in stress proteins to scope-for-growth measurements in Cu-exposed mussels, which relates to fitness and could have consequences at the population level. Integrated measures have been developed, such as scope-for-growth, which is an indication of energy status based on the concept that energy needed for detoxification will reduce the amount available for growth. The vast literature on biochemical biomarkers is the subject of many books but is barely covered here, where the focus is on organism-level responses. These processes, including respiration, osmoregulation, energy metabolism, excretion, growth, reproduction, behavior, etc., must function appropriately for individuals and populations to thrive. While most studies have been in laboratory organisms (which may be exposed to far higher concentrations of chemicals than occur naturally) some studies have measured these processes in organisms from contaminated sites. Automated monitoring systems have been developed to measure rates of physiological processes and certain types of behavior (Depledge et al. 1995).

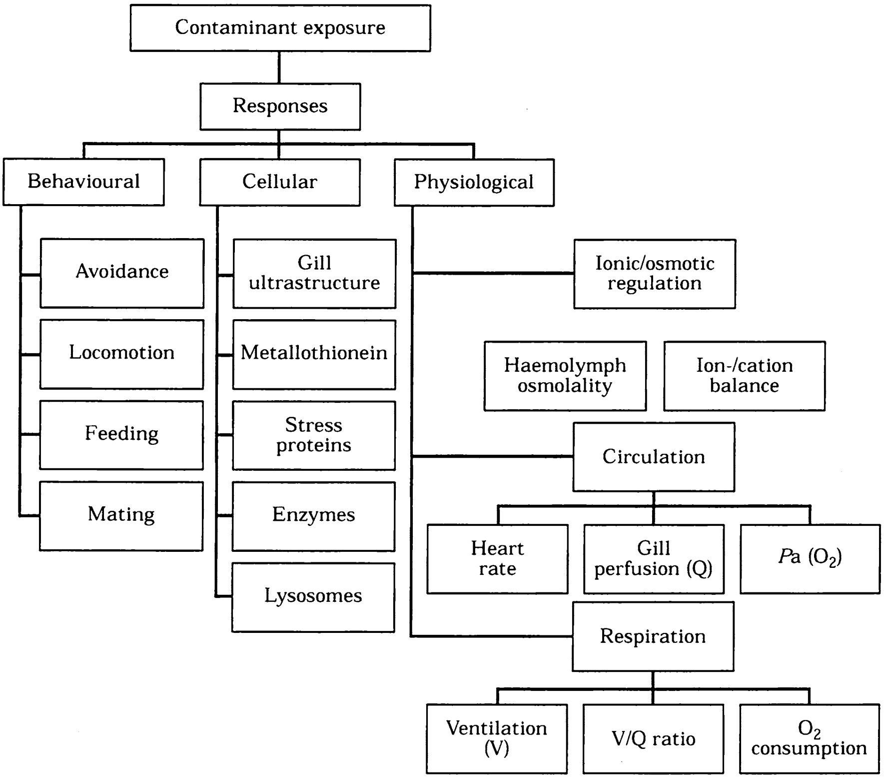


Fig. 1.8 Compensatory responses of *Carcinus maenas* following exposure to copper (Reprinted from: Hebel et al. 1997: 178, courtesy Elsevier Publishing Company)

1.2.3 Linked Responses – Metals

Comprehensive linked responses of many systems to the same stressor can be found. For example, Hebel et al. (1997) examined in a holistic way the responses of the crab *Carcinus maenas* to copper, including behavioral (locomotion, feeding, mating), physiological (osmoregulation, circulation, respiration), and cellular responses (lysosomal changes, induction of metallothioneins (MT) and stress proteins, and altered enzyme activity) (Fig. 1.8). A proposed sequence of responses is as follows: when the contaminant is first detected, the crab may attempt to avoid it and leave the area. If the chemical interferes with chemoreception, other behaviors such as reduced feeding will follow. Copper enters the crab via the gills, causing changes in cardiac and ventilatory activity. Effects on osmoregulation may follow, and some days later gill structural damage will occur, further impairing osmoregulation. The crab may increase its ventilation to compensate, but oxygen uptake will probably decline nevertheless. Detoxification mechanisms will be activated such as formation of granules to excrete excess metal. At the molecular level MT synthesis and stress proteins will be increased, which may or may not allow repair of damage and restoration of normal function.

1.2.4 Field Studies

Integrated field approaches focused on ecological effects are essential complements to laboratory studies and can produce insights into effects at the population and community level. Field experiments can investigate contaminated environments, but only under very restricted conditions can scientists release known amounts of chemicals in the field to observe effects in a controlled experimental way. Attempts to bring the field closer to the lab include studies on multiple species in microcosms or mesocosms, which can be used to reveal community effects of contaminants. They allow for replication, so dose-response relationships under controlled conditions can be ascertained. They can account for differential sensitivity of different species, and biological interactions. There is much to be learned from such approaches. However, dosing of complicated mesocosms with known concentrations of specific chemicals does not really replicate the natural environment in which organisms are subjected to many different pollutants at different concentrations, and where some biota may have evolved increased resistance to some contaminants. Thus, there remains uncertainty with ecological risk assessments and with upscaling mesocosm studies to be consistent with field situations.

It is generally difficult to attribute problems seen in the field to particular contaminants since field sites generally have multiple contaminants. In some rare cases initial observations on natural populations in the field called attention to toxic effects of certain chemicals. This was the case with tributyltin (TBT) effects on oysters in Europe (see Chap. 8). Since abnormalities produced by TBT are unlike those produced by other chemicals, making the connection between effects observed (abnormal shells in oysters, imposex in snails) and the particular chemical (TBT) was easier (Alzieu et al. 1986).

At the level of the organism, life history strategies are related to the severity of effects of contaminants. Differential sensitivity can be related to physiological differences, generation time, and life cycle characteristics, which can all affect initial effects and the ability to recovery from the effects. Species that are “r-selected,” that tend to be opportunistic, short-lived, and produce large numbers of offspring, can exploit changing environments, including contaminated ones. High metabolic rates can lead to more rapid biotransformation. In contrast, “k-selected” species, which are long-lived, slow to mature, and have relatively few offspring, are suited to stable environments and less likely to be resistant to or to evolve resistance to contaminants. Long-lived species tend to be at higher trophic levels and accumulate greater body burdens of persistent contaminants over a long period of time. Their slow reproduction makes population recovery from declines very slow. Slow reproduction, combined with high bioaccumulation of contaminants makes them particularly vulnerable to reproductive effects. Maternal transfer of lipophilic contaminants (e.g., PCBs, DDT) into the yolk of developing oocytes, exposes the next generation even before they are hatched (Rowe 2008).

1.2.5 Oil

Early studies after oil spills focused on short-term monitoring and tests of acute toxicity in the laboratory. Approaches to studying sublethal effects in the laboratory include the use of the water-soluble fraction of oil or of particular PAHs. However, after the *Exxon Valdez* spill in Alaska it became clear that long-term impacts at the population level, as well as interactions among species and abiotic variables, need to be considered (Peterson et al. 2003).

Since the 1970s, it has been known that application of dispersants increases toxicity by increasing hydrocarbon exposure to water column species. Many scientists are concerned about the likelihood of severe, acute impacts on Gulf species exposed to Corexit® and oil in the water column. For vulnerable species such as seagrass, corals, plankton, shrimp, crabs, and small fish, acute effects can be lethal, particularly during the spring spawning season (Rhoton et al. 1998; Bhattacharyya et al. 2003; Chapman et al. 2007; Anderson et al. 2009; Couillard et al. 2005; Ramachandran et al. 2004; Fisher and Foss 1993). Coral larvae are extremely sensitive to the combined effects, with 0 % fertilization in the presence of dispersant and dispersed oil, compared with 98 % fertilization in the presence of oil alone (Negri and Heyward 2000; Shafir et al. 2007; Epstein et al. 2000).

1.2.6 CECs

Nanoparticles are likely to have enhanced toxicity due to their size, which facilitates movement across cellular membranes and into organelles. Inside cells, NPs can stimulate the formation of reactive oxygen species (ROS) that interfere with structural integrity of DNA, proteins, and cell membranes (Moore 2006). Studies on fish and invertebrates reveal that uptake and harmful effects are possible (Gagné et al. 2008; Griffitt et al. 2008). Fullerenes and nanotubes produced adverse effects on adult fish (Smith et al. 2007), and metal NPs caused deleterious effects in several fish species (Asharani et al. 2008) and invertebrates (Griffitt et al. 2008; Gagné et al. 2008). Most nanoparticle types present in the aquatic environment, such as titanium dioxide, have low acute toxicity, but display sublethal effects (Jovanovic and Palic 2012).

Noise pollution can cause lethal and sublethal effects. Most animals are alarmed by the sounds, which may damage internal organs especially ears, and cause a panic response. Normal communication between marine animals can be disrupted by noise. Scientists are working on the question of which frequencies and at what levels noise negatively effects marine life. The death of animals, especially cetaceans, often occurs hours after exposure to extreme underwater noise. For example, whales die after beaching themselves shortly after a tactical sonar exercise; this is a rather common occurrence. Such beachings have been reported in Greece, Madeira, Hawaii, Spain and the coastal US- areas where sonar exercises are common. In March 2000, at least 17 whales stranded themselves in the Bahamas, and a federal investigation identified testing of a U.S. Navy active sonar system as the cause. Other taxa are also sensitive. Giant squid were found dead along the shores of Spain in 2001 and 2003 following the use of air guns by offshore vessels; examinations indicated that the deaths were related to excessive sound exposure. André et al. (2011) examined the effects of low frequency sound exposure – similar to what the giant squid would have experienced – in four cephalopod species, and found that all of the exposed squid, octopus and cuttlefish exhibited massive acoustic trauma in the form of severe lesions in their auditory structures.

1.2.7 Nutrients

The two major symptoms of eutrophication are hypoxia and harmful algal blooms, both of which can destroy aquatic life in affected areas. Hypoxia occurs after algae die, sink to the bottom, and are decomposed by bacteria, using up the available dissolved oxygen. Salinity and temperature differences between surface and deep waters lead to stratification, limiting the ability to replenish oxygen from surface waters and creating conditions that lead to formation of a hypoxic or “dead” zone. Hypoxia is a chronic stress for organisms in or near the bottom, and there have been numerous studies of this stress alone and combined with effects of chemical contaminants. Hypoxia occurs when the dissolved oxygen (DO) falls below ≤ 2 ml of O_2/l, which may cause benthic species to abandon burrows for the sediment-water interface, eventually dying when DO falls below 0.5 ml/l. Dead zones in the coastal oceans have increased greatly since the 1960s, including continental seas, such as the Baltic Sea, Kattegat, Black Sea, and East China Sea, as well as the Gulf of Mexico.

Pelagic species experience habitat compression when hypoxia makes deeper, cooler water unavailable in the summer or overlaps with nursery habitat. Blue marlin, other billfish and tropical tuna are rapid swimmers that need high dissolved oxygen, and the expansion of dead zones shrinks the useable habitat for these valuable pelagic fishes. Ecosystems exposed to long periods of hypoxia have low secondary production and little or no benthic fauna. Factors determining the degree of degradation are the duration of exposure and DO concentration. It may take years to recover from severe hypoxia and the species that establish during recovery will likely not be the same as the pattern of species loss during DO depletion (Diaz and Rosenberg 2008). Hypoxia can interact with chemical contaminants to increase stress. Particularly sensitive ecosystems include submerged aquatic vegetation (e.g., eel grass), which dies as excessive algal growth reduces light penetration. Coral reefs are another particularly sensitive ecosystem, where excess nutrients alter the normal balance between corals and algae. Eutrophication and herbivore overfishing promote seaweed overgrowth and reef degradation. Harmful algal blooms (HAB) of species that produce toxins, can cause fish kills, human illness through shellfish poisoning, and death of marine mammals and birds.

1.2.8 Climate Change

The warming of the ocean will have numerous effects on all organisms, most basically elevating their metabolic rates, which ultimately determine life history traits, population growth, and ecosystem processes. Elevated metabolic rates create increased demand for oxygen at the same time that the warmer water can hold less oxygen. Variation in temperature can also affect biological processes such as the abundance and distribution of plankton. As the ocean surface warms, it becomes more stratified or confined to layers that mix less than they did in the past, which will reduce overall ocean productivity, because nutrients in surface water get depleted and plankton productivity depends on upwelling of deeper water to replenish nutrients. The annual primary production of the world has decreased since the 1980s (Hoegh-Guldberg and Bruno 2010). Studies suggest that increasing acidity reduces the availability of iron, an element crucial to phytoplankton production. Because iron already is limited in marine waters, increased acidity may have serious implications (Shi et al. 2010).

Among the most sensitive groups of organisms are organisms such as sea grasses, mangroves, salt marsh grasses, oysters, and corals, which form the habitat for thousands of other species. Intertidal mangroves and salt marshes are threatened by rising sea levels, and will have to migrate inland or elevate in order to prevent themselves from being submerged. Current and future CO_2 levels will result in changes in ocean temperature and chemistry beyond those that corals have experienced. Some scientists suggest that conditions have already reached a “tipping point” for coral survival, and corals now are less able to recover from additional change (Eakin et al. 2009; Anthony et al. 2008). They are considered one of the most sensitive ecosystems to climate change and can be likened to the canary in the coal mine. Coral reefs have been in existence for over 500 million years, but their continued persistence is in doubt. With increases in ocean temperature, corals begin to bleach (Fig. 1.9). Bleaching occurs when the corals lose their symbiotic relationship with single-celled photosynthetic algae, zooxanthellae, which provide corals with food and receive protection and nutrients needed for photosynthesis. Zooxanthellae are sensitive to temperature changes, and when they die or leave, bleached corals are generally unable to meet their energy requirements. Coral death by bleaching and diseases due to increased heat and irradiation, as well as the decline in calcification caused by ocean acidification are the most important large-

Fig. 1.9 Normal and bleached areas of coral (From NOAA)

scale threats. Since the 1980s, major bleaching events have increasingly occurred across the globe – for example, in 1998, 80 % of the coral reefs in the Indian Ocean bleached, causing 20 % mortality.

Unlike rising temperature, research on ocean acidification is relatively recent. However, changing pH levels can cause dissolution of a coral's calcium carbonate skeleton and impair calcification. Increasing CO_2 may be an additional stress driving a shift from corals to seaweeds on reefs. Coral (*Acropora intermedia*) mortality in contact with a common coral-reef seaweed (*Lobophora papenfussii*) increased two- to threefold between background CO_2 (400 ppm) and a level projected for late twenty-first century (1,140 ppm). The interaction between CO_2 and seaweeds on coral mortality was attributed to a chemical competitive mechanism. Thus, coral reefs may become more susceptible to seaweed proliferation under ocean acidification (Diaz-Pulido et al. 2011).

Polar ecosystems are also vulnerable to effects of climate change. Their temperatures are increasing more rapidly than elsewhere (>5 times the global average). Researchers have documented that warming ocean currents have accelerated melting of the Arctic sea ice sheet and the decline and breakup of Antarctic ice shelves. Greenland is now losing an estimated 100 billion tons of ice annually as a result of accelerated melting. Sea levels are now projected to rise much faster than predicted by the Intergovernmental Panel on Climate Change in 2007, because of this acceleration, further threatening coastal habitats (Schofield et al. 2010). As the temperature has risen, plankton blooms, typical of the region, have decreased, and the plankton community has shifted from large species to smaller ones. The shift in phytoplankton biomass and size has affected the zooplankton. Krill, which are inefficient at grazing on small phytoplankton, are declining, while salps, which are efficient, are increasing. Krill also depend on sea ice for their reproduction. Furthermore, other species that depend on the ice, like Adelie penguins, are also decreasing, while other penguin species have increased (Schofield et al. 2010).

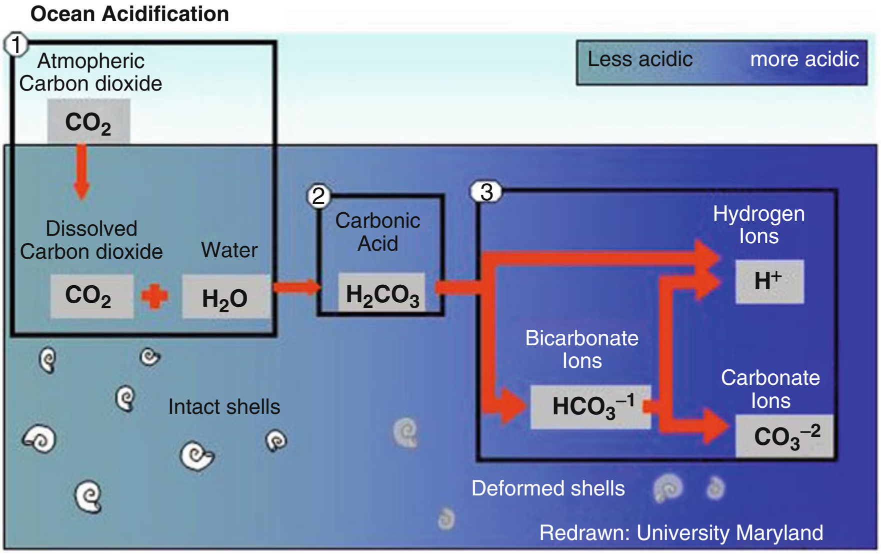


Fig. 1.10 Ocean acidification (Taken from Chesapeake Quarterly, Maryland Sea Grant College Program)

Along with physiological effects on organisms, temperature stress affects predator/prey interactions. Many intertidal organisms already live very close to their thermal tolerance limits. At cooler sites, mussels and rocky shore barnacles were able to live high on the shore, well beyond the range of their aquatic predators. However, as temperatures rose, they were forced to live at lower shore levels, placing them at the same level as predatory sea stars (Harley 2011). Daily high temperatures during the summer months have increased by almost 3.5° C in the last 60 years, causing the upper limits of the habitats to retreat 50 cm down the shore, while effects of predators, and the position of the lower limit, have remained constant.

The increased acidity of the oceans is expected to harm a wide range of ocean life – particularly those with shells (Fig. 1.10). Many organisms use calcium and carbonate ions from seawater to produce calcium carbonate for shells.

Some acidity is natural in some regions, even without added CO_2 from human activities. Water off the Pacific coast of the United States already has a low carbonate saturation state. When surface winds blow the top layer of water out from coastal regions, deeper water with high acidity (“corrosive water”) can upwell, and produce deleterious effects. The common mineral forms of calcium carbonate are aragonite and calcite. Aragonite calcifiers (corals, pteropods, bivalves) are expected to be more strongly affected than calcite calcifiers (coralline algae, sea urchins) because of differences in the solubility of the mineral: aragonite is more soluble than calcite. The resulting disruption to the ocean ecosystem could have widespread effects and further deplete already struggling fisheries worldwide. Research suggests that larval mollusks and other calcifying organisms are already experiencing these effects.

Delicate corals may face an even greater risk than shellfish because they require very high levels of carbonate to build their skeletons. Acidity slows reef-building, which could lower the resiliency of corals and lead to their erosion. Coral reefs are home for many other forms of ocean life, so their loss would reverberate throughout the marine environment and have profound social impacts in the tropics – especially on fishing and tourism. The loss of coral reefs would also reduce the protection that they offer coastal communities against storms surges and hurricanes, which might become more severe with warmer air and sea surface temperatures.

1.2.9 Marine Debris/Litter

Effects of marine litter are primarily physical rather than chemical. Marine debris takes a toll on the marine environment by affecting animals through ingestion or entanglement; it is estimated that up to 100,000 marine mammals, including endangered species, are killed each year by marine debris. Many marine animals consume flotsam by mistake, as it often looks similar to their natural prey. Sea turtles, for example, may mistake plastic bags or balloons for jellyfish. Marine debris is ingested by marine animals, including fishes, birds, sea turtles and marine mammals. Avery-Gomm et al. (2012) quantified the stomach contents of 67 Northern fulmars (*Fulmarus glacialis*) from beaches in the eastern North Pacific in 2009–2010 and found that 92.5 % of the birds had ingested an average of 36.8 pieces, or 0.385 g of plastic. Plastic ingestion in these fulmars is among the highest recorded globally. Compared to earlier studies in the North Pacific, these findings indicate an increase in plastic ingestion over the past 40 or so years. Plastic debris may become lodged in digestive tracts, blocking the passage of food and causing death through starvation. Tiny floating particles also resemble zooplankton, which can lead filter feeders to consume them and cause them to enter the food chain. In addition, hydrophobic pollutants collect on the surface of plastic debris, thus making plastic a source of toxicity, by transferring chemicals such as PCBs into the food web. Worldwide efforts are underway to monitor and remove marine debris, as well as to prevent further pollution by controlling litter or trash at its source. One unexpected finding was that the litter provided a habitat for the marine insect *Halobates sericeus*. These sea skaters, relatives of pond water skaters, inhabit water surfaces and lay eggs on flotsam (floating objects). Goldstein et al. (2012) found that they have exploited the plastic garbage as new surfaces for their eggs, leading to a rise in egg densities in the North Pacific Subtropical Gyre.

1.2.10 Survival in Contaminated Environments

The combined effects of many anthropogenic stressors pose major challenges to organisms in estuaries, and reduces biological diversity in contaminated environments.

The species that remain have some degree of inherent resistance to the contaminants they are subject to, and have developed adaptive strategies to reduce pollution impacts – such as increased resistance via plasticity or via selection over many generations. Numerous studies have been done examining the processes by which organisms detoxify metals (e.g., metallothioneins) and organic contaminants (CYP, or cytochrome P-450 system). The CYP system is important in metabolizing organic contaminants such as PAHs (Stegeman and Lech 1991). Other resistance mechanisms include reducing uptake of contaminants by being less permeable, and mucus production – excess mucus is shed from an organism, taking with it whatever was irritating the organism. Changing the energy budget is another common strategy – since they must spend more energy on detoxification, animals generally reduce their respiration and activity rates to maintain their energy balance.

While some organisms are able to develop tolerance to pollutants in their environment, this comes at a cost. A reduction of marine pollution in estuaries and oceans should be a long-term goal of policy-makers in order to protect the health of the oceans, their inhabitants, and humans.

References

Alzieu C, Sanjuan J, Deltreil JP, Borel M (1986) Tin contamination in Arcachon Bay: effects on oyster shell anomalies. Mar Pollut Bull 17:494–498

Anderson DM, Glibert P, Burkholder JM (2002) Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries 25:704–726

Anderson BS, Arenella-Parkerson D, Phillips BM, Tjeerdema RS, Crane D (2009) Preliminary investigation of the effects of dispersed Prudhoe Bay Crude Oil on developing topsmelt embryos, *Atherinops affinis*. Environ Pollut 157:1058–1061

André M et al (2011) Low-frequency sounds induce acoustic trauma in cephalopods. Front Ecol Environ 9:489–493

Ankley GT, Di Toro DM, Hansen DJ, Berry WJ (1996) Technical basis and proposal for deriving sediment quality criteria for metals. Environ Toxicol Chem 15:2056–2066

Anthony KRN, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O (2008) Ocean acidification causes bleaching and productivity loss in coral reef builders. Proc Natl Acad Sci USA 105:17442–17446

Asharani PV, Wu YL, Gong Z, Vallyavettil S (2008) Toxicity of silver nanoparticles in zebrafish models. Nanotechnol 19:255102. doi:10.1088/0957-4484/19/25/255102

Avery-Gomm S, O'Hara PD, Kleine L, Bowes V, Wilson LK, Barry KL (2012) Northern fulmars as biological monitors of trends of plastic pollution in the eastern North Pacific. Mar Pollut Bull 64:1776–1781

Banks JL, Ross DJ, Keough MJ, Eyre BD, Macleod CK (2012) Measuring hypoxia induced metal release from highly contaminated estuarine sediments during a 40 day laboratory incubation experiment. Sci Total Environ 420:229–237

Bell PR (1992) Eutrophication and coral reefs – some examples in the Great Barrier Reef lagoon. Wat Res 26:553–568

Berry WJ et al (1996) Predicting the toxicity of metals-spiked laboratory sediments using acid-volatile sulfide and interstitial water normalization. Environ Toxicol Chem 15:2067–2079

Bhattacharyya S, Klerks PL, Nyman JA (2003) Toxicity to freshwater organisms from oils and oil spill chemical treatments in laboratory microcosms. Environ Pollut 122:205–215

Browne MA, Crump P, Nivens SJ, Teuten E, Tonkin A, Galloway T, Thompson R (2011) Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ Sci Technol 45:9175–9179

Cai WJ et al (2011) Acidification of subsurface coastal waters enhanced by eutrophication. Nat Geosci 4:766–770. doi:10.1038/ngeo1297

Calabrese EJ, Baldwin LA (1997) A quantitatively-based methodology of the evaluation of chemical hormesis. Hum Ecol Risk Assess 3:545–554

Camilli R, Reddy CM, Yoerger DR, Van Mooy BA, Jakuba MV, Kinsey JC, McIntyre CP, Sylva SP, Maloney JV (2010) Tracking hydrocarbon plume transport and biodegradation at Deepwater Horizon. Science 330:201–204

Chapman H, Purnell K, Law RJ, Kirby MF (2007) The use of chemical dispersants to combat oil spills at sea: a review of practice and research needs in Europe. Mar Pollut Bull 54: 827–838

Cleveland CJ (Lead Author), NOAA (Content Source), Saundry P (Topic Editor) (2010) Exxon Valdez oil spill. In: Cleveland CJ (ed) Encyclopedia of earth. Environmental Information Coalition, National Council for Science and the Environment, Washington, DC

Cleveland D, Long SE, Pennington PL, Cooper E, Fulton MH, Scott GI, Brewer T, Davis J, Petersen EJ, Wood L (2012) Pilot estuarine mesocosm study on the environmental fate of silver nanomaterials leached from consumer products. Sci Total Environ 421–422:267–272

Couillard CM, Lee K, Légaré B, King TL (2005) Effect of dispersant on the composition of the water-accommodated fraction of crude oil and its toxicity to larval marine fish. Environ Toxicol Chem 24:496–1504

Depledge M, Aagaard A, Györkös P (1995) Assessment of trace metal toxicity using molecular, physiological, and behavioral biomarkers. Mar Pollut Bull 31:19–27

Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. Science 321:926–929

Diaz-Pulido G, Gouezo M, Tilbrook B, Dove S, Anthony KR (2011) High CO_2 enhances the competitive strength of seaweeds over corals. Ecol Lett 14:156–162

Eakin CM, Lough JM, Heron SF (2009) Climate variability and change: monitoring data and evidence for increased coral bleaching Stress. In: Van Oppen MJH, Lough JM (eds) Coral bleaching: patterns, processes, causes and consequences. Springer, Berlin, pp 41–67

Epstein N, Bak RP, Rinkevich B (2000) Toxicity of third generation dispersants and dispersed Egyptian crude oil on Red Sea coral Larvae. Mar Pollut Bull 40:497–503

Feely RA, Sabine CL, Lee K, Berelson W, Kelpas JA, Fabry VJ, Millero FJ (2004) Impact of anthropogenic CO_2 on the $CaC0_3$ system in the oceans. Science 305:362–366

Fisher WS, Foss SS (1993) A simple test for toxicity of Number 2 fuel oil and oil dispersants to embryos of grass shrimp, *Palaemonetes pugio*. Mar Pollut Bull 26:385–391

Friedrich T et al (2012) Detecting regional anthropogenic trends in ocean acidification against natural variability. Nat Clim Change. doi:10.1038/NCLIMATE1372

Gagné F, Auclair J, Turcotte P, Fournier M, Gagnon C, Sauvé S, Balise C (2008) Ecotoxicity of CdTe quantum dots to freshwater mussels: impacts on immune system, oxidative stress and genotoxicity. Aquat Toxicol 86:333–340

Goldstein MC, Rosenberg M, Cheng L (2012) Increased oceanic microplastic debris enhances oviposition in an endemic pelagic insect. Biol Lett. doi:10.1098/rsbl.2012.0298

Griffitt RJ, Luo J, Gao J, Bonzonga J-C, Barber DS (2008) Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. Environ Toxicol Chem 27:1972–1978

Harley CD (2011) Climate change, keystone predation, and biodiversity loss. Science 334:1124–1127

Hazen T et al (2010) Deep-sea oil plume enriches indigenous oil-degrading bacteria. Science 330:204–208

Hebel DK, Jones MB, Depledge MH (1997) Responses to crustaceans to contaminant exposure: a holistic approach. Estuar Coast Shelf Sci 44:177–184

Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world's marine ecosystems. Science 328

Jovanović B, Palić D (2012) Immunotoxicology of non-functionalized engineered nanoparticles in aquatic organisms with special emphasis on fish–review of current knowledge, gap identification, and call for further research. Aquat Toxicol 118–119:141–151

Kessler JD et al (2011) A persistent oxygen anomaly reveals the fate of spilled methane in the deep Gulf of Mexico. Science 331:312–315

Kujawinski E, Soule MC, Valentine D, Boysen AK, Longnecker K, Redmond MC (2011) Fate of dispersants associated with the Deepwater Horizon oil spill. Environ Sci Technol 45:1298–1306

Kukulka T, Proskurowski G, Morét-Ferguson S, Meyer DW, Law KL (2012) The effect of wind mixing on the vertical distribution of buoyant plastic debris. Geophys Res Lett 39(7):L07601. doi:10.1029/2012GL051116

Laughlin RB, Ng J, Guard HE (1981) Hormesis: a response to low environmental concentrations of petroleum hydrocarbons. Science 213:705–707

Miller RJ, Bennett S, Keller AA, Pease S, Lenihan HS (2012) TiO_2 nanoparticles are phototoxic to marine phytoplankton. PLoS One 7(1):e30321. doi:10.1371/journal.pone.0030321

Moore MN (2006) Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environ Int 32:967–976

National Transportation Safety Board (1990) Marine accident report: grounding of the U.S. Tankship Exxon Valdez: on Bligh Reef, Prince William Sound, near Valdez, Alaska, 24 March 1989. Washington, DC, NTSB. NTSB/MAR-90/04, 255 p

Negri AP, Heyward AJ (2000) Inhibition of fertilization and larval metamorphosis of the coral *Acropora millepora* (Ehrenberg 1834) by petroleum products. Mar Pollut Bull 41:420–427

Peterson CH, Rice SD, Short JW, Esler D, Bodkin JL, Ballachey BE, Irons DB (2003) Long-term ecosystem response to the Exxon Valdez oil spill. Science 302:2082–2086

Ramachandran SD, Hodson PV, Khan CW, Lee K (2004) Oil dispersant increases PAH uptake by fish exposed to crude oil. Ecotoxicol Environ Saf 59:300–308

Rhoton SL, Perkins RA, Richter ZD, Behr-Andres C, Lindstrom JE, Braddock JF (1998) Toxicity of dispersants and dispersed oil to an Alaskan marine organism. In: International oil spill conference, Proceedings of the 1998 Oil Spill Conference, American Petroleum Institute, Washington, DC, pp 8485–8488

Rowe C (2008) "The calamity of so long life": life histories, contaminants, and potential emerging threats to long-lived vertebrates. BioScience 58:623–631

Sanders BM, Martin LS, Nelson WG, Phelps DK, Welch W (1991) Relationships between accumulation of 60 kDa stress protein and scope-for-growth in *Mytilus edulis* exposed to a range of copper concentrations. Mar Environ Res 31:81–97

Scarlett A, Galloway TS, Canty M, Smith EL, Nilsson J, Rowland SJ (2005) Comparative toxicity of two oil dispersants, Superdispersant-25 and Corexit 9527, to a range of coastal species. Environ Toxicol Chem 24:1219–1227

Schofield O, Ducklow HW, Martinson DG, Meredith MP, Moline MA, Frazer WR (2010) How do polar marine ecosystems respond to rapid climate change? Science 328:1520–1523

Shafir S, van Rihn J, Rinkevich B (2007) Short and long term toxicity of crude oil and oil dispersants to two representative coral species. Environ Sci Tech 41:5571–5574

Shi D, Xu Y, Hopkinson BM, Morel FMM (2010) Effect of ocean acidification on iron availability to marine phytoplankton. Science 327:676–679

Smith CJ, Shaw BJ, Handy RD (2007) Toxicity of single-walled carbon nanotubes to rainbow trout, (*Oncorhynchus mykiss*): respiratory toxicity, organ pathologies, and other physiological effects. Aquat Toxicol 82:94–109

Stebbing ARD (1981) Hormesis – stimulation of in *Campanularia flexuosa* (hydrozoa) by copper, cadmium and other toxicants. Aquat Toxicol 1:227–238

Stegeman J, Lech JJ (1991) Cytochrome P-450 monooxygenase systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. Environ Health Perspect 90:101–109

Sunda WG, Lewis JA (1978) Effect of complexation by natural organic ligands on the toxicity of copper to the unicellular alga *Monochrisis lutheri*. Limnol Oceanogr 23:870–876

Sunda WG, Engel DW, Thuotte RM (1978) Effect of chemical speciation on toxicity of cadmium to the grass shrimp, *Palaemonetes pugio*: importance of free cadmium ion. Environ Sci Technol 12:409–413

U.S.E.P.A (2010) EPA response to BP spill in the Gulf of Mexico. http://www.epa.gov/bpspill/dispersants-qanda.html#general2

Valentine D, Mezić I, Maćešić S, Črnjarić-Žic N, Ivić S, Hogan PJ, Fonoberov VA, Loire S (2012) Dynamic autoinoculation and the microbial ecology of a deep water hydrocarbon irruption. Proc Nat Acad Sci USA. doi:10.1073/pnas.1108820109

Chapter 2
Feeding and Digestion

Abstract Obtaining food for energy is essential for all living things that don't photosynthesize. Reduced feeding and digestion are commonly observed after exposure to a variety of pollutants. Alterations in feeding, nutrient assimilation, and energetics in many species could not only impact their own population dynamics, but also could have community-wide repercussions. Decreased feeding is not only a general response to contaminants, but also can result in a "positive feedback" situation, since poor nutrition resulting from decreased feeding can in turn make animals more susceptible to contaminants (Dissanayake et al. Aquat Toxicol 89:40–46, 2008). These authors advised that "ecotoxicological studies need to take into account the nutritional state of the test organism to achieve the full assessment of contaminant impact." On the other hand, it is also likely that decreased feeding will reduce further uptake of contaminants. This is particularly true for animals at higher trophic levels, which acquire much of their body burden of contaminants from their food. Additional discussion of pollution effects on feeding is covered in Chap. 9, Behavior.

Keywords Assimilation • Consumption • Enzymes • Feces • Filtration • Foraging • Growth • Gut fluid • Intestinal transport

2.1 Feeding

2.1.1 Crustaceans

Reduced food consumption is an almost universal response in crustaceans to many toxicants (Taylor et al. 1993; Maltby and Crane 1994; Wallace et al. 2000). However, increased feeding rates have occasionally been observed, e.g., in amphipods exposed to the chlorinated pesticide lindane (Blockwell et al. 1998).

J.S. Weis, *Physiological, Developmental and Behavioral Effects of Marine Pollution*,
DOI 10.1007/978-94-007-6949-6_2,

Metals

Chronic exposure to copper (>85 μg l^{-1}) and zinc (>106 μg l^{-1}) reduced growth of shrimp larvae (*Farfantepenaeus paulensis*) because of reduced feeding (Santos et al. 2000). Both metals reduced the number of *Artemia* captured by the shrimp larvae during 30 min. Oxygen consumption also was reduced by about 30 % in all concentrations. Similarly, gut fullness of shrimp, *Metapenaeus ensis,* larvae feeding on the diatom *gracilis* was reduced by a 2-h exposure to copper at 0.25 mg l^{-1} (Wong et al. 1993).

In contrast, gut fullness of larvae was not affected even after 24-h exposure to chromium or nickel at high concentrations close to the 48-h LC_{50} (5.41 and 1.28 mg l^{-1}, respectively). However, postlarval shrimp exposed for 24-h to those concentrations of Cr, Cu or Ni consumed fewer *Artemia* nauplii. While younger stages are generally more sensitive than older stages, this obviously depends on the species and toxicant. Blue crabs, *Callinectes sapidus*, were fed grass shrimp *Palaemonetes pugio* contaminated with 1.8 μg TBT, 0.09 μg DBT (dibutyltin) and 0.03 μg MBT (monobutyltin) g^{-1} wet weight tissue. Feeding rates for exposed and control crabs were equal during the 16-day test (Rice et al. 1989). Growth, molting success and feeding rates were not affected. Catabolism of TBT reduced tissue concentrations of TBT, thereby increasing the tolerance of blue crabs to TBT.

Organics

Jensen and Carroll (2010) examined feeding of copepods exposed to the water-soluble fraction (WSF) of crude oil. Feeding was inhibited in *Calanus finmarchicus* exposed to 0.4 μg l^{-1} of the WSF, showing that adults are sensitive to exposure to crude oil well below saturation level. Effects of PCBs on estuarine shrimps were investigated by Nimmo et al. (1975). Toxicity tests showed estuarine species to be sensitive at low concentrations in water, with shrimps (*Penaeus duorarum, P. aztecus*, and *Palaemonetes pugio*) affected at or near 1 μg l^{-1}. Exposed shrimp later became lethargic and stopped feeding. It is possible that the lethargy was due to non-polar narcosis, which could have caused the reduced feeding. It is also possible that the reduced activity (lethargy) was due to low energy levels from reduced feeding.

Hypoxia

Feeding by the mud crab *Neopanope sayi* and juvenile blue crabs *Callinectes sapidus*, decreased during hypoxia, suggesting that short hypoxic episodes may create predation refuges for their prey (Sagasti et al. 2001). This was supported by mesocosm studies by Seitz et al. (2003) using blue crabs and *Macoma balthica* clams. Predation on clams was significantly lower under low DO (<2 mg O_2 l^{-1})

than under normoxia. Thus, under short-term hypoxia, both crab feeding efficiency and trophic transfer from *M. balthica* to blue crabs were reduced. Changes in clam burial depth due to oxygen levels were determined by establishing normoxic and low DO levels in replicate aquaria. Burial depth after 48 h exposure did not differ as a function of oxygen level. None of the clams died after 2 days in low DO, 27 % died after 6 days, and 90 % died after 21 days. Authors concluded that short-term hypoxia therefore reduces the ability of crabs to forage upon clams efficiently and increases clam survival, whereas long-term hypoxia may increase the availability of clams to predators through mortality and movement to the surface. Bell et al. (2003) used biotelemetry and measurements of dissolved oxygen to monitor the feeding and movement responses of free-ranging blue crabs *Callinectes sapidus* to episodic hypoxic events and subsequent relaxation events within the Neuse River Estuary, North Carolina, USA. Although crabs did feed in water with DO as low as 1.01 mg l^{-1}, the feeding declined slightly in mild (2–4 mg l^{-1}) and severe hypoxia (<2 mg l^{-1}). Crabs reduced the proportion of time spent feeding during hypoxic conditions. However, the proportion of time crabs spent feeding did not increase and crabs did not reinvade deeper water habitats when DO increased, as had been hypothesized. No significant difference occurred in the feeding rates of blue crabs exposed to normoxia, 119 and 73 Torr O_2, but these rates were significantly higher than that of blue crabs exposed to 50 Torr O_2. [Unfortunately, different investigators use different measurements for DO. Torr is a measurement of pressure, defined as $^{1}/_{760}$ of one atmosphere; a pressure of 1 Torr is approximately equal to one mm of mercury. In this book the units utilized by the investigator will be used.] Feeding rates in the lesser blue crab (*C. similis*) exposed to 50 and 25 Torr O_2 were significantly lower than in crabs exposed to 119 Torr O_2 and normoxia (Das and Stickle 1993). The feeding rate of crabs after being exposed to hypoxia for 10 days increased sharply upon transfer to normoxic water; however, this may have been due to partial starvation during hypoxia.

Climate Change/Acidification

Effects of 650, 1,250 and 3,500 μatm CO_2 on feeding of shore crabs *Carcinus maenas*, were examined after exposure of both the predators and their prey, the blue mussel *Mytilus edulis*, for 10 weeks. Intermediate levels had no significant effect, but the highest level reduced feeding by 41 % (Appelhans et al. 2012). Active extracellular pH compensation by means of bicarbonate accumulation was observed in the crabs.

In contrast to the effects of hypoxia and acidification on shore crabs, the copepod, *Centropages tenuiremis*, increased both its feeding and respiration rate at elevated CO_2 (1,000 μatm), and associated acidity (pH 7.83), (Fig. 2.1) except for an initial acclimation period, when it fed less (Li and Gao 2012). The authors suggest that copepods increase their respiration and feeding in response to acidification in order to balance the energy costs associated with increased acidity and CO_2.

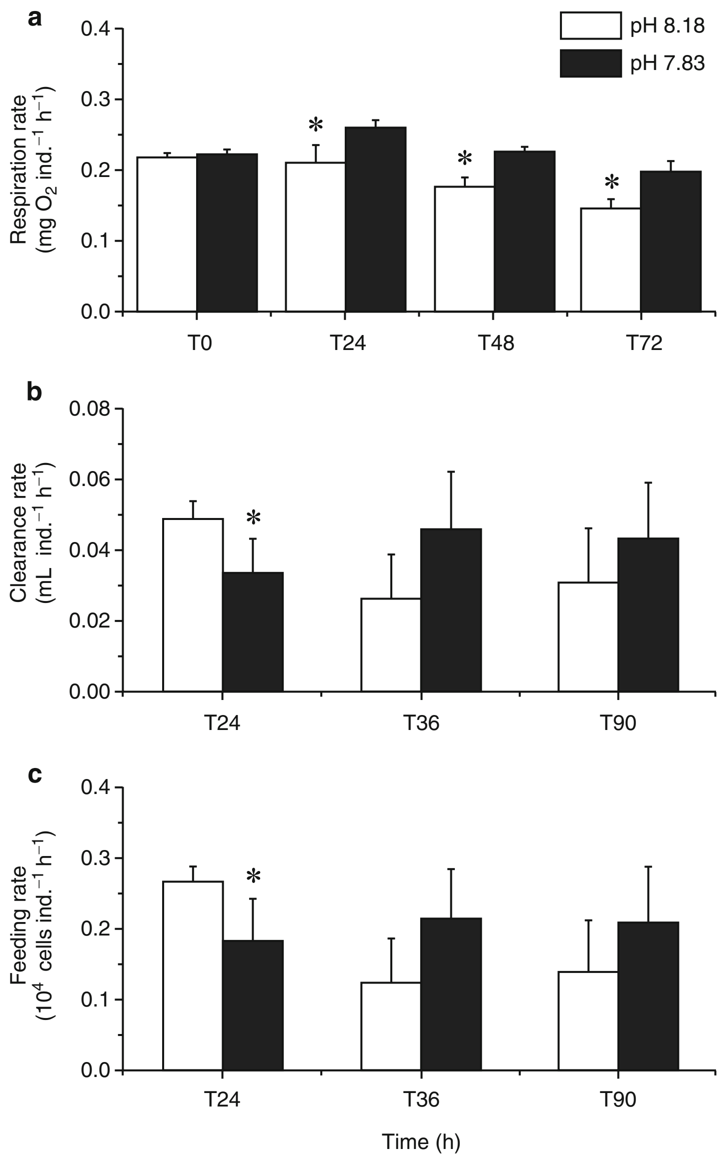


Fig. 2.1 Rates of respiration and feeding by *C. tenuiremis* under ambient (pH 8.18) and acidified (pH 7.83) conditions. (**a**) Respiration, (**b**) clearance, and (**c**) feeding rates of *C. tenuiremis*. *above the columns = significant ($p < 0.05$) differences between the two pH treatments. Vertical bar = SD (From Li and Gao 2012: 702, reprinted courtesy of Elsevier Publishing Co)

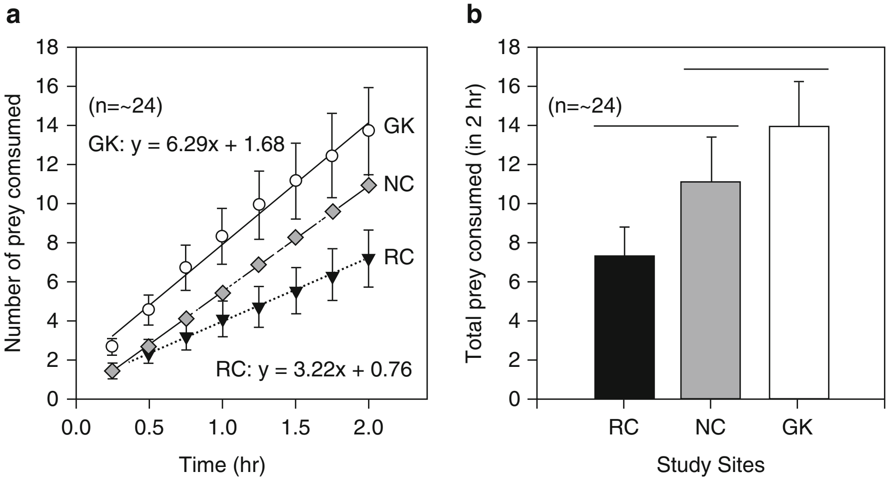


Fig. 2.2 Comparison of (**a**) number of prey (*Artemia francescana*) consumed as a function of time, and (**b**) total prey consumed over a 2-h trial for grass shrimp from Great Kills (GK – *open circle* and *bar*), Nassau Creek (NC – *half tone diamond* and *bar*) and Richmond Creek (RC – *full tone triangle* and *bar*). Mean ± SE. Results of linear regressions for GK and RC in panel A. Lines above bars in B = non-significance ($p > 0.05$) (Reprinted from Perez and Wallace 2004: 84, courtesy Springer Publishing Co)

Impacts in Polluted Sites

A number of studies have assessed feeding rates in animals living in contaminated sites. Perez and Wallace (2004) found that grass shrimp (*Palaemonetes pugio*) from a clean reference site (Great Kills) captured brine shrimp at about twice the rate as grass shrimp from more contaminated sites (Fig. 2.2).

Grass shrimp from the clean site that were maintained in the laboratory for 8 weeks with sediment and water from the contaminated site reduced their feeding to that typical of shrimp from that site, showing that the behavioral difference was caused by the environment. Videotape analysis indicated that reduced feeding was due to shrimp using a less efficient grab type of capture, rather than a lunge or pursuit type of attack. Khoury et al. (2009) compared feeding rate (number of scoops of substrate) of fiddler crabs (*Uca pugnax*) from a contaminated site and a reference site. Crabs from the reference site performed twice as many scoops (on the same sediment) than crabs from the contaminated site. Blue crabs (*Callinectes sapidus*) from a contaminated site captured fewer active prey (killifish or juvenile blue crabs) compared with crabs from a cleaner reference site, but ate comparable amounts of less active prey (fiddler crabs and mussels) (Reichmuth et al. 2009). Gut content analysis showed that blue crabs from the contaminated site ate less fish and crabs but much more detritus, algae, and sediment than crabs from the cleaner site. These food items are not typical for this predatory species. Transplanting "polluted" crabs to the clean site or maintaining them in the laboratory with food from the clean site, caused a reversal in behavior – "polluted" crabs increased their feeding on juvenile blue crabs. Conversely, transplanting reference site crabs to the polluted site or maintaining them in the lab on food from the polluted site caused them to decrease their feeding; this was correlated with bioaccumulation of mercury.

Cellular activity, immune function, cardiac activity, and foraging behavior were studied in green crabs, *Carcinus maenas,* collected from a PAH-contaminated site and two comparatively clean field sites and compared with responses of crabs exposed in the laboratory to the PAH pyrene as a model organic contaminant (200 μg l^{-1}) for 28 days (Dissanayake et al. 2010). Cellular function (hemocyte membrane integrity) and immune function (phagocytosis), were decreased by pyrene exposure in the laboratory. In the field, however, no significant cellular or physiological impacts were seen in the contaminated site, but foraging behavior was significantly reduced, demonstrating that feeding behavior is a more sensitive response (Fig. 2.3). Crabs from the contaminated site (PLYM) took significantly longer than other field-collected and laboratory-exposed crabs to approach a cockle and break the shell, causing longer prey handling time, with both contaminated groups showing significantly longer handling times.

A precise method for quantifying feeding of *C. maenas* in polluted sediments, using the polychaete *Hediste* (*Nereis*) *diversicolor* as food, was developed (Moreira et al. 2006b). Organisms were deployed at several reference and contaminated sites, and reduced feeding (16.3–72.7 %) was observed at all contaminated sites.

Litter

Marine debris can become part of the diet of animals. Small plastic fragments are available to invertebrates because they are in the same size range as their normal food items. Many of these small fragments come from fishing debris, which accumulates in areas used by commercially important marine life. Of 120 specimens of Norway lobster, *Nephrops norvegicus* collected from the Clyde Sea, Scotland, 83 % contained plastic in their stomachs. This plastic consisted mainly of monofilament strands of different colors and thickness (Murray and Cowie 2011). *Nephrops* fed fish seeded with strands of polypropylene rope were able to ingest but not to excrete the strands. The study showed that some filaments are unable to pass through the gastric mill system (composed of one median and two serrated lateral teeth) and into the pyloric stomach for eventual elimination via the hindgut. The long-term effects of this build-up are unknown and should be investigated.

2.1.2 Mollusks

Mollusks also reduce their feeding activity after exposure to a variety of toxicants. Shipp and Grant (2006) and Krell et al. (2011) developed a short-term in situ toxicity assay based on the post-exposure feeding of the mudsnail *Hydrobia ulvae*. Growth

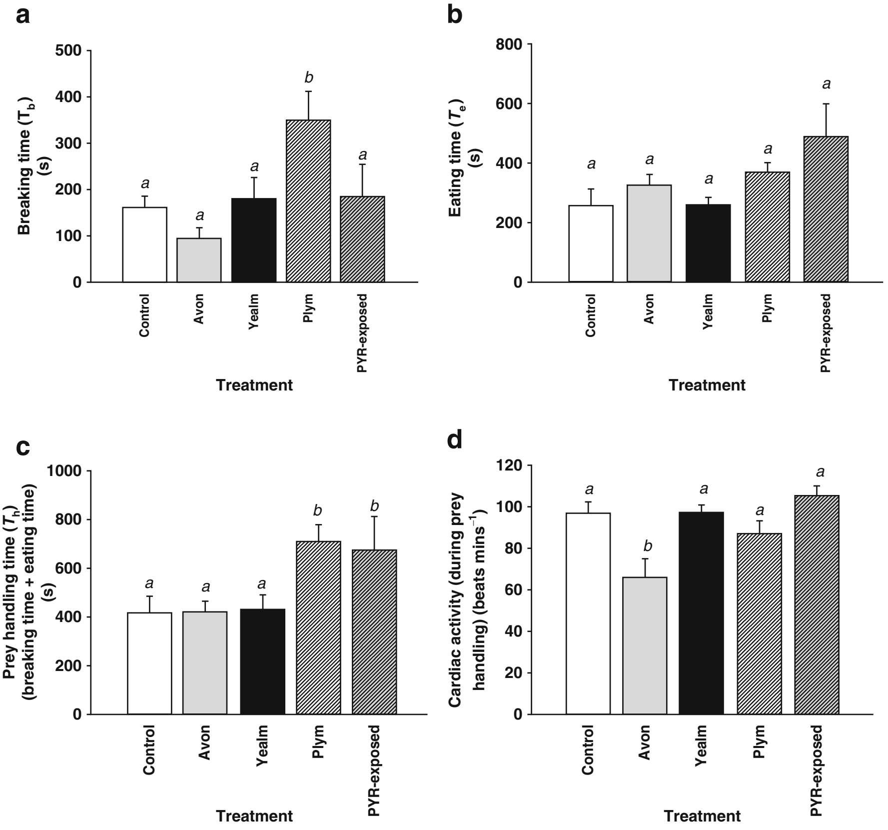


Fig. 2.3 Foraging behaviours (mean + SE) in adult *Carcinus maenas*. (**a**). Breaking time of shelled prey, (**b**) eating time of flesh, (**c**) handling time, and (**d**) cardiac activity during prey handling time (beats/min). *Different letters* = significant differences (Reprinted from Dissanayake et al. 2010, 70: 368–373, 73: 96, courtesy of Elsevier Publishing Co)

over 28 days in *H. ulvae* was reduced at all sites where other studies had detected adverse ecological effects. Feeding rate after 24 h also was decreased at moderately contaminated sites where sediments were not acutely toxic, and feeding was a very good predictor of 28-day growth. Methodologies to quantify post-exposure egestion as a surrogate of feeding were also developed. Reduced feeding in these bioassays was a good predictor of growth.

Metals

Feeding behavior of snails and bivalves has been analyzed in the presence of metal contaminants. Filtration rates of the mussel *Mytilus edulis* and clam *Mya arenaria* were reduced by exposure to chromium (1 mg l^{-1}), to sediments from a

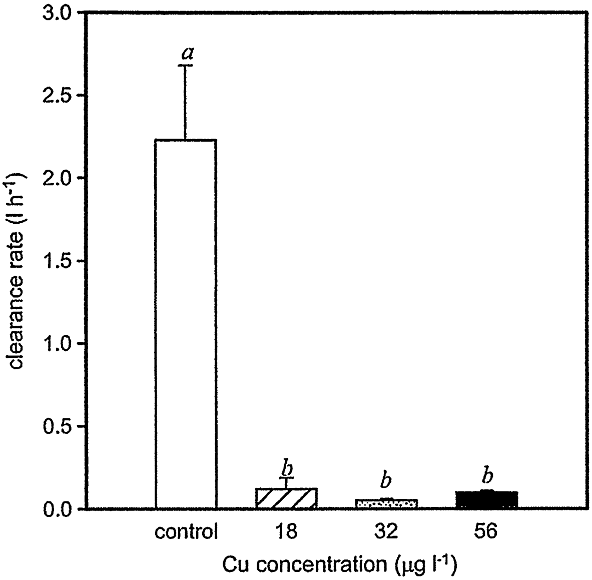


Fig. 2.4 Clearance rates (M ± SE) of *M. edulis* following 5 days exposure to copper. *Different letters* = significant differences (Reprinted from Al-Subiai et al. 2011: 1916, reprinted courtesy of Elsevier Publishing Co)

Cr-contaminated site, or to artificial sediments enriched in Cr (Capuzzo and Sasner 1977). Both dissolved and particulate Cr ($CrCl_3$) reduced filtration rates; *Mya* was less affected than *Mytilus* by particulate Cr. *M. edulis* were exposed for 5 days to Cu (18–56 μg l^{-1}). While molecular biomarkers were affected at 56 μg l^{-1} Cu, the clearance rate showed a significant decrease at concentrations of 18 μg l^{-1} (Fig. 2.4) (Al-Subiai et al. 2011), supporting previous observations that feeding is more sensitive than biochemical biomarkers.

Mollusks are particularly sensitive to copper, which is used as a molluscicide. Effects of 20 μg Cu^{2+} l^{-1} and lowered salinity (20 psu) were studied on the grazing snail *Trochus maculatus* and the macroalgae, *Gracilaria tenuistipitata* and *Enteromorpha intestinalis* (Elfwing and Tedengren 2002). The two factors were applied both separately and in combination to evaluate interactions. Results indicated that moderate salinity reduction and ecologically relevant amounts of Cu reduced snail grazing but not algal productivity, and thus could promote algal growth and potential dominance on coral reefs.

Organics

Oil and Dispersants

Exposure of mussels (*M. edulis*) to an unresolved complex mixture (UCM) of aromatic hydrocarbons isolated from crude oil reduced feeding rate by 40 %

(Donkin et al. 2003). The feeding rate of mussels collected from polluted sites increased when they were placed in clean water, suggesting depuration of toxicants. Water into which mussels from an oil-polluted site had depurated contained a UCM, and tissue extracts of mussels from polluted sites reduced the feeding activity of juvenile mussels. Extracts of mussels from an oil-polluted site were fractionated by HPLC, and a fraction comprising a monoaromatic UCM, reduced feeding of juvenile mussels by 70 %.

Since dispersants are often used following oil spills, there is concern that they may exert toxic effects. The objective of dispersant use is to increase the amount of oil that mixes into the water column, reducing the chances that a slick will contaminate the shoreline or come into contact with birds, marine mammals, or other organisms on the surface or shoreline. By promoting dispersion into the water, however, dispersants increase the potential exposure of biota to both oil and the dispersants themselves. Feeding rates of *M. edulis* exposed to two common oil dispersants were measured by Scarlett et al. (2005). Effects were assessed at dispersant concentrations of 50 mg^{-1} for 48 h. Feeding was reduced dramatically by both dispersants, with SD-25 reducing feeding to 9.8 % of control levels and Corexit® 9527 reducing feeding rates to only 2.6 % of controls.

Pesticides

Filtration rate of adult Pacific oysters *Crassostrea gigas* in response to different concentrations of lindane (gamma-hexachlorocyclohexane [γ-HCH]) for 12 days was investigated (Anguiano et al. 2007). Oysters were exposed to ten different concentrations (<10.0 mg l^{-1}) of γ-HCH. After 4 h of exposure to 0.3 and 0.7 mg l^{-1} γ-HCH, filtration rates were reduced compared with controls to 65.8 and 38.2 %, respectively. After 11 days of exposure, filtration rates were reduced to 60.4 and 30.9 % at concentrations of 0.1 mg l^{-1} and higher. This study showed the filtration rate to be more sensitive than genotoxicity and cytotoxicity.

Hypoxia

Significant differences were noticed in the feeding rate of the oyster drill *Stramonita haemastoma* exposed to hypoxia. Feeding rate in *S. haemastoma* declined linearly with declining oxygen concentration (Das and Stickle 1993).

Contaminants of Emerging Concern

Since mussels take up 100-nm polystyrene (PS) beads, effects of 30-nm PS on the feeding behavior of the blue mussel (*M. edulis*) were studied by Wegner et al. (2012) by exposing mussels to different nano PS and different concentrations of algae (*Pavlova lutheri*). In all treatments, mussels produced pseudofeces. Mussels reduced their filtering activity when nano PS was present, but still reduced the nano

PS concentration in the water and accumulated it. Authors felt that chronic effect studies are needed to further investigate effects of nanoplastics on *M. edulis* and possible consequences for its predators.

Polluted Sites

Growth in *Hydrobia ulvae* was reduced at metal-contaminated field sites, which was associated with reduced feeding rates. Feeding rate after 24 h also was decreased at moderately contaminated sites, which was a very good predictor of 28-day growth (Shipp and Grant 2006).

2.1.3 Fishes

Fish feeding, like that of crustaceans and mollusks, is generally reduced after exposure to a variety of contaminants. These responses are discussed in greater detail under "prey capture" in the behavior chapter.

Metals

Weis and Khan (1990) found that exposure of adult mummichogs (*F. heteroclitus*) to 10 $\mu g\ l^{-1}$ of either $HgCl_2$ or meHg for 1 week reduced feeding rate. In addition, feeding of mummichog larvae was examined after embryonic exposure to 5 or 10 $\mu g\ l^{-1}$. After hatching, larvae were maintained in clean water. Feeding by early larvae was reduced by the embryonic exposure, but approximately 1 week after hatching feeding was comparable to controls, showing that this effect was temporary (Weis and Weis 1995a). The exposure may have caused retardation of neurological development that was subsequently compensated for. After exposure during both embryonic and larval stages, deleterious effects on feeding were greater than after embryonic exposure alone, i.e. lower concentrations were seen to reduce feeding (Zhou et al. 2001).

Organics

Embryonic exposure of mummichogs to environmentally relevant concentrations of PCBs similarly reduced prey capture of larvae (Couillard et al. 2011). The lowest observed effective dose was 5.0 pg PCB126 egg^{-1}. Prey capture efficiency (number of *Artemia* captured per feeding strike) was reduced at ≥ 10.0 pg egg^{-1}. In microcosm experiments, juvenile spot (*Leiostomus xanthurus*) removed fewer harpacticoid copepods from PAH-contaminated sediments than from reference sediments (Marshall and Coull 1996). This may reflect avoidance of the contaminated sediments or a decreased feeding response due to toxicity.

Acidification

Nowicki et al. (2012) found CO_2 level did not significantly affect foraging in juvenile anemonefish, but there was an interaction with temperature. At high temperature (31.5 °C) and control or moderate (530 μatm) CO_2 food consumption and foraging activity were reduced, while high temperature and high CO_2 (960 μatm) increased feeding. Maintaining food consumption and foraging activity in high temperature and CO_2 may reduce energy efficiency if the thermal optimum for food assimilation and growth has been exceeded. The authors concluded that the interaction of rising temperatures and CO_2 will have deleterious effects on this species by mid-century.

Contaminants of Emerging Concern (CECs)

Feeding was inhibited by the anti-depressant fluoxetine, (a selective serotonin reuptake inhibitor, SSRI) in hybrid striped bass (*Morone saxatilis* x *M. chrysops*)., with significant effects observed after only 6 days at the lowest concentration, 23.2 μg l^{-1} (Gaworecki and Klaine 2008). Increased time to capture food was correlated with decreases in brain serotonin activity, which also decreased in a time- and concentration-dependent manner.

Hypoxia

Growth rates of both winter flounder *Pseudopleuronectes americanus* and summer flounder *Paralichthys dentatus* were generally reduced as DO decreased, particularly at DO levels of 50–70 % air saturation, and as temperature increased (Stierhoff et al. 2006). Summer flounder were more tolerant of low DO than winter flounder in this laboratory experiment. A significant relationship between feeding rate and growth indicated that reduced feeding was the major cause of growth reduction. Effects of moderate hypoxia and oscillating DO on feeding and growth of European sea bass (*Dicentrarchus labrax*) were investigated (Thetmeyer et al. 2001). Fish were exposed to one of three oxygen regimes (40 % air saturation; oscillations between 40 and 86 % with a period of 770 min; 86 % as a control) for 1 month. Fish in hypoxia consumed less food, had reduced growth, and a lower condition factor. Fish in oscillating conditions were intermediate. Growth was correlated with food intake, suggesting that reduced growth is primarily due to reduced appetite. Juvenile turbot *Scophthalmus maximus* were fed to satiation at O_2-concentrations of 3.5, and 5.0 mg l^{-1} and 7.2 mg l^{-1} (normoxia) (Pichavant et al. 2000). Both food intake and growth were significantly lower under reduced DO. During the first 2 weeks of the experiment, food intake was halved in hypoxic conditions, and there were large differences among treatments in feed conversion ratio. When juvenile turbot, *Scophthalmus maximus,* and European sea bass, *Dicentrarchus labrax,* were fed to satiation, food intake and growth were depressed under hypoxia (3.2 and

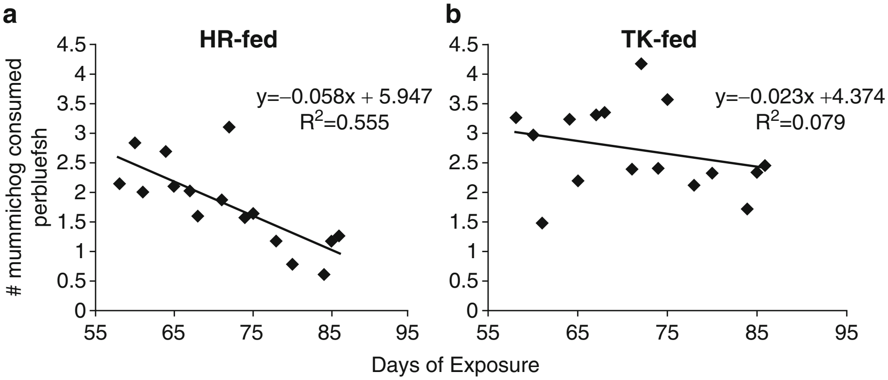


Fig. 2.5 Mean number of mummichogs consumed per bluefish for each trial. (**a**). HR-fed (polluted site) regression analysis (p = 0.001). (**b**). TK-fed (reference) regression analysis (p = 0.291) (Reprinted from Candelmo et al. 2010: 1031, courtesy Springer Publishing Co)

4.5 mg O_2 l^{-1}) (Pichavant et al. 2001). Growth was comparable between fish in hypoxia that were fed to satiation and fish in normoxia that were fed restricted rations. Decreased food intake is a mechanism by which prolonged hypoxia reduces growth, and may be a way to reduce energy and oxygen demand under hypoxic conditions.

Negative effects of hypoxia on fish feeding can sometimes be compensated for by increased availability of benthic prey during periods of hypoxia. For example, in a field study, spot (*Leiostomus xanthurus*) and hogchoker (*Trinectes maculatus*) showed evidence of optimal prey exploitation during or right after hypoxic events in Chesapeake Bay (Pihl et al. 1992). In most instances gut contents contained larger, deeper-burrowing prey during periods of low oxygen than during normal oxygen levels. Spot consumed a greater biomass (45–73 %) of polychaetes than other prey, with crustaceans initially also constituting a main dietary component. The deep-burrowing anemone, *Edwardsia elegans*, was an important prey species for spot, particularly in deeper hypoxic areas. Prey consumed by 10-to 15-cm-long spot increased significantly in size during some hypoxic events, suggesting a sublethal effect of hypoxia causing large benthic species to move up closer to the sediment surface where they are more available to fish predators.

Polluted Environment

Young-of-the-year bluefish *Pomatomus saltatrix*, that were fed diets in the laboratory of contaminated food (mummichogs and menhaden from Hackensack Meadowlands, an estuary with multiple contaminants including Hg and PCBs), gradually showed reduced appetite and consumed less food than fish that had been fed diets of the same species from a reference estuary (Candelmo et al. 2010) (Fig. 2.5). Bluefish fed contaminated diets grew more slowly, probably due to the reduced food intake.

2.1.4 Other Taxa

Metals

Coral feeding on zooplankton can be inhibited by metal exposure. Peng et al. (2004) found that Cu-exposed corals, *Subergorgia suberosa* were unable to catch or consume brine shrimp effectively. The rate of successful feeding for control polyps was 85 %, but was 57 % at 0.2 μg Cu l^{-1} and only 24 % at 0.5 μg Cu l^{-1}. This is one of the most sensitive responses to Cu. Other metals (Zn, Cd, Pb) did not produce this sublethal effect.

Organics

The deposit feeding lugworm, *Arenicola cristata*, exposed to Kepone ($>$2.8 μg l^{-1}) showed a significant reduction in sediment processing (feeding) (Rubenstein 1979). The reduction of sediment reworking by these deposit feeders could affect sediment-water column dynamics and alter benthic food chains.

Effects of oil on the sea anemone *Actinia equina* were investigated by Ormond and Caldwell (1982). After 7 weeks exposure to 2.5 ml l^{-1} crude oil, anemones were frequently observed with tentacles expanded and mouth open, but the response to food offered to the tentacles was slow or absent. In separate tests it was found that crude oil presented on filter paper to the anemones could act as a feeding inducer, but that it interfered with or diluted the action of natural feeding inducers present in fish muscle extract.

Corals normally acquire food by a combination of filter feeding by the tentacles of the polyps at night and by photosynthesis by the symbiotic dinoflagellates (zooxanthellae) in cells lining the polyp's gut during the day. When filter feeding, tentacles capture planktonic prey, which are stunned or killed by the nematocysts on the tentacles. Corals also produce mucus, which can trap planktonic prey. Dinoflagellates in the genus *Symbiodinium* occur as endosymbionts, forming a mutualistic relationship with their coral host (Baker 2003). They provide the coral with fixed carbon for energy, remove waste products, and enhance calcification. The host coral polyp provides its zooxanthellae with protection and with carbon dioxide and nutrients in its waste that can be used for photosynthetic processes. This symbiotic relationship allows corals to thrive because of the tight coupling of resources and the advantage of combining filter feeding at night and algal photosynthesis in the day. However, the relationship can be disrupted by a number of stresses, including various pesticides. Irgarol is now used in antifouling paints as a substitute for tributyltin, which has been banned in many countries. Irgarol 1051 was detected by Owen et al. (2002) in marinas, harbors and coastal waters of Florida, Bermuda and St. Croix, with concentrations ranging between 3 and 294 ng l^{-1}. Incubation experiments with isolated zooxanthellae from the coral *Madracis mirabilis* showed no incorporation of $H^{14}CO_3^-$ from seawater (no photosynthesis) after 4–8 h exposure to Irgarol 1051 concentrations as low as 63 ng l^{-1}. Reduction in net photosynthesis of intact corals was found at concentrations of 100 ng l^{-1} with little or no photosynthesis at concentrations exceeding 1,000 ng l^{-1} after 2–8 h exposure. These data suggest Irgarol 1051 is prevalent in tropical marine ecosystems and is a potent inhibitor of coral photosynthesis at environmentally relevant concentrations.

Similarly, Photosystem II (PSII) herbicides used in agriculture and antifouling paints can affect corals and their symbiotic dinoflagellates. Jones (2006) reviewed ecotoxicological studies and found that PSII herbicides readily penetrate coral tissues and within minutes reduce the photochemical efficiency of the algal symbionts, with photosynthesis being affected at low concentrations (i.e. in the ng l^{-1} range). At these levels and over short exposure periods, effects are reversible when corals are returned to clean seawater. However, with higher concentrations or longer exposure periods, there is long-term reduction of the photochemical efficiency of the algae, which can result in the loss of the symbionts (bleaching, see below) a stress response that requires months for recovery.

Markey et al. (2007) investigated various pesticides for effects on the coral *Acropora millepora*. Most had few visible effects on adults after 96 h exposure to 10 μg l^{-1}, with the exception of profenofos, which caused polyp retraction, bleaching, and a slight reduction in photosynthetic efficiency of the algal symbionts. The fungicide MEMC (2-methoxyethylmercuric chloride) caused polyps to become withdrawn and photosynthetic efficiency was slightly reduced at 1.0 μg l^{-1}. At 10 μg l^{-1} MEMC, branches bleached and some host tissue died.

Climate Change

Corals

The symbiotic relationship of corals and zooxanthellae is vulnerable to stresses such as elevated temperature, which causes the corals to bleach, i.e. lose their zooxanthellae (see Fig. 1.9). While bleaching can also be caused by intense irradiance, chemical stresses (see above), freshwater inflow, and sedimentation, elevated temperature is the primary cause of mass bleaching events (Kleppel et al. 1989). Most reef-building corals normally contain around $1–5 \times 10^6$ zooxanthellae cm^{-2} of live surface tissue and 2–10 pg of chlorophyll *a* per zooxanthella. Photosynthetic pathways in zooxanthellae are impaired at temperatures above 30 °C, which could activate the separation of coral and algae. When corals bleach they generally lose 60–90 % of their zooxanthellae and each remaining zooxanthella may lose 50–80 % of its photosynthetic pigments (Glynn 1996). The pale appearance of bleached corals is due to the calcareous skeleton showing through the tissues that have lost pigmented zooxanthellae. Temperature shocks can also reduce zooxanthellae through loss of cell adhesion, the detachment of coral endodermal cells from their zooxanthellae and eventual expulsion. If bleaching is not too severe and if it decreases over time, the corals may regain their symbiotic algae within several weeks or months. If zooxanthellae loss continues and dinoflagellate populations do not recover, the coral eventually dies because filter feeding is not adequate to meet its nutritional needs. However the coral plays a significant role in recovery and resilience, and some species can adjust. Bleached and recovering *Montipora capitata* corals could meet all their daily metabolic energy requirements by increasing their feeding rates and CHAR (percent contribution of heterotrophically acquired carbon to daily animal respiration), whereas *Porites compressa* and *Porites lobata* could not. Therefore coral species with high-CHAR capability are more resilient to bleaching, and may become the dominant coral species on reefs in the future (Grottoli et al. 2006).

It had been hoped that corals living inside marine protected areas (MPAs) where there is no fishing would also be more resilient to effects of increased temperatures. To determine whether coral deaths caused by bleaching were lower inside MPAs, Selig et al. (2012) compared over 8,000 coral reef surveys by divers with satellite measurements of ocean surface temperatures. They found that although MPAs could help coral populations recover from temperature-induced mortality in some situations, it did not appear to be a general solution. In general, corals living inside MPAs were just as susceptible to warming as unprotected corals.

While there is some specialization of particular hosts for particular *Symbiodinium* species, many corals associate with more than one type of *Symbiodinium*, and a particular *Symbiodinium* may associate with a variety of hosts (Baker 2003). This flexibility allows corals to function well in different settings (e.g., shallow, high-light situations versus deep water low-light conditions). Physiologically distinct lines of *Symbiodinium* may have different thermal tolerance. Oliver and Palumbi (2011) found that the coral–algal symbiosis adapts to particular temperature environments through changes in the algal symbiont. It has been hypothesized that bleaching allows the coral to be repopulated with zooxanthellae that are more temperature-resistant. Thus, the coral/algal association may be able to adapt within a coral's lifetime. Corals in warmer environments tend to host *Symbiodinium* that are more thermally tolerant.

Sponges

Elevated temperature also affects feeding in sponges. Massaro et al. (2012) examined the effects of thermal stress on feeding in the Great Barrier Reef sponge, *Rhopaloeides odorabile*, focusing on filtration efficiencies and choanocyte chamber characteristics. When temperature reached 31 °C, flow rate, filtration efficiency, and choanocyte chamber density and size were reduced, thus reducing food intake.

Echinoderms

Acidification altered feeding and growth in sea stars *Asterias rubens* exposed to 650, 1,250 and 3,500 μatm. Appelhans et al. (2012) exposed both the predators and their prey, the blue mussel *Mytilus edulis*, over 10 weeks and subsequently performed feeding assays. Intermediate acidification levels had no significant effect on growth or consumption, but produced a slight increase in feeding and growth. The highest acidification level reduced feeding and growth rates by 56 %. Mussels exposed to elevated pCO_2 were preferred by previously untreated *A. rubens.* A trend toward a lower shell mass in mussels in increasing seawater pCO2 was observed, and the breaking resistance of shells was significantly lowered by ~20 % at 3,500 μatm. Despite the decrease in the breaking resistance of the shell, mussels did not become more susceptible to crab predation under high acidification levels, even though crabs consume them by breaking the shells. Breaking the shell is not the feeding mode of the sea star, which pries open the valves; however, the mussel's adductor muscle (which holds the shells together) was not affected by the acidification. Thus, the altered predation by the sea star predators is not explained by the physical changes (or lack thereof) in the mussels.

Hypoxia/Nutrients

Benthic polychaetes have variable tolerance to low DO. *Loima medusa*, a common species in estuarine habitats where summer hypoxic events often occur can tolerate anoxia or severe hypoxia (7 % air saturation at 26 °C) for 3–5 days (Llanso and Diaz 1994). Under low DO (<14 % air saturation) feeding stops, although tube irrigation continues and periodic protrusions from the tube are common. Most worms come out to the sediment surface. The prevalence of *L. medusa* in deep estuarine channels may be partially explained by its tolerance to prolonged periods of hypoxia.

Excess nutrients (N), rather than hypoxia, can affect the susceptibility of corals to bleaching. Increased dissolved inorganic nitrogen (DIN) has been linked to a reduction of the temperature threshold of coral bleaching. Wiedenmann et al. (2013) found that increased DIN and decreased phosphate increased the susceptibility of corals to bleaching. Analyses suggested that the imbalanced supply of DIN results in phosphate starvation of the symbiotic algae. A model was developed that assumes that a transition of zooxanthellae from a nutrient-limited to a nutrient-starved (in this case phosphate) state leads to changes in the lipid composition of the algal membranes. Under stress, the altered photosynthetic membranes and photosystems would impair photosynthesis and cause the breakdown of the symbiosis and loss of zooxanthellae. These results suggest that a balanced reduction of N input in coastal waters could help mitigate effects of increasing temperatures on coral reefs.

Other Cnidarians (e.g. jellyfish) appear to be quite tolerant of low DO, which can give them an advantage over more sensitive taxa. Low DO (~2 mg l^{-1}) greatly increased predation on fish larvae (naked goby *Gobiosoma bosc*) by sea nettles (*Chrysaora quinquecirrha*) but decreased predation by juvenile striped bass (*Morone saxatilis*) (Breitburg et al. 1997). Predation by the sea nettle increased for fish larvae, decreased for fish eggs, and was not strongly affected for copepods (mostly *Acartia tonsa*) at low DO. Changes in predator–prey interactions reflected species differences in tolerance to low DO and its effects on escape behavior of prey and on swimming and feeding behaviors of predators. Because of the variation in effects, low DO has the potential to alter the relative importance of different pathways of energy flow in estuarine systems. Larvae of the red sea bream *Pagrus major* in four size classes were used as prey in a short-term predation experiment with moon jellyfish *Aurelia aurita*. No change in the bell contraction rate of the jellyfish was observed at the DO levels tested (1, 2 and 4 mg/l, and 5.5–6.0 mg/l), suggesting tolerance to low DO (Shoji et al. 2005). Over 80 % of the 2.5 and 4.1-mm larvae were eaten at all DO concentrations during 15-min trials. The 6.2 and 8.6-mm larvae were able to escape due to their developed swimming ability at the two higher DO concentrations, but they suffered increased predation at the two lower DO levels. Similarly, ctenophores (comb jellies) also are quite tolerant of hypoxia. Laboratory clearance rates of *Mnemiopsis leidyi* feeding on bay anchovy (*Anchoa mitchilli*) eggs and yolk sac larvae, and naked goby (*Gobiosoma bosc*) larvae were as high at low DO (1.5 mg l^{-1}) as at high DO concentrations (7 mg l^{-1}) (Kolesar et al. 2010). Years of field sampling at two sites revealed that ctenophore densities remained high in the bottom even at low DO levels.

Polluted Sites

Sediments from estuaries in Southwest Portugal classified as undisturbed and impacted were tested on the polychaete *Hediste diversicolor* (Moreira et al. 2006a). A significant depression in post-exposure feeding (from 30 to 70 %) was consistently seen in all impacted sediments, supporting the sensitivity and responsiveness of feeding as a sublethal endpoint. Along with a reduced energy intake, increased anaerobic metabolism (enhancement of lactate dehydrogenase activity), suggested a rapid need for additional energy to ameliorate chemical stress.

This section has shown that reduced feeding (or reduced energy uptake from photosynthetic symbionts) is a very common response to a variety of pollutant stresses. Reduced energy intake can be the initial impetus for a variety of subsequent responses, including respiration, growth, etc. that will be discussed in subsequent chapters.

2.2 Digestion and Assimilation

Ingested pollutants can alter digestive physiology even before they are assimilated – while still in the gut fluids they can affect gut motility, enzyme activities, or absorption (De La Ruelle et al. 1992). This is termed “pre-assimilatory toxicity.” Post-assimilatory toxicity occurs after the pollutant has been incorporated into tissues; this may damage gut tissues and interfere with digestive enzyme synthesis or release, and interfere with absorption, transport and subsequent assimilation of nutrients (and pollutants), and thus impact energy reserves (Seebaugh 2010). Most studies, however, do not attempt to distinguish between pre- and post-assimilatory toxicity. Impacts on digestive function may depend on the manner of exposure.

2.2.1 Crustaceans

Metals

Digestive enzymes tend to be inhibited by metals (Li et al. 2008). Sarojini et al. (1992) reported that amylase, lipase, and protease activities of the stomach, midgut, and hindgut of the prawn *Caridina rajadhari* were reduced after exposure to tributyltin. Cd exposure (0.05 mg l^{-1}) reduced food assimilation efficiency and fecal pellet production in the mysid *Leptomysis* (Gaudy et al. 1991). Exposures ranged from 0.01 to 0.2 mg l^{-1}. Reduced food intake, combined with decreases in fecal pellet production and reduced assimilation efficiency reflected a significant decrease in energy which authors felt would lead to an unbalanced energy budget and lower reproductive potential. Hydrolase activity increased initially at 0.2 mg Cd l^{-1}, but after 48 h it declined, reaching very low values at 72 h. The unbalanced energy budget was an overall consequence of the inability to utilize food.

Cd in prey altered assimilation efficiency of Cd in the grass shrimp, *P. pugio* (Seebaugh and Wallace 2004; Seebaugh et al. 2006). Cd assimilation was positively correlated with gut residence time in shrimp collected along a pollution gradient. Increased gut residence time can, in turn, influence pollutant assimilation. Ingestion of a pulse of Cd reduced protease activities and fecal elimination rate (Seebaugh 2010). Digestive protease activities could have been influenced by pre-assimilatory interactions between Cd in the gut fluids and enzyme-secreting cells, or they could have resulted from impacts on stored or circulating enzymes. Ingestion of a pulse of Cd can influence protease activities and fecal elimination rate (Seebaugh 2010). Thus, previous exposure to dietary metals can induce changes in digestive physiology and affect digestive enzymes that may influence future digestion and assimilation.

Grass shrimp fed Cd-containing polychaetes did not show a change in carbon assimilation efficiency, minimum gut residence time, or gut pH, but did show a dose-dependent decrease in feces elimination rate and an increase in protease activities, but the latter was not dose-dependent (Seebaugh et al. 2012a). Studies with dye-labeled food suggested that the reduced feces elimination rate was not due to reduced food intake, but rather to reduced feces packaging and transport, possibly by affecting the muscles responsible for peristalsis.

Grass shrimp were fed Hg-contaminated oligochaetes (*Tubifex* worms exposed to 0.007, 0.014, or 0.028 lM Hg for 96 h) over a 15-day period and analyzed for Hg, Cd, and carbon assimilation efficiencies (AE) as well as end points related to digestion (Seebaugh et al. 2012b). Hg AE by pre-exposed shrimp reached a plateau (approximately 53 %), whereas Cd AE varied (approximately 40–60 %) in a manner that was not dose-dependent. Carbon AE did not differ among treatments. Gut residence time and feces elimination were not impacted. Extracellular protease activity varied but did not exhibit dose-dependency. pH increased over the range of Hg pre-exposures within the gut, and Hg assimilation had a negative relationship to hydrogen ion concentrations. Thus, previous Hg ingestion can elicit post-assimilatory impacts on digestive physiology, which may, in turn, influence subsequent Hg assimilation.

Organic Pollutants – Pesticides

There have been few studies on effects of organic pollutants on digestion. Fisher and Clark (1990) examined kepone and assimilation in grass shrimp, *P. pugio*. Grass shrimp and their food were exposed to a Kepone concentration of 0.04 μg l^{-1}. A first-order pharmacokinetic equation was used to model accumulation kinetics during 16-day uptake and 21-day clearance. Doubling the contaminated food ration caused a significant increase in the whole-body Kepone concentration. Shrimp fed either a 4 or 8 % ration of uncontaminated food and exposed to Kepone in water bioconcentrated Kepone equally. When shrimp were exposed to contaminated water and food, Kepone accumulation from each source was additive, but the food was very important in determining final body burdens. Dietary Kepone represented approximately 24 and 33 % of the total body burden accumulated by shrimp fed 4 and 8 % food rations, respectively, but assimilation efficiencies of Kepone from the food were low.

Horst et al. (2007) found that exposure to the juvenile hormone analog methoprene (50 μg l^{-1}) caused up-regulation of genes in the hepatopancreas of the lobster, *Homarus americanus*, for the enzymes betaine-homocysteine *S*-methyltransferase (BHMT) and other enzymes of the methionine cycle. Increased levels of enzymes associated with protein turnover, including trypsin, ubiquitin conjugating enzyme, and ubiquitin carboxyl terminal hydrolase were also observed.

Polluted Sites

Grass shrimp (*P. pugio*) from polluted sites had reduced digestive protease activity compared to shrimp from a reference site. Casein hydrolysis rates were negatively correlated with gut residence time and inversely related to AE of Cd (Seebaugh 2010), which would affect future assimilation of pollutants. However, carbon assimilation was not affected, suggesting that the shrimp could compensate for metal-induced post-assimilatory toxicity to maintain assimilation of nutrients (Seebaugh 2010). It appears that gut plasticity (increasing gut residence time) allows shrimp in contaminated sites to maintain adequate assimilation of essential nutrients, but may increase the risk of dietary exposure to specific pollutants. There was a trend of increasing gut residence time with increasing dietary Cd but not Hg or carbon. Increased gut residence time can compensate for reduced digestive enzyme activities. Fecal elimination rate was also not affected by field exposure, which also may be a compensatory response to impacts of pollutants. Seebaugh et al. (2011) found that digestive protease activities decreased markedly in grass shrimp from impacted field sites relative to reference shrimp, and suggested that digestive plasticity (increasing gut residence time) may be important in compensating for post-assimilatory digestive toxicity (reduced protease activities) in order to maintain nutrient assimilation. Stress-induced variability in digestive function may, in turn, enhance the assimilation of non-essential elements, such as Cd.

2.2.2 Mollusks

Pesticides

Field-collected oysters, *C. gigas* from areas in Northwest France highly contaminated with urea herbicides showed elevated mortality. Laboratory exposures to diuron and isoproturon, 0.5 and 1 μ gl^{-1} produced histopathology in the digestive system (atrophy of the digestive tubule epithelium) (Buisson et al. 2008), which would affect digestion and assimilation.

Contaminants of Emerging Concern

Canesi et al. (2012) exposed mussels (*M. edulis*) to nanoparticles (NPs) and found that, due to the physiological mechanisms involved in the feeding process, NP agglomerates/aggregates taken up by the gills were directed to the digestive gland, where intracellular uptake of nanosized materials induced lysosomal perturbations and oxidative stress. This could be a major mechanism of action underlying the potential toxicity of NPs in marine invertebrates.

Acidification

Juvenile *Mytilus galloprovincialis* under conditions of −0.3 and −0.6 pH units for 78 days showed increased absorption efficiency and ammonium excretion, and increased scope for growth and tissue dry weight, suggesting that this species is tolerant to acidification (Fernandez-Reiriz et al. 2012). Feeding itself was unaffected.

2.2.3 Fishes

Metals

Socci and Farmanfarmaian (1985) investigated effects of inorganic Hg, methylmercury, and Cd (<5.0 mg l^{-1}) on intestinal absorption of amino acids (l-leucine and l-methionine) by the toadfish, *Opsanus tau*, using an *in vitro* system. At 2.5 mg l^{-1} inorganic Hg inhibited leucine uptake, while meHg inhibited uptake at 5 mg l^{-1}. For methionine, inorganic Hg reduced intestinal uptake at 5 mg l^{-1}, while meHg inhibited it at 2.5 mg l^{-1}. It is of interest that for leucine, $HgCl_2$ was a more potent inhibitor of intestinal uptake, which is unusual, in that meHg is generally the more toxic form of the metal.

A high concentration (6.8 mg l^{-1}) of Cd affected the histology and enzyme activities of the alimentary tract and liver of the fish, *Heteropneustes fossilis* (Sastry and

Gupta 1979). Three phosphatases studied were significantly inhibited in the liver and intestine. Pepsin activity was elevated in the stomach, but trypsin was inhibited in the intestine. Inhibition was also noted in the activities of aminotripeptidase and glycylglycine dipeptidase. High concentrations (0.3 mg l^{-1}) of $HgCl_2$ also affected digestive enzymes in this fish (Gupta and Sastry 1981). The activities of alkaline phosphatase and glucose-6-phosphatase decreased, while acid phosphatase activity was elevated above normal. Significant decreases were observed in activities of all the digestive enzymes except pepsin.

These experiments are decades old, and used high concentrations of metals, well above those encountered in the field; thus, there is a need for new studies on effects of lower levels of contaminants on fish digestive and assimilative activities.

Organic Pollutants – Pesticides

DDT exposure (0.05 or 0.1 mg l^{-1} for 24 h) of mummichogs (*Fundulus heteroclitus*) impaired intestinal absorption of amino acids (Miller and Kinter 1977). The authors thought this was due to impairment of membrane transport and speculated that this could lead to reduced nutrition and growth.

Hypoxia

Juvenile cod (*Gadus morhua*) were exposed to low DO to investigate digestion and metabolism (Jordan and Steffenson 2007). Reduced oxygen (6.3 kPa PO_2) depressed the usual postprandial (after feeding) increase in oxygen consumption. The specific dynamic action (production of heat associated with the ingestion of food) lasted over twice as long, perhaps to compensate for the reduced oxygen availability. The percentage of energy associated with digestion and assimilation was greater in hypoxia, occupying most of the scope for activity and leaving little energy for other activities On the other hand, postprandial blood flow to the gut during hypoxia was not proportionately reduced in sea bass, as predicted by Axelsson et al. (2002). Although post-prandial absolute blood flow decreased during hypoxia, the relative proportion of cardiac output reaching the gut did not decrease. This was unlike the situation in non-feeding fish.

2.2.4 Other Taxa

Metals

Chen et al. (2002) assessed potential impacts of Cu on digestive enzyme activities in a wide range of benthic invertebrates (echinoderms, mollusks, polychaetes, echiurids, and hemichordates), by monitoring enzyme activities in their gut fluids during *in vitro* titrations with dissolved Cu, which mimics Cu solubilization from sediments. Increasing Cu inhibited digestive protease activities at values that varied from 8 μM for an echinoderm to 0.4 M for an echiuran. Threshold Cu concentrations were similar for different digestive enzymes, suggesting the same inhibition mechanism. Copper was less effective at inhibiting enzymes at lower pH, suggesting that H^+ can compete with Cu ion for binding to active sites or that enzyme conformation is less vulnerable to Cu inhibition at lower pH. The results suggest that animals with low enzyme activity and high gut pH are more vulnerable to Cu, although they solubilize less sedimentary Cu than animals with high enzyme activity and low gut pH.

Seick et al. (1999) examined how cadmium pre-exposure (3 and 30 μg Cd g^{-1} dry wt. sediment) and gut passage time interact to determine cadmium absorption efficiency (Cd-AE) in the polychaete *Capitella* sp. A 5-day pre-exposure to Cd did not affect egestion rates during either the pre-exposure period or the chase phase. Overall, Cd-AE increased with increasing gut passage time in worms that were not pre-exposed, but pre-exposure to cadmium reversed the relationship between gut passage time and Cd-AE. Thus, worm physiology may be especially important in controlling metal bioavailability in deposit-feeding organisms and should be considered in sediment quality approaches.

Organics

Schweitzer et al. (2000) examined dietary assimilation of PCBs and maternal transfer in sea urchins. Adult *Lytechinus pictus* were allowed to graze on sediment spiked with radiolabeled PCB, 2,2′,4,4′-tetrachlorobiphenyl for 35 days. *L. pictus* was found to have quite high extraction efficiency; approx. 62 % of the PCB sediment concentration was removed while passing through the gut. Maternal transfer was not a more sensitive exposure route to developing embryos than direct water exposure. Both adults and embryos were resilient to this PCB at environmentally relevant sediment concentrations. Low toxicity of PCBs allows for significant bioaccumulation in sea urchins.

Hypoxia

Specific feeding rate, growth, and production efficiency were measured on individuals of the polychaete *Capitella* species 1 to determine whether previously measured declines in growth rates in response to hypoxia were due to decreased feeding, decreased conversion efficiency, or both (Forbes et al. 1994). The relationship between feeding rate and growth was influenced by oxygen concentration such that in relatively nitrogen-poor sediment, greater growth rates were observed at lower DO. Measurements of growth and feeding rates indicated that the effect of DO was due to a decrease in the efficiency with which ingested sediment was converted to tissue under low N, high DO conditions. Authors suggested that the decreased conversion rate of ingested sediment to body volume under the higher DO regime reflected an aerobic metabolic system poised to rapidly exploit available oxygen supplies.

Along with tolerance to hypoxia in their feeding responses, gelatinous taxa also appear to have high tolerance in their digestive processes. Ctenophore (*Mnemiopsis leidyi*) digestion rates were unchanged at oxygen concentrations of 1 mg l^{-1} (Decker et al. 2004). Gelatinous species, which are more tolerant of hypoxia than fishes, may be able to inhabit regions of low oxygen that are avoided by zooplanktivorous fishes that have higher oxygen requirements. This could lead to dominance of gelatinous predators in areas affected by hypoxia and might alter energy pathways in these systems.

Polluted Sites

The polychaete *Nereis diversicolor* from a polluted estuary (Loire) and a relatively clean site (Bay of Bourgneuf) were compared (Kalman et al. 2009). Significant inhibition of the digestive enzymes amylase and carboxymethylcellulase were recorded in individuals from the Loire compared to the reference site. Feeding and egestion rates were also depressed in worms from the Loire compared to the reference site. This impairment was accompanied by changes in digestive enzyme activities, which could explain the generally poorer condition of worms in the Loire estuary.

2.3 Conclusions

Most animals respond to most contaminants with a reduction in feeding and digestion. Decreased food intake places energetic demands on the organism, which may be responsible for decreases in other physiological functions (e.g. respiration) that will be discussed in the following chapters. In many cases when food intake is reduced, animals reduce their activity in order to conserve energy; this in turn may make it harder to find and get food – which intensifies the problem in positive feedback situation. The taxonomic group that would seem to be at greatest risk of mortality from reduced energy intake would appear be the corals, which get most of their energy requirements from their photosynthetic symbionts, which are very sensitive to toxicants and temperature. Coral bleaching can frequently lead to mortality.

References

Al-Subiai N, Moody AJ, Mustafa SA, Jha AN (2011) A multiple biomarker approach to investigate the effects of copper on the marine bivalve mollusc, *Mytilus edulis*. Ecotoxicol Environ Saf 74:1913–1920

Anguiano G, Llera-Herrera R, Rojas E, Vazquez-Boucard C (2007) Subchronic organismal toxicity, cytotoxicity, genotoxicity, and feeding response of pacific oyster (*Crassostrea gigas*) to lindane (γ-HCH) exposure under experimental conditions. Environ Toxicol Chem 26:2192–2197

Appelhans YS, Thomsen J, Pansch C, Melzner F, Wahl M (2012) Sour times: seawater acidification effects on growth, feeding behaviour and acid–base status of *Asterias rubens* and *Carcinus maenas*. Mar Ecol Prog Ser 459:85–98

Axelsson M, Altimiras J, Claireux G (2002) Post-prandial blood flow to the gastrointestinal tract is not compromised during hypoxia in the sea bass *Dicentrarchus labrax*. J Exp Biol 205:2891–2896*

Baker A (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology and biogeography of *Symbiodinium*. Annu Rev Ecol Evol Syst 34:661–689

Bell GW, Eggleston DB, Wolcott TG (2003) Behavioral responses of free-ranging blue crabs to episodic hypoxia. II. Feeding. Mar Ecol Prog Ser 259:227–235

Blockwell SJ, Taylor EJ, Jones I, Pascoe D (1998) The influence of fresh water pollutants and interaction with *Asellus aquaticus* (L) on the feeding activity of *Gammarus pulex* (L). Arch Environ Contam Toxicol 34:41–47

Breitburg DL, Loher T, Pacey CA, Gerstein A (1997) Varying effects of low dissolved oxygen on trophic interactions in an estuarine food web. Ecol Monogr 67:489–507

Buisson S, Bouchart V, Guerlet E, Malas JP, Costil K (2008) Level of contamination and impact of pesticides in cupped oyster, *Crassostrea gigas*, reared in a shellfish production area in Normandy (France). J Environ Sci Health B 43:655–664

Candelmo A, Deshpande A, Dockum B, Weis P, Weis JS (2010) The effect of contaminated prey on feeding, activity, and growth of young-of-the-year bluefish, *Pomatomus saltatrix*, in the laboratory. Estuar Coast 33:1025–1038

Canesi L, Ciacci C, Fabbri R, Marcomini A, Pojana G, Gallo G (2012) Bivalve molluscs as a unique target group for nanoparticle toxicity. Mar Environ Res 76:16–21

Capuzzo JM, Sasner JJ (1977) The effect of chromium on filtration rates and metabolic activity of *Mytilus edulis* and *Mya arenaria*. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Physiological responses of Marine Biota to pollutants. Academic, New York, pp 225–237

Chen Z, Mayer LM, Weston DP, Bock MJ, Jumars PA (2002) Inhibition of digestive enzyme activities by copper in the guts of various marine benthic invertebrates. Environ Toxicol Chem 21:1243–1248

Couillard CM, Légaré B, Bernier A, Dionne Z (2011) Embryonic exposure to environmentally relevant concentrations of PCB126 affect prey capture ability of *Fundulus heteroclitus* larvae. Mar Environ Res 71:257–265

Das T, Stickle WB (1993) Sensitivity of crabs *Callinectes sapidus* and *C. similis* and the gastropod *Stramonita haemastoma* to hypoxia and anoxia. Mar Ecol Prog Ser 98:263–274

De la Ruelle M, Hajjou M, Van Herp F, Le Gal Y (1992) Aminopeptidase activity from the hepatopancreas of *Procambarus clarkii*. Biochem Syst Ecol 20:331–337

Decker MB, Breitburg D, Purcell JE (2004) Effects of low dissolved oxygen on zooplankton predation by the ctenophore *Mnemiopsis leidyi*. Mar Ecol Prog Ser 280:163–172

Dissanayake A, Galloway TS, Jones MB (2008) Nutritional status of *Carcinus maenas* (Crustacea: Decapoda) influences susceptibility to contaminant exposures. Aquat Toxicol 89:40–46

Dissanayake A, Piggott C, Baldwin C, Sloman KA (2010) Elucidating cellular and behavioural effects of contaminant impact (polycyclic aromatic hydrocarbons, PAHs) in both laboratory-exposed and field-collected shore crabs, *Carcinus maenas* (Crustacea: Decapoda). Mar Environ Res 70:368–373

Donkin P, Smith EL, Rowland SJ (2003) Toxic effects of unresolved complex mixtures of aromatic hydrocarbons accumulated by mussels, *Mytilus edulis*, from contaminated field sites. Environ Sci Technol 37:4825–4830

Elfwing T, Tedengren M (2002) Effects of copper and reduced salinity on grazing activity and macroalgae production: a short-term study on a mollusc grazer, *Trochus maculatus*, and two species of macroalgae in the inner Gulf of Thailand. Mar Biol 140:913–919

Fernandez-Reiriz M, Range P, Alvarex-Salgado X, Espinosa J, Labarta U (2012) Tolerance of juvenile *Mytilus galloprovincialis* to experimental seawater acidification. Mar Ecol Prog Ser 454:65–74

Fisher DJ, Clark JR (1990) Bioaccumulation of Kepone by grass shrimp (*Palaemonetes pugio*): importance of dietary accumulation and food ration. Aquat Toxicol 17:167–186

Forbes TL, Forbes VE, Depledge MH (1994) Individual physiological responses to environmental hypoxia and organic enrichment: implications for early soft-bottom community succession. J Mar Res 52:1081–1100

Gaudy R, Guérin J-P, Kerambrun P (1991) Sublethal effects of cadmium on respiratory metabolism, nutrition, excretion and hydrolase activity in *Leptomysis lingvura* (Crustacea: Mysidacea). Mar Biol 109:493–501

Gaworecki KM, Klaine SJ (2008) Behavioral and biochemical responses of hybrid striped bass during and after fluoxetine exposure. Aquat Toxicol 88:207–213

Glynn PW (1996) Coral reef bleaching: facts, hypotheses and implications. Glob Change Biol 2:495–509

Grottoli A, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. Nature 440:1186–1189

Gupta PK, Sastry KV (1981) Effect of mercuric chloride on enzyme activities in the digestive system and chemical composition of liver and muscles of the catfish, *Heteropneustes fossilis*. Ecotoxicol Environ Saf 5:389–400

Horst MN, Walker AN, Bush P, Wilson T, Chang ES, Miller T, Larkin P (2007) Pesticide induced alterations in gene expression in the lobster, *Homarus americanus*. Comp Biochem Physiol Part D Genomics Proteomics 2:44–52

Jensen LK, Carroll J (2010) Experimental studies of reproduction and feeding for two Arctic-dwelling *Calanus* species exposed to crude oil. Aquat Biol 10:261–271

Jones R (2006) The ecotoxicological effects of Photosystem II herbicides on corals. Mar Pollut Bull 51:495–506

Jordan AD, Steffenson JF (2007) Effects of ration size and hypoxia on specific dynamic action in the cod. Physiol Biochem Zool 80:176–185

Kalman J, Palais F, Amiard JC, Mouneyrac C, Muntz A, Blasco J, Riba I, Amiard-Triquet C (2009) Assessment of the health status of populations of the ragworm *Nereis diversicolor* using biomarkers at different levels of biological organization. Mar Ecol Prog Ser 393:55–67

Khoury J, Powers E, Patniak P, Wallace W (2009) Relating disparity in competitive foraging behavior between two populations of fiddler crabs to the subcellular partitioning of metals. Arch Environ Contam Toxicol 56:489–499

Kleppel GS, Dodge RE, Reese CJ (1989) Changes in pigmentation associated with the bleaching of stony corals. Limnol Oceanogr 34:1331–1335

Kolesar SE, Breitburg DL, Purcell JE, Decker MB (2010) Effects of hypoxia on *Mnemiopsis leidyi*, ichthyoplankton and copepods: clearance rates and vertical habitat overlap. Mar Ecol Prog Ser 411:173–188

Krell B, Moreira-Santos M, Ribeiro R (2011) An estuarine mudsnail in situ toxicity assay based on postexposure feeding. Environ Toxicol Chem 30:1935–1942

Li W, Gao K (2012) A marine secondary producer respires and feeds more in a high CO_2 ocean. Mar Pollut Bull 64:699–703

Li N, Zhao Y, Yang J (2008) Effects of water-borne copper on digestive and metabolic enzymes of the giant freshwater prawn *Macrobrachium rosenbergii*. Arch Environ Contam Toxicol 55:86–93

Llanso RJ, Diaz RJ (1994) Tolerance to low dissolved oxygen by the tubicolous polychaete *Loimia medusa*. J Mar Biol Assoc UK 74:143–148

Maltby L, Crane M (1994) Responses of *Gammarus pulex* (Amphipoda: Crustacea) to metalliferous effluents: identification of toxic components and importance of interpopulation variation. Environ Pollut 84:45–52

Markey KL, Baird AH, Humphrey C, Negri AP (2007) Insecticides and a fungicide affect multiple coral life stages. Mar Ecol Prog Ser 330:127–137

Marshall KR, Coull BC (1996) PAH effects on removal of meiobenthic copepods by juvenile spot (*Leiostomus xanthurus*: Pisces). Mar Pollut Bull 32:22–26

Massaro AJ, Weisz JB, Hill MS, Webster NS (2012) Behavioral and morphological changes caused by thermal stress in the Great Barrier Reef sponge *Rhopaloeides odorabile*. J Exp Mar Biol Ecol 416–417:55–60

Miller DS, Kinter WB (1977) DDT inhibits nutrient absorption and osmoregulatory function in *Fundulus heteroclitus*. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Physiological responses of Marine Biota to pollutants. Academic, New York, pp 63–74

Moreira SM, Lima I, Ribeiro R, Guilhermino L (2006a) Effects of estuarine sediment contamination on feeding and on key physiological functions of the polychaete *Hediste diversicolor*: laboratory and in situ assays. Aquat Toxicol 78:186–201

Moreira SM, Moreira-Santos M, Guilhermino L, Ribeiro R (2006b) An in situ postexposure feeding assay with *Carcinus maenas* for estuarine sediment-overlying water toxicity evaluations. Environ Pollut 139:318–329

Murray F, Cowie PR (2011) Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). Mar Pollut Bull 62:207–1217

Nimmo DR, Hansen DJ, Couch JA, Cooley NR, Parrish PR, Lowe JI (1975) Toxicity of Aroclor® 1254 and its physiological activity in several estuarine organisms. Arch Environ Toxicol Chem 3:22–39

Nowicki JP, Miller GM, Munda PL (2012) Interactive effects of elevated temperature and CO_2 on foraging behavior of juvenile coral reef fish. J Exp Mar Biol Ecol 412:46–51

Oliver TA, Palumbi SR (2011) Many corals host thermally resistant symbionts in high-temperature habitat. Coral Reefs 30:241–250

Ormond RF, Caldwell S (1982) The effect of oil pollution on the reproduction and feeding behaviour of the sea anemone *Actinia equine*. Mar Pollut Bull 13:118–122

Owen R, Knap A, Toaspern M, Carbery K (2002) Inhibition of coral photosynthesis by the antifouling herbicide Irgarol 1051. Mar Pollut Bull 44:623–632

Peng S, Huang J, Xiong T, Huang M (2004) Effects of four heavy metals on toxicity and feeding behavior of soft coral (*Subergorgia suberosa*). J Oceanogr Taiwan Strait 23:293–301

Perez MH, Wallace WG (2004) Differences in prey capture in grass shrimp *Palaemonetes pugio*, collected along an environmental impact gradient. Arch Environ Contam Toxicol 46:81–89

Pichavant K et al (2000) Effects of hypoxia on growth and metabolism of juvenile turbot. Aquaculture 108:103–114

Pichavant K, Person-Le-Ruyet J, Le Bayon N, Severe A, Le Roux A, Boeuf G (2001) Comparative effects of long-term hypoxia on growth, feeding and oxygen consumption in juvenile turbot and European sea bass. J Fish Biol 59:875–883

Pihl L, Baden SP, Diaz RJ, Schaffner LC (1992) Hypoxia-induced structural changes in the diet of bottom-feeding fish and Crustacea. Mar Biol 112:349–361

Reichmuth JM, Roudez R, Glover T, Weis JS (2009) Differences in prey capture behavior in populations of blue crab (*Callinectes sapidus* Rathbun) from contaminated and clean estuaries in New Jersey. Estuar Coast 32:298–308

Rice SD, Short JW, Stickle WB (1989) Uptake and catabolism of tributyltin by blue crabs fed TBT contaminated prey. Mar Environ Res 27:137–145

Rubenstein NI (1979) A benthic bioassay using time-lapse photography to measure the effect of toxicants on the feeding behavior of lugworms (Polychaeta: Arenicolidae). In: Vernberg WB, Thurberg FP, Calabrese A, Vernberg FJ (eds) Marine pollution: functional responses. Academic Press, New York, pp 341–351

Sagasti A, Schaffner LC, Duffy JE (2001) Effects of periodic hypoxia on mortality, feeding and predation in an estuarine epifaunal community. J Exp Mar Biol Ecol 258:257–283

Santos MH, da Cunha N, Bianchini A (2000) Effects of copper and zinc on growth, feeding and oxygen consumption of *Farfantepenaeus paulensis* postlarvae (Decapoda: Penaeidae). J Exp Mar Biol Ecol 247:233–242

Sarojini R, Reddy PS, Nagabhushanam R (1992) Acute and chronic tributyltin-induced alterations in digestive enzymes of the prawn, *Caridina rajadhari*. Uttar Pradesh. J Zool 12:20–24

Sastry KV, Gupta PK (1979) The effect of cadmium on the digestive system of the teleost fish, *Heteropneustes fossilis*. Environ Res 19:221–230

Scarlett A, Galloway TS, Canty M, Smith EL, Nilsson J, Rowland SJ (2005) Comparative toxicity of two oil dispersants, Superdispersant 25 and Corexit 9527, to a range of coastal species. Environ Toxicol Chem 24:1219–1227

Schweitzer LE, Bay SM, Suffet IH (2000) Dietary assimilation of a polychlorinated biphenyl in adult sea urchins (*Lytechinus pictus*) and maternal transfer to their offspring. Environ Toxicol Chem 19:1919–1924

Seebaugh DR (2010) Relationships between pollutant-induced digestive toxicity and the assimilation and subcellular partitioning of elements by grass shrimp *Palaemonetes pugio*. PhD dissertation, City University of New York, New York

Seebaugh DR, Wallace WG (2004) Importance of metal-binding proteins in the partitioning of Cd and Zn as trophically available metal (TAM) in the brine shrimp *Artemia franciscana*. Mar Ecol Prog Ser 272:215–230

Seebaugh DR, Estephan A, Wallace WG (2006) Relationship between cadmium assimilation by grass shrimp (*Palaemonetes pugio*) and trophically available cadmium in amphipod (*Gammarus lawrencianus*) prey. Bull Environ Contam Toxicol 76:16–23

Seebaugh DR, L'Amoreaux WJ, Wallace WG (2011) Digestive toxicity in grass shrimp collected along an impact gradient. Aquat Toxicol 105:609–617

Seebaugh DR, Wallace WG, L'Amoreax WJ, Stewart GM (2012a) Carbon assimilation and digestive toxicity in naïve grass shrimp (*Palaemonetes pugio*) exposed to dietary cadmium. Bull Environ Contam Toxicol 88:449–455

Seebaugh DR, Wallace WG, L'Amoreax WJ, Stewart GM (2012b) Assimilation of elements and digestion in grass shrimp pre-exposed to dietary mercury. Arch Environ Contam Toxicol 63:230–240

Seick H, Decho AW, Forbes VE (1999) Effects of chronic metal exposure and sediment organic matter on digestive absorption efficiency of cadmium by the deposit-feeding polychaete *Capitella* species. Environ Toxicol Chem 18:1289–1297

Seitz R, Marshall LS Jr, Hines AH, Clark KL (2003) Effects of hypoxia on predator–prey dynamics of the blue crab *Callinectes sapidus* and the Baltic clam *Macoma balthica* in Chesapeake Bay. Mar Ecol Prog Ser 257:179–188

Selig ER, Casey KS, Bruno JF (2012) Temperature-driven coral decline: the role of marine protected areas. Glob Chang Biol 18:1561–1570. doi:10.1111/j.1365-2486.2012.02658.x

Shipp E, Grant A (2006) *Hydrobia ulvae* feeding rates: a novel way to assess sediment toxicity. Environ Toxicol Chem 25:3246–3252

Shoji J, Masuda R, Yamashita Y, Tanaka M (2005) Predation on fish larvae by moon jellyfish *Aurelia aurita* under low dissolved oxygen concentrations. Fish Sci 71:748–753

Socci R, Farmanfarmaian A (1985) Effect of heavy metals on the intestinal absorption of L-leucine and L-methionine in the toadfish, *Opsanus tau*. In: Vernberg FJ, Thurberg FP, Calabrese A, Vernberg WB (eds) Marine pollution and physiology: recent advances. University of South Carolina Press, Columbia, pp 267–279

Stierhoff KL, Targett TE, Miller KL (2006) Ecophysiological responses of juvenile summer and winter flounder to hypoxia: experimental and modeling analyses of effects on estuarine nursery quality. Mar Ecol Prog Ser 325:255–266

Taylor EJ, Jones DP, Maund SJ, Pascoe D (1993) A new method for measuring the feeding activity of *Gammarus pulex* (L.). Chemosphere 26:1375–1381

Thetmeyer H, Waller U, Black KD, Inselmann S, Rosenthal H (2001) Growth of European sea bass (*Dicentrarchus labrax* L.) under hypoxic and oscillating oxygen conditions. Aquaculture 174:355–367

Wallace WG, Hoexum Brouwer T, Brouwer M, Lopez GR (2000) Alterations in prey capture and induction of metallothioneins in grass shrimp fed cadmium-contaminated prey. Environ Toxicol Chem 19:962–971

Wegner A, Besseling E, Foekema EM, Kamermans P, Koelmans AA (2012) Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). Environ Toxicol Chem 31:2490–2497

Weis JS, Khan AA (1990) Effects of mercury on the feeding behavior of the mummichog, *Fundulus heteroclitus* from a polluted habitat. Mar Environ Res 30:243–249

Weis JS, Weis P (1995) Effects of embryonic exposure to methylmercury on larval prey capture ability in the mummichog, *Fundulus heteroclitus*. Environ Toxicol Chem 14:153–156

Wiedenmann J, D'Angelo C, Smith EG, Hunt AN, Legiret F-E, Postle AD, Achterberg EP (2013) Nutrient enrichment can increase the susceptibility of reef corals to bleaching. Nat Clim Change 3:160–164

Wong CK, Chu KH, Tang KW, Tam TW, Wong LJ (1993) Effects of chromium, copper and nickel on survival and feeding behaviour of *Metapenaeus ensis* larvae and postlarvae (Decapoda: Penaeidae). Mar Environ Res 36:63–78

Zhou T, Scali R, Weis JS (2001) Effects of methylmercury on ontogeny of prey capture ability and growth in three populations of larval *Fundulus heteroclitus*. Arch Environ Contam Toxicol 41:47–54

Chapter 3
Respiration and Metabolism

Abstract Respiration includes the transport of oxygen from the outside of the organism into the cells and the transport of carbon dioxide in the opposite direction. Cellular respiration, which takes place within cells, consists of the metabolic processes by which energy is obtained by breaking down glucose through enzymatic pathways (glycolysis and the Krebs cycle), creating water, carbon dioxide and ATP. Respiration responds directly to metabolic needs. Most toxicants studied have been found to reduce the metabolic rate and thus, the respiration of many organisms. Many studies have relied primarily on a single metric, oxygen consumption, to determine changes in metabolic rates. In some cases, however, lowered oxygen consumption can be attributed to reduced ventilation of the gills or to gill damage, and in other cases the toxic mechanism is disruption of the enzymes of cellular respiration. Relatively few studies have related effects on respiration to carbon assimilation through measures of feeding and excretion or have examined total effects on the carbon, nitrogen, or energy budget.

Keywords Anaerobic • CO_2 • Energy • Gills • Glycolysis • Oxygen • Respiratory enzymes

3.1 Crustaceans

3.1.1 Metals

Cadmium

Early studies showed that Cd (0.1 and 0.5 mg l^{-1}) reduced the metabolic rate (O_2 consumption) in both larval and adult fiddler crabs, *Uca pugilator* (Vernberg et al. 1974), and grass shrimp (Hutcheson et al. 1985). When grass shrimp

J.S. Weis, *Physiological, Developmental and Behavioral Effects of Marine Pollution*, DOI 10.1007/978-94-007-6949-6_3, © Springer Science+Business Media Dordrecht 2014

(*Palaemonetes pugio*) were placed in reduced DO (4.6 mg l^{-1}) they reduced their respiration rate and activity level (an adaptive response) and when Cd was present, respiration and activity were reduced even further. Similarly, Barbieri (2007) found that exposure of pink shrimp (*Farfantepenaeus paulensis*) to Zn (0.31 mg l^{-1}) or to Cd (0.18 mg l^{-1}) inhibited oxygen consumption. However, lobsters exposed to much lower concentrations of Cd (3 μg l^{-1}) had elevated gill oxygen consumption and increased ATPase activity (Thurberg et al. 1977). This may have been an example of hormesis (the tendency for low levels of contaminants to have "positive" effects, while at high levels, processes are inhibited). Gaudy et al. (1991) found that temperature affected the responses of the mysid *Leptomysis lingvura* to Cd. At 18 °C, respiration rate was affected only by concentrations greater than 0.05 mg Cd l^{-1}. Exposure to 0.1 mg Cd l^{-1} depressed the respiration rate more significantly at 20 °C than at 10 °C. Thus it is important to consider temperature and oxygen level when examining respiratory responses to contaminants.

Mercury

St-Amand et al. (1999) exposed zoea larvae of the shrimp *Pandalus borealis* to inorganic Hg (0–160 μg Hg l^{-1}) for 27 h. and measured oxygen consumption, potential respiration (respiratory electron transfer system activity, ETSA), and swimming activity. ETSA was unchanged after 27 h exposure to 160 μg Hg l^{-1}, while oxygen consumption and swimming activity decreased, showing that Hg disturbed a part of the respiration process without changing the activity of the enzymes involved in ETSA.

Copper and Zinc

Cu and Zn (0.4 mg l^{-1}) impaired respiratory function of the crab *Cancer pagurus* after 7 days exposure, but only during hypoxia (Spicer and Weber 1991). Exposure did not produce significant changes in ventilation or perfusion rates, although there was some indication that cardiac output may increase in respiratory-impaired individuals. They considered that respiratory impairment was due to an increase in the diffusion barrier thickness of the gills. Similar responses were observed by Nonnotte et al. (1993) in *Carcinus maenas* in 0.5 mg l^{-1} Cu. Gill structural alterations such as hyperplasia, vacuolization and necrosis were found after only 5–6 days, causing thickening of the gill epithelium, which restricted respiratory gas exchange. In a review, Spicer and Weber (1992) concluded that the essential metals Cu and Zn act on the respiratory system primarily by disrupting gill function, causing development of internal hypoxia, while the more toxic Hg and Cd interfere with the respiratory system at every level of organization including cellular respiration itself.

3.1.2 Organics

Oil

Effects of oil and its constituent hydrocarbons vary considerably among different crustaceans, with a number of studies showing increased metabolic rates in response to exposure. Studies have generally used individual hydrocarbons (e.g., naphthalene) or the water soluble fraction (WSF) of oil, which lowered respiration in the shrimp *Crangon* (Edwards 1978). In adult Northern shrimp *Pandalus borealis,* energy balance (scope for growth) declined after exposure to WSF (20–36 μg l^{-1}), but this was due to reduced food intake and remained positive at all oil concentrations (Stickle et al. 1987). These investigators measured the costs of respiration and ammonia excretion and found that energy budget costs did not change significantly in response to differing levels of fuel oil, while feeding rate was dose dependent. In fact, at low levels of exposure, feeding rates increased (evidence of hormesis), while at high levels feeding rate decreased. Since metabolic costs remained relatively constant, food consumption alone determined energy budget in this study. Nitrogen excretion accounted for only 10–20 % of metabolic costs, while oxygen metabolism accounted for 80–90 % of costs. Blue crabs (*C. sapidus*) exposed to WSF increased their energy expenditure and decreased their scope for growth, due (again) primarily to reduced feeding. The crabs reduced their energy intake without reducing maintenance costs and had reduced growth and longer intermolt periods at 800 μg l^{-1} (Wang and Stickle 1987). In both studies, metabolic costs of respiration remained relatively constant while feeding rate changed in response to contamination. Lobster larvae (*Homarus americanus*) exposed to 0.25 mg l^{-1} South Louisiana crude oil showed reduced respiration rate and O:N ratios (Capuzzo and Lancaster 1981). Low O:N ratios suggest that the larvae were deriving their energy from catabolism of protein, rather than carbohydrates or lipids. Energy metabolism did not return to control values after 1 week in clean water.

Atlantic rock crab (*Cancer irroratus)* in ^{14}C-naphthalene-labeled oiled sea water readily accumulated the isotope into hemolymph (Vandermeulen et al. 1980). Respiration was lowered in crabs in 11.0 mg l^{-1}, and returned to control levels when crabs were returned to clean sea water. The hemocyanin-O_2 binding potential and the structural integrity of hemocyanin were unaltered, suggesting that disruption of hemocyanin-O_2 binding is not a mechanism of hydrocarbon respiratory toxicity. Naphthalene (0.12 mg l^{-1}) exposure in the shrimp *Metapenaeus affinis*, caused reduced oxygen consumption, protein content, organic carbon, and weight gain, but lipid content increased (Ansari et al. 2010). In contrast, oxygen consumption increased in 8 mg l^{-1}-naphthalene-treated mud crabs, *Scylla serrata* (Vijayavel and Balasubramanian 2006), while activity of the respiratory enzymes lactate dehydrogenase, isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, and α-ketoglutarate dehydrogenase decreased in the hepatopancreas, ovary, and gills at all tested concentrations. Thus, some of the oxygen brought into the animal was not used in cellular respiration. Authors explained these somewhat contradictory effects as follows: When the animal is under stress, it consumes more oxygen to generate more energy and more water to utilize the oxygen, leading to enhanced uptake of naphthalene, which bioaccumulates to high concentrations. Normally there is equilibrium between the amount of oxygen generated and the activity of mitochondrial enzymes. Under stress, the equilibrium is disturbed which, as oxygen consumption increases, leads to the reduction in activity of the respiratory enzymes. Damage to mitochondrial membranes causes inhibition of mitochondrial enzymes, which reduces substrate oxidation and the rate of transfer to molecular oxygen, thus reducing the energy produced. Mitochondrial damage by naphthalene leads to decreased respiration, partial uncoupling of oxidative phosphorylation, and reduced ATP production.

Naphthalene at the much lower concentration of 0.2 mg l^{-1} also increased oxygen consumption in adult mysids *Neomysis americana* (Smith and Hargreaves 1985). In another example of increase in metabolic rate, Laughlin and Linden (1983) found that exposure to high levels of WSF of crude oil (200 and 1,000 μg l^{-1}) increased metabolic rate and ammonia excretion in another mysid, *N. integer*. Effects were influenced by temperature, with the greatest effect at 21.5 °C, the highest temperature tested. It is possible that temperature may partially explain some of the contradictory results in direction of response. Differences have been found in sensitivity of juvenile vs. adult shore (green) crabs (*C. maenas*) (Dissanayake et al. 2008). Sublethal exposure to 200 μg l^{-1} pyrene elevated the basal heart rate and decreased respiration rate of juveniles, but had no overall impact on adults, confirming that juveniles are more susceptible than adults. The authors felt their results had implications for environmental risk assessment, since basing "safe" concentrations on the tolerances of adults fails to protect more sensitive life stages.

Pesticides and Other Organic Chemicals

Energy metabolism of the mysid *Mysidopsis bahia* was altered by exposure to the pesticide fenthion. Juveniles increased their respiration, which reduced the amount of energy available for growth, resulting in reduced growth (McKenney and Matthews 1990). Fenvalerate, a pyrethroid insecticide, when present in sediment at 10 μg kg^{-1}, also reduced weight gain in *P. pugio* larvae and juveniles due to altered energy metabolism. Affected larvae contained significantly less N than controls, while exposed postlarvae contained significantly less carbon and less energy (McKenney et al. 1998) (Fig. 3.1). Thiobencarb, a carbamate insecticide, at 100 μg l^{-1} also stimulated the respiratory rate in *M. bahia*, thus reducing the amount of energy available for growth. Higher O:N ratios suggested a greater reliance on energy-rich lipid substrates resulting in less lipid being available for gamete production (McKenney 1985).

Verslycke et al. (2004) studied cellular respiratory responses of the mysid *Neomysis integer* as well as scope for growth (SFG) and cellular energy allocation

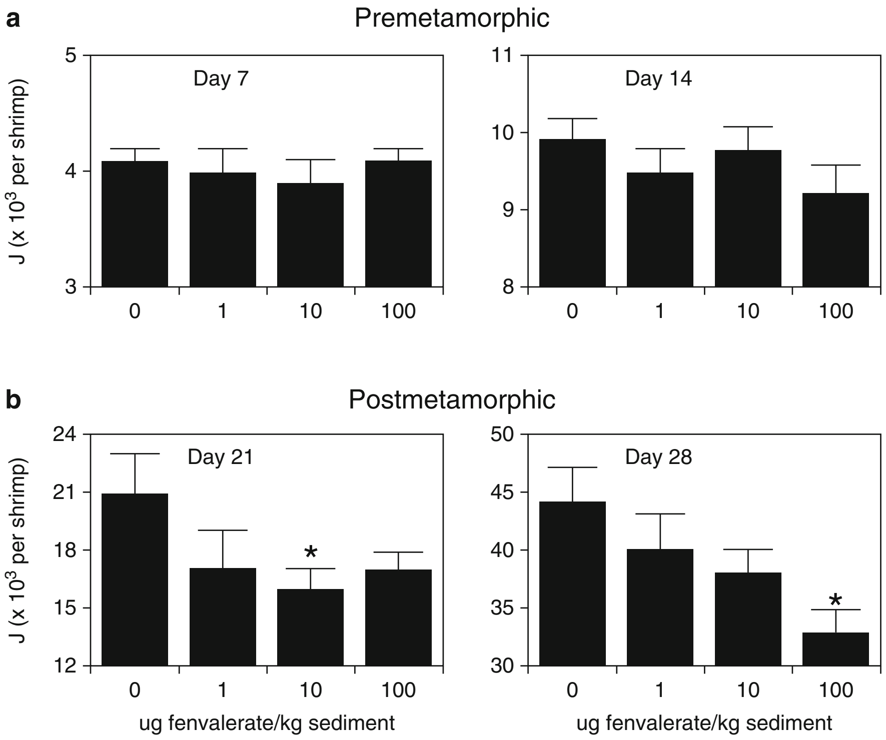


Fig. 3.1 Energy content (J × 10^3 per shrimp) for *P. pugio* larvae (**a**) and post-larvae (**b**) as influenced by exposure from day of hatch to sediment with fenvalerate. Values = means ± SE; asterisks = significant differences ($p < 0.05$) from control (Reprinted from McKenney et al. 1998: 469, courtesy of Springer Publishing Co)

(CEA). Both assays are based on the concept that energy in excess of that required for normal maintenance will be available for growth and reproduction. Mysids were exposed to environmentally realistic concentrations of the organophosphate pesticide chlorpyrifos. Results of both assays were significantly correlated, and both were significantly affected by chlorpyrifos (Fig. 3.2). CEA was more sensitive and was reduced at lower concentrations (0.038 and 0.056 $\mu g\ l^{-1}$) than SFG.

Effects of PCBs on fiddler crab *Uca pugilator* respiration were variable – at some temperatures 50 $\mu g\ l^{-1}$ PCBs increased metabolic rate and at other temperatures it decreased it (Vernberg et al. 1978), showing again that temperature may be responsible for some of the disparate results obtained in different studies. Effects of pentachlorophenol on the metabolic rate of grass shrimp depended on the molt cycle stage (Cantelmo et al. 1978), with molting stages much more sensitive than intermolt animals. This study also found pentachlorophenol inhibition of respiratory enzymes in blue crabs, including fumarase, succinate dehydrogenase, malate dehydrogenase, glucose-6-phosphate dehydrogenase, pyruvate kinase, and lactic dehydrogenase.

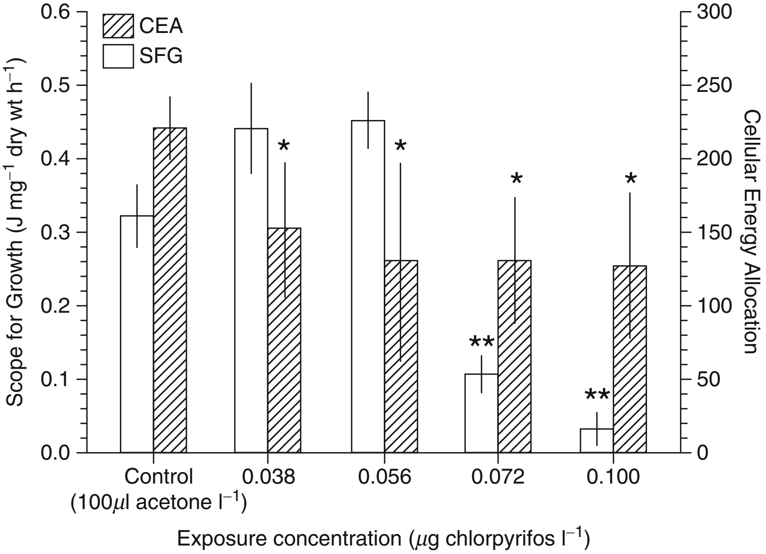


Fig. 3.2 Scope-for-growth (SFG) and cellular energy allocation (CEA) of *N. integer* following exposure to chlorpyrifos. Data pooled from SFG calculated at 48, 96, and 168 h and CEA calculated at 48 and 168 h. Error bars = SD. * = significantly different from control ($p < 0.05$, **$p < 0.01$) (Reprinted from Verslycke et al. 2004: 10, courtesy Elsevier Publishing Co)

3.1.3 Hypoxia

Most organisms reduce their respiration in response to hypoxia, which is an adaptive response. Bridges and Brand (1980) found a respiratory overshoot or oxygen debt in *Corystes cussivelaunus* and *Galathea strigosa* after exposure to a hypoxic stress. This oxygen debt depended on the length and severity of the hypoxia, and the oxygen debt/deficit ratio was smaller in *Corystes* than in *Galathea*. Hemolymph lactate levels rose rapidly during hypoxia in both species but returned to control levels more rapidly in *Corystes* when returned to normoxia. Studies of lactate levels in other crustaceans showed that lactate declines during recovery from hypoxia more rapidly in burrowing crustaceans (which may normally be exposed to low DO more often) compared to non-burrowing species. Examples of burrow-dwelling crustaceans are the mud shrimps *Upogebia stellata* and *U. deltaura* whose burrows are usually hypoxic and hypercapnic. Burrow water pO_2 in the parts of the burrow normally occupied by the mud-shrimp was between 80 and 110 Torr (1 Torr = $^1/_{760}$ of one atmosphere), but was much lower (10–45 Torr) in the deepest, poorly-irrigated parts. They irrigate their burrows by pleopod beating, which draws oxygenated water into the burrow. When exposed to hypoxia, these species could maintain their rates of oxygen consumption over a wide range of pO_2 (30–50 Torr) (Astall et al. 1997). The shrimp *Calocaris macandreae* inhabits complex burrows in marine muddy sediments which are subject to severe hypoxia. In the laboratory, it was highly tolerant of anoxia ($LT_{50} = 43$ h) and accumulated L-lactate as the end-product of anaerobic metabolism (Anderson et al. 1994). Metabolic recovery was slow; hemolymph lactate concentrations returned to normal after approximately 40 h. Anaerobic metabolism appeared to be initiated only during exposure to severe hypoxia (<7 Torr).

Taylor and Spicer (1987) investigated the prawns, *Palaemon elegans* from intertidal pools, and *P. serratus* from subtidal areas. *P. elegans* had greater tolerance of severe hypoxia than *P. serratus*. Exposure to moderate hypoxia (30 Torr) resulted in little change in the concentration of L-lactate in the blood or in the tissues of either species, but when in extreme hypoxia (10 or 5 Torr), there was a progressive increase in the concentration of L-lactate in the blood and tissues of both species, which returned to normal levels more rapidly in *P. elegans* after return to normoxic conditions. Under hypoxia, both species showed an increase in blood glucose and a reduction in the glycogen content of the tissues, which returned to normal levels within 6 h of return to normoxic conditions. However, during exposure to severe hypoxia under both laboratory and field conditions, *P. elegans* frequently exhibited partial emersion by moving into shallow water, usually lying on their sides at the water's edge (Taylor and Spicer 1988). The P_{O2} at which this response occurred depended on temperature; at higher temperatures they exhibited the response at higher oxygen tensions. When exposed to anoxic conditions in the laboratory *P. elegans* often left the water and emerged on rocks. In these prawns, the total oxygen content of the blood was higher and lactate lower than that of either fully immersed or totally emersed prawns. Similarly, when exposed to hypoxia, the green crab, *Carcinus maenas* also can emerge and aerate its branchial chambers by reversing the direction of their irrigation (Taylor et al. 1973). At low oxygen tensions submerged crabs underwent a progressive bradycardia – heart rate became significantly lower than the rate in normoxia. However, emersion into air resulted in aeration of the branchial chambers and an immediate return of heart beat towards that in normoxic seawater. The results provide indicate a respiratory role for the emersion response.

Glucose, lactate, and ammonia concentrations of Norway lobster, *Nephrops norvegicus* were studied in normoxia and in various hypoxia levels for periods up to 3 weeks. Increases in circulating glucose and lactate took place in oxygen tensions less than 30 Torr, indicating aerobic metabolism down to this oxygen tension (Hagerman et al. 1990). In moderate hypoxia (half saturation), lobsters synthesised hemocyanin; in more severe hypoxia some hemocyanin catabolism occurred.

3.1.4 Climate Change/Ocean Acidification

The copepod, *Centropages tenuiremis*, under 1,000 μatm CO_2 increased respiration and feeding, except for an initial acclimating period, when feeding was less (Li and Gao 2012). The authors suggested that the copepods increased their respiration and feeding in response to acidification in order to balance energy costs.

The shrimp *Metapenaeus joyneri* exposed to both hypercapnia (1 kPa) at two temperatures (15 and 20 °C) demonstrated physiological effects, (i.e. lower hemolymph osmolality and higher pH) and reduced metabolic scope (the difference between active and routine metabolism), compared with control individuals at 0.04 kPa (Dissanayake and Ishimatsu 2011). Authors suggested that synergistic factors may cause organisms to shift their energy utilization towards up-regulation of maintenance functions (e.g. osmoregulation) causing a decrease in aerobic scope and energy-demanding activities.

3.2 Mollusks

3.2.1 Metals

Oysters, *Crassostrea virginica*, were exposed to Cu (50–100 $\mu g\ l^{-1}$) or Cd (100–600 $\mu g l^{-1}$) for up to 14 days. Cu produced increases in oxygen consumption at 100 $\mu g\ l^{-1}$, as did Cd at 600 $\mu g l^{-1}$ (Engel and Fowler 1979). Gills accumulated both metals as a function of time of exposure, and Cu produced cellular swelling and mitochondrial damage in gill tissue. Brown and Newell (1972) found that Cu reduced oxygen consumption in *M. edulis*, while Thurberg et al. (1974) reported that silver stimulated respiration of surf clams (*Spisula solidissima*) after exposure to 0.05 and 0.10 $\mu g\ l^{-1}$ Ag. Thus, different metals produced different responses in respiratory processes.

Brown mussels, *Perna perna*, exposed to Cu at 25 and 50 $\mu g\ l^{-1}$ increased their mucus secretion rate, nitrogen excretion rates and oxygen consumption rates (Vosloo et al. 2012). The increased respiratory rates at the higher metal concentrations were related to the induced stress response (Fig. 3.3).

Elfwing and Tedengren (2002) compared responses of two intertidal oysters (*Saccostrea cucullata* and *Crassostrea lugubris*) and a subtidal species, *C. belcheri* to 12 h exposure to 20 $\mu g\ l^{-1}$ Cu. While oxygen consumption, ammonia excretion, clearance rate, and absorption efficiency were reduced, the intertidal species were more tolerant than the subtidal species, presumably because they live in a more variable environment.

Manila clams (*Ruditapes philippinarum*) were exposed to 10 and 40 $\mu g\ l^{-1}$ of Cu (96 h) and responses characterized using NMR-based metabolomics (Zhang et al. 2011). At both concentrations, metabolic changes were seen in intermediates of the Krebs cycle and amino acids, such as increases in homarine and branched chain amino acids, and decreases in succinate, alanine and dimethylamine in gills. Similar studies with arsenic by Wu et al. (2013) with different salinities revealed that in salinity of 31.1 ppt, As decreased levels of amino acids (glutamate, β-alanine, etc.), and increased betaine and fumarate. Metabolic biomarkers of decreased threonine, histidine, ATP and fumarate were found in As-treated clams at medium salinity (23.3 ppt). However, in low salinity (15.6) only elevated ATP and depleted succinate

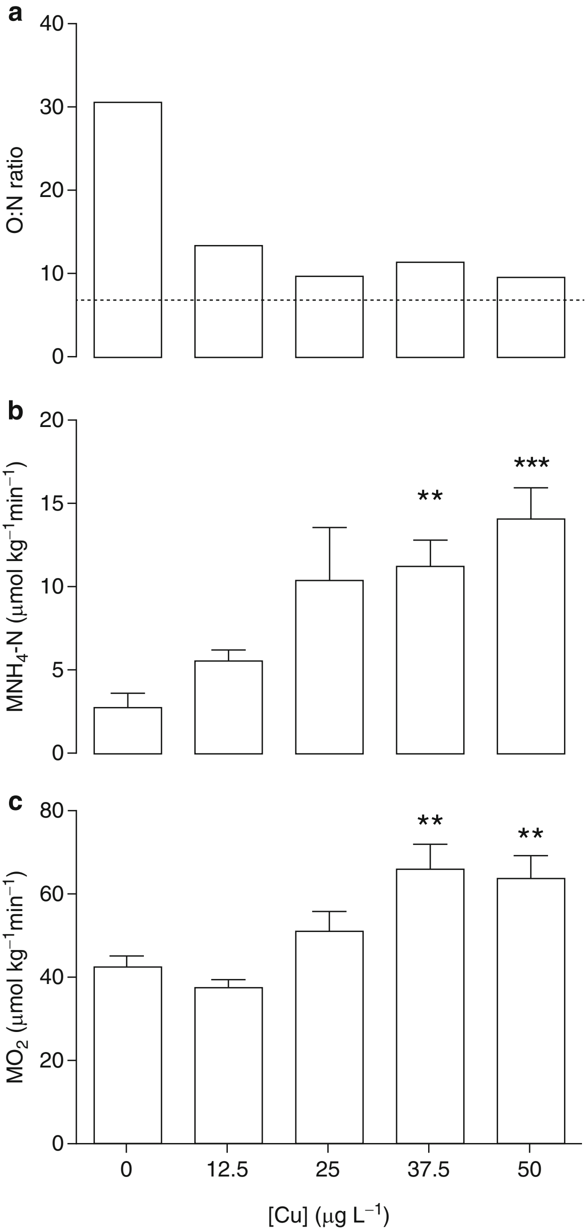


Fig. 3.3 (**a**) Atomic equivalents of oxygen consumed and nitrogen excreted (O:N ratio), (**b**), mass-specific ammonia excretion rates, and (**c**) mass-specific oxygen consumption rates for mussels exposed to copper for 24 h. Error bars = SEM, * = statistical difference between exposed and controls (**$p < 0.01$, ***$p < 0.0001$) (Reprinted from Vosloo et al. 2012: 5, courtesy of Elsevier Publishing Co)

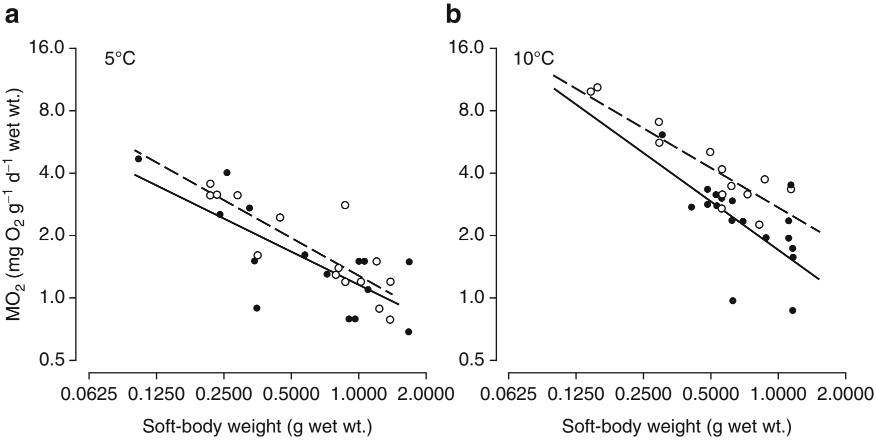


Fig. 3.4 Relationship between oxygen consumption and wet soft-body weight of control (*open circle*) and Cd-exposed (*solid circle*) *Nucella lapillus* at 5 °C (**a**) and 10 °C (**b**). Significant regression lines ($p < 0.05$) for control (*dashed line*) and treatment (*solid line*) are shown (Reprinted from Leung et al. 2000, courtesy Cambridge University Press)

were detected. Thus, As induced osmotic stress and disturbed energy metabolism in normal and medium salinities, but caused only disturbance in energy metabolism under low salinity.

Leung et al. (2000) found metabolic depression in Cd-exposed (500 $\mu g\ l^{-1}$) dog-whelks, *Nucella lapillus*. (Fig. 3.4). Reduced metabolism was considered a strategy to minimize Cd uptake and toxicity while meeting the extra energy demands for detoxification and maintenance. They also observed mucus secretion and necrotic cells on the surface of the gills, which would also depress O_2 uptake. Reduction in metabolic O_2 may be directly linked to Cd-induced mucus production, structural damage to gills, and reduction in oxygen carrying capacity of hemocyanin. These effects were observed at 10 °C but not at 5 °C.

Mussels, *Perna viridis*, exposed to Cd or Zn similarly exhibited reduced oxygen consumption and ammonia excretion (Cheung and Cheung 1995). Mudsnails (*Hydrobia ulvae*) exposed to 100 and 200 $\mu g\ l^{-1}$ Cd had altered carbon and energy balance as well as growth rate and increased carbon loss but no change in egestion rates (Forbes and Depledge 1992). The changes in the partitioning of carbon loss from body stores suggested a partial shift from aerobic to anaerobic metabolic pathways. Oysters (*C. virginica*) exposed to Cd showed suppressed anaerobic metabolism during anoxia. In controls, ATP turnover during anoxia was sustained by anaerobic glycolysis with negligible contributions from ATP breakdown, but in Cd-exposed oysters ATP breakdown contributed significantly to turnover rate. Thus, while control oysters could maintain ATP levels and tissue energy status during anoxia by glycolysis, Cd-exposed oysters had disturbed energy balance as indicated by the depletion of ATP, decline in adenylate energy charge and increase in ADP/ATP ratios (Fig. 3.5) (Ivanina et al. 2010).

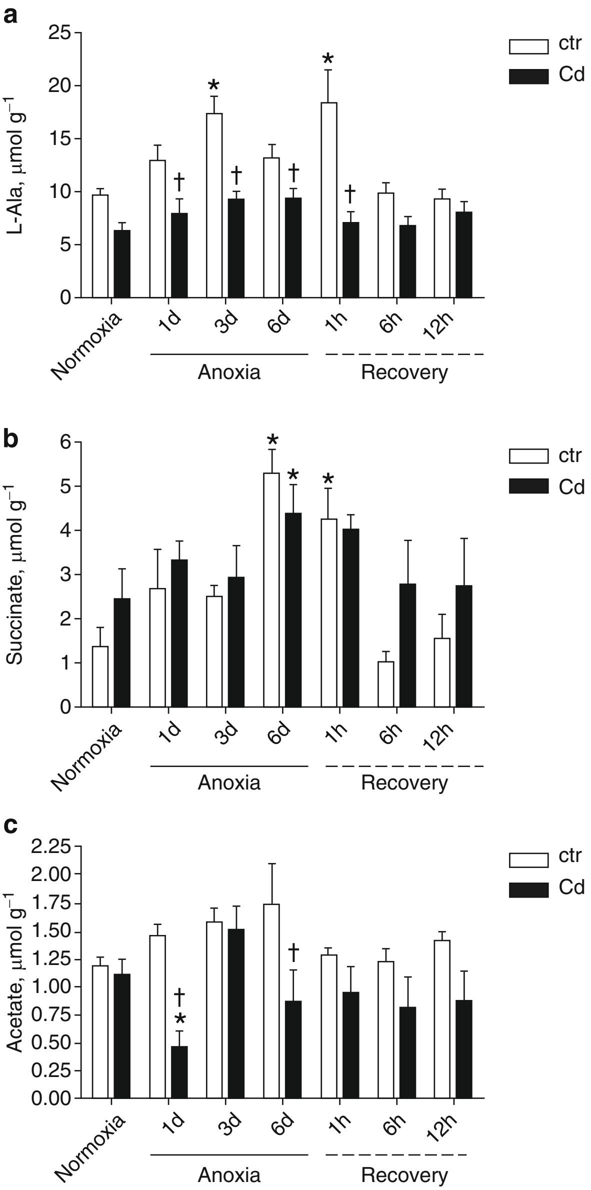


Fig. 3.5 Changes in the concentrations of anaerobic end products in hepatopancreas of *C. virginica* during intermittent anoxia and recovery. (**a**) L-Alanine, (**b**) succinate, and (**c**) acetate. * = significantly different from respective normoxic value ($p < 0.05$). Daggers = difference between control and Cd-exposed ($p < 0.05$) (Reprinted from Ivanina et al. 2010: 334, courtesy Elsevier Publishing Co)

Mercury was associated with respiratory impairment in cockles *Cerastoderma edule* at field sites in Portugal (Nilin et al. 2012). The energy content was negatively correlated with both Hg concentration in tissues and survival in air. Hexavalent Cr (0.1, 1, and 10 μg l^{-1}) had multiple effects on gills of the mussel *M. galloprovincialis*. Exposure induced progressive changes in morphology and immunoreactivity (Ciacci et al. 2012). Cr(VI) increased the activities of the glycolytic enzymes GST and GSR, indicating modulation of carbohydrate metabolism. Changes in transcription of genes for various enzymes were also observed, indicating that Cr affected functional and molecular parameters in gills.

Tributyltin increased the respiration rate in mussels (*Mytilus edulis*) in a dose-dependent manner from 0.5 to 10 μg TBT g^{-1} in tissues. The maximum rate was about twice the control level. Feeding rates were reduced at 3–4 μg g^{-1}, indicating a severe reduction in scope for growth, which is consistent with field observations of growth reduction. Effects of dibutyltin (DBT) were an order of magnitude less toxic (Widdows and Page 1993).

3.2.2 Organics

Oil

The scope for growth (SFG – derived by subtracting the energy used and excreted from the energy absorbed from food) represents the energy available for reproduction and somatic growth. Stickle et al. (1985) found *Mytilus edulis* exposed to the WSF of crude oil reduced their scope for growth with increasing WSF concentrations until it was 0 at 1,163 μg^{-1} aromatic hydrocarbons. The major reason for the reduction, however, was found – again- to be reduced food intake.

Pesticides

Effects of the piscicide, 3-trifluoromethyl-4-nitrophenol, were studied in two marine mollusks by *in vivo* ^{31}P nuclear magnetic resonance spectroscopy, a technique for examination of cellular respiratory toxicity since inorganic phosphate, phosphoarginine, and adenosine 5′-triphosphate levels, and the arginine kinase rate constant, can be measured (Viant et al. 2001). These parameters were measured in red abalone (*Haliotis rufescens*) and owl limpets (*Lottia gigantea*) during 5-h exposures to 3 mg l^{-1} 3-trifluoromethyl-4-nitrophenol, followed by 5-h recovery. In the abalone, phosphoarginine decreased by 50 %, inorganic phosphate increased by 900 %, and the arginine kinase rate constant quadrupled compared to controls, which is consistent with an uncoupling of oxidative phosphorylation. Limpets were less affected, showing no change in phosphoarginine, an increase of only 200 % in inorganic phosphate, and an approximate doubling of the arginine kinase rate constant. Adenosine 5′-triphosphate levels remained constant in all control and exposed mollusks.

As biological pesticides have become more prevalent, products based on the bacterium *Bacillus thuringiensis* (Bt) are among the most common biopesticides. Manachini et al. (2013) studied the effect of a commercial Bt product on physiological responses and energy budgets of two intertidal bivalves in the Mediterranean, the native *Mytilaster minimus* and the invasive *Brachidontes pharaonis*. They simulated worst case scenarios using the average dose applied to fields (45 μl l^{-1}) and a hypothetical accumulation dose (90 μl l^{-1}). Feeding rates declined while respiration rates and cardiac activity increased, resulting in reduced energy budgets. *B. pharaonis* was affected to a greater degree than *M. minimus*, but neither species showed altered excretion rates. Whether such "worst case" concentrations would occur in nature is unknown.

3.2.3 Contaminants of Emerging Concern

Triclosan (5-Chloro-2-(2,4-dichlorophenoxy) phenol) is an antibacterial compound used in pharmaceuticals and personal care products. Alterations in lysosomal stability can be induced by various stressors. When stable, lysosomes accumulate and retain the dye neutral red for extended periods of time, but when destabilized, they coalesce to form larger structures and the neutral red will leak into the cytosol of the cell through damaged membranes. Lysosomal changes following the addition of neutral red dye can be directly related to the degree of stress being imposed. Triclosan produced a significant reduction of neutral red retention time by *Perna perna* at 12 ng l^{-1}, levels considered similar to environmental levels (Fig. 3.6) (Cortez et al. 2012).

3.2.4 Hypoxia

Wang et al. (2011) maintained mussels, *Perna viridis*, for 4 weeks under different DO (1.5, 3.0 and 6.0 mg O_2 l^{-1}) and salinity (15, 20, 25 and 30) regimes. Clearance rate, absorption efficiency, respiration rate and scope for growth decreased with decreasing salinity and DO, while excretion rate increased with decreasing salinity and increasing DO. The O:N ratio was <10 at salinities of 15 and 20 at all DO levels. SFG was negative in most cases, except for those exposed to 6.0 mg O_2 l^{-1} or at a salinity of 30 with lower DO.

Exposure to hypoxic sea water (pO_2 of 50 mmHg) and hyposalinity (20 %) caused the heart rate of the limpet *Patella granularis* to decline rapidly (Marshall and McQuaid 1993). In hypoxia the normal heart rate (50 beats/min) fell initially to 15–30 beats/min, and 2 h later, cardiac arrest occurred. When oxygen tension and salinity were returned to normal, heart rate became significantly elevated. In *Siphonaria capensis*, a pulmonate, exposure to reduced oxygen tension and salinity induced a regular, although often delayed (after 2 h) bradycardia (<10

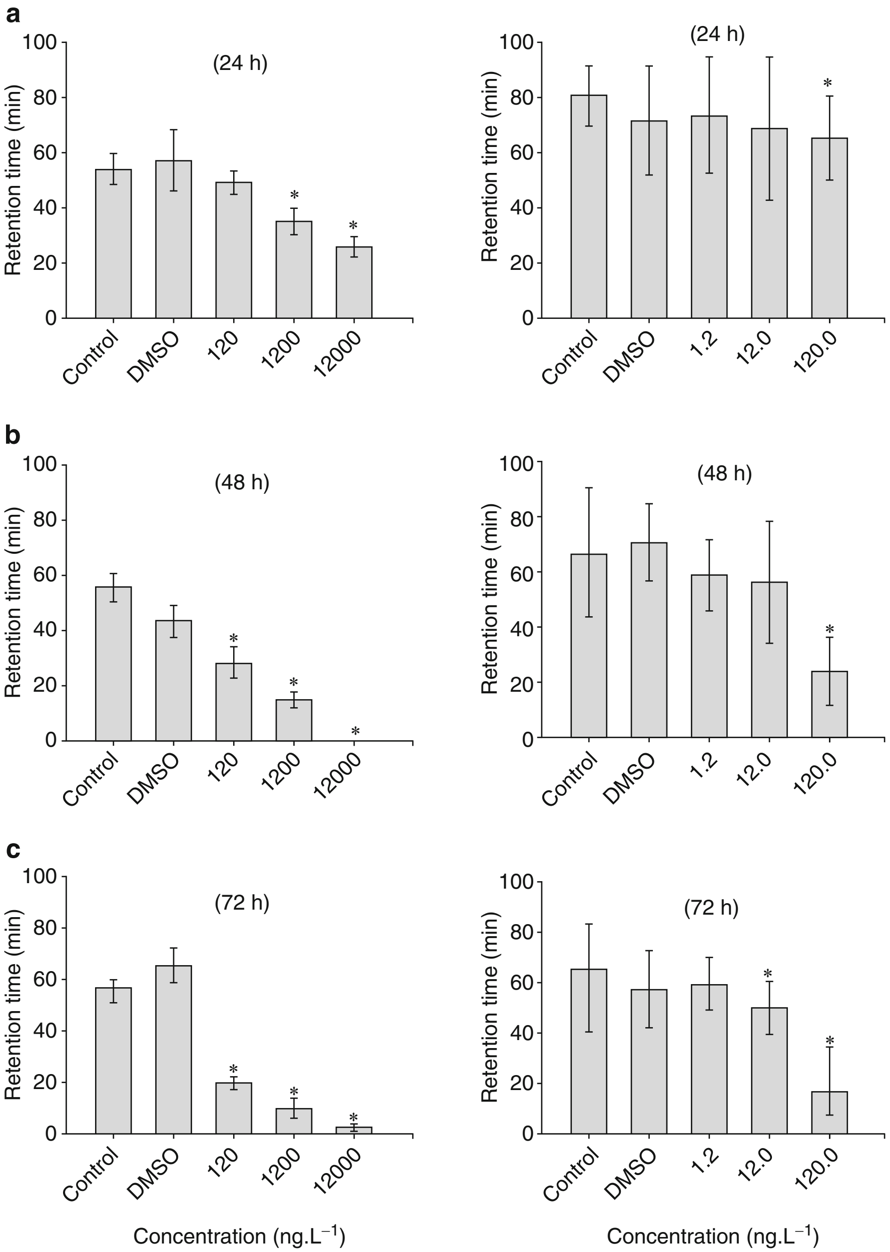


Fig. 3.6 Results of the first Neutral Red Retention Time Assay (mean ± SD). (**a**) Exposure time of 24 h; (**b**) exposure time of 48 h; (**c**) exposure time of 72 h. * = significant difference from control ($p < 0.05$) (Reprinted from Cortez et al. 2012: 148, courtesy of Elsevier Publishing Co)

beats/min). No significant cardiac overshoot was observed for this limpet. Different heart beat patterns by the limpet species may be linked with respiratory/metabolic responses.

Kozuki et al. (2013) studied after-effects of hypoxia (DO of 0.5 mg l^{-1}) on the manila clam *Ruditapes philippinarum.* After 3 days of hypoxia, no substantial change in mortality, glycogen content, or clearance rate was observed in the period following the hypoxia. However, after another 3 days of hypoxia following recovery from the first exposure, mortality rate increased, glycogen content declined, and clearance rate decreased to 77 % of controls and was unable to recover following the second exposure.

Hypoxia can interact with metals. Ivanina et al. (2011) studied the combined effects of Cd and intermittent anoxia on anaerobic metabolism and energy status of oysters (*C. virginica*). Anaerobic metabolism (indicated by accumulation of L-alanine, succinate and acetate) was suppressed in Cd-exposed oysters. In control oysters, ATP turnover rate during anoxia was mostly sustained by anaerobic glycolysis with negligible contributions from ATP and PLA breakdown. In contrast, in Cd-exposed oysters ATP breakdown was a major factor. Thus, while controls could maintain ATP levels and tissue energy status during prolonged anoxia, Cd-exposed oysters had disturbed energy balance, as indicated by the depletion of ATP, a decline in adenylate energy charge, and increase in ADP/ATP ratios. This energy deficiency combined with suppression of anaerobic metabolism may strongly affect oysters in estuaries where metal pollution co-occurs with hypoxia. However, some acclimation can occur. Hypoxia-acclimated oysters retained normal standard metabolic rates at 5 % O_2 (in contrast to a decline during acute hypoxia). Oysters spent more time actively ventilating in hypoxia than normoxia, causing enhanced Cd uptake (Ivanina et al. 2011). Cd (50 μg l^{-1}) led to a decrease in tissue glycogen stores, increase in free glucose, and elevated activity of glycolytic enzymes (hexokinase and aldolase) indicating a greater dependence on carbohydrate catabolism.

3.2.5 Climate Change/Ocean Acidification

Juvenile clams *Ruditapes decussatus* were exposed to reduced pH (−0.4 and −0.7 pH units) compared with control seawater. After 87 days, clearance, ingestion and respiration rates decreased, and ammonia excretion increased (Fernández-Reiriz et al. 2011). Reduced ingestion combined with increased excretion is associated with lowered energy input, which will likely contribute to slower growth. It is interesting to note that in this relatively long-term exposure, there was no adaptation to the changed environment.

High CO_2 levels (pH ~7.5, pCO_2 ~3,500 μatm) increased juvenile mortality rates and inhibited both shell and soft-body growth of juvenile *C. virginica* compared to control conditions (pH ~8.2, pCO_2 ~380 μatm) (Beniash et al. 2010).

Furthermore, elevated CO_2 raised the standard metabolic rate which was considered to be due to the higher energy cost of homeostasis. Dickinson et al. (2012) studied interactive effects of salinity and CO_2 on energy homeostasis in juvenile *C. virginica,* exposed for 11 weeks to 30 or 15 salinity at current atmospheric P_{CO2} (~400 μatm) or ~700–800 μatm. Elevated P_{CO2} and/or low salinity led to increased mortality, reduction of tissue energy stores (glycogen and lipid) and negative soft tissue growth, indicating energy deficiency. Tissue ATP levels were not affected by changing salinity and P_{CO2}, suggesting that juvenile oysters maintain their cellular energy status at the expense of lipid and glycogen stores. At the same time, no compensatory up-regulation of carbonic anhydrase activity was found. Metabolic profiling revealed altered metabolite status; specifically, acetate levels were lower in high CO_2 individuals at low salinity.

Navarro et al. (2013) evaluated the impact of medium-term exposure to elevated $p\mathrm{CO}_2$ (750–1,200 ppm and 380 control) on juvenile mussels, *Mytilus chilensis,* over 70 days in a mesocosm system. Reduced clearance was observed after 35 days; absorption rate and absorption efficiency were reduced at high $p\mathrm{CO}_2$ levels. In addition, oxygen uptake fell significantly, indicating metabolic depression. These physiological responses resulted in reduced energy available for growth (scope for growth) with negative consequences for aquaculture during medium-term exposure to acid conditions.

Melatunan et al. (2011) investigated respiration rates, adenylate energy charge, nucleotide concentrations, and metabolite concentrations in the periwinkle, *Littorina littorea* maintained for 30 days at pH of 8.0 or 7.6 and at temperatures of 15 or 20 °C. Snails in reduced pH decreased respiration rate by 31 %, but only by 15 % in reduced pH plus elevated temperature. Decreased respiration was associated with metabolic reduction and an increase in end-product metabolites, indicating increased reliance on anaerobic metabolism. There was an interactive effect of low pH with elevated temperature on total adenylate nucleotides, which was apparently compensated for by the maintenance of adenylate energy charge via AMP deaminase activity. The findings suggest that snails have complex responses, with likely negative effects on growth.

The deep-sea bivalve *Acesta excavata* was subjected from 1 to 96 h to elevated CO_2 (pH 6.35, $P_{CO2} = 33{,}000$ μatm), corresponding to conditions reported from natural CO_2 seeps (Hammer et al. 2011). During exposure there was a drop in hemolymph and intracellular pH. During recovery, intracellular pH returned to control values, while extracellular pH remained significantly lower. Oxygen consumption initially dropped by 60 %, but then increased during the later stages of exposure, indicating some recovery. These results of extreme, although brief, exposures suggest that some species of deep-sea organisms can compensate for elevated CO_2.

Seibel et al. (2012) showed that high $P\mathrm{CO}_2$ reduced oxygen consumption in the pelagic pteropod, *Limacina helicina forma antarctica*, by ~20 %. The rates measured at 180–380 μatm were significantly higher than those measured at elevated CO_2 levels (789–1,000 μatm). However, metabolic plasticity was noted, and the response to CO_2 was dependent on the baseline level of metabolism and on food intake. In contrast, Maas et al. (2012) studied oxygen consumption and ammonia excretion of five pteropod species, collected from the tropical Pacific. When exposed to elevated CO_2 (1,000 ppm), pteropods that naturally migrate into oxygen minimum zones (*Hyalocylis striata, Clio pyramidata, Cavolinia longirostris* and *Creseis virgula*) were not affected. However, *Diacria quadridentata*, which does not migrate, responded to high CO_2 with reduced oxygen consumption and ammonia excretion, similar to *Limacina*, indicating that the natural environment of a species influences its resilience to ocean acidification. Since over the past half-century the Norwegian Sea has experienced a progressive freshening with time, Manno et al. (2012) investigated the combined effects of ocean acidification and freshening on *Limacina retroversa*, the dominant pteropod in sub polar areas. Living *L. retroversa* were exposed to four different pH values ranging from the pre-industrial level to the forecasted end of century level. Each pH was combined with a salinity gradient. Mortality increased only when both pH and salinity were reduced. The combined effects also affected the ability to swim upwards, suggesting that energy costs of maintaining ion balance (low salinity) and avoiding sinking combined with the energy cost necessary to counteract shell dissolution (high pCO_2), exceed the available energy budget.

3.2.6 Polluted Environment

Mytilus galloprovincialis were caged in impacted and reference sites along the coast of Sicily, Italy. PAHs were elevated in the digestive gland of mussels from the industrial areas compared with control. Digestive gland metabolic profiles showed changes in metabolites involved in energy metabolism. Changes in lactate and acetoacetate indicate increased anaerobic metabolism and alteration in lipid metabolism, respectively, suggesting that the mussels in the contaminated sites were affected by adverse environmental conditions (Fasulo et al. 2012). Physiological parameters were measured on natural populations of cockles *Cerastoderma edule* from different sites in Portugal (Nilin et al. 2012). The energy content was negatively correlated with both Hg concentration in cockle tissues and survival in air, but was positively correlated with the condition index. Interestingly, there was a positive correlation between survival in air and tissue mercury concentration.

Scope for Growth and physiological rates were measured in wild mussels from many sites by the Spanish Marine Pollution monitoring program. The integration of biological and chemical data suggests that organochlorine compounds, particularly chlordanes and DDTs, may have a negative effect on SFG, although such effects are smaller than those associated with biological parameters such as mussel age and condition, which act as confounding factors when attempting to determine the effect of chemicals on SFG (Albentosa et al. 2012). However, one would think that condition index was an effect of contaminant exposure, rather than a cause of SFG.

3.3 Fishes

3.3.1 Metals

Chronic exposures (30–90 days) of juvenile striped bass (*Morone saxatilis*) to low levels of Cd (0.5–5 μg l^{-1}) or inorganic Hg (1–10 μg l^{-1}) depressed gill-tissue respiration, but did not cause changes in two liver enzymes monitored. Chronic exposures (60 days) of winter flounder (*Pseudopleuronectes americanus*) to Cd, inorganic Hg, or Ag produced contrasting effects. At 10 μg l^{-1}, Cd depressed gill-tissue respiration, while Hg increased it and altered plasma protein levels, while Ag caused no detectable change in gill-tissue respiration (Calabrese et al. 1977). Gharaei et al. (2011) examined blood biochemical parameters including GLU (glucose), LDH (lactate dehydrogenase), AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase) and cortisol in juvenile Beluga sturgeon (*Huso huso*) fed 32 days on diets with meHg: (low = 0.76 mg kg^{-1}; medium = 7.88 mg kg^{-1}; and high = 16.22 mg kg^{-1}). Significant increases were observed in all parameters, except ALP, which decreased compared to controls. These results suggest that long-term dietary meHg affects metabolic enzyme activity and glucose levels in this fish. Yadetie et al. (2013) analyzed transcriptional changes in the liver of cod (G*adus morhua*) treated with meHg (0.5 and 2 mg/kg of body weight) for 14 days. From the observed transcriptional changes, the main pathways affected by the treatment were energy metabolism, oxidative stress response, immune response and cytoskeleton remodeling. The activity of many genes in oxidative stress pathways such as glutathione metabolism were altered. There were disproportionate numbers of genes coding for enzymes involved in metabolism of amino acids, fatty acids and glucose. The effects observed on transcripts coding for enzymes of energy pathways suggests meHg disruption of nutrient metabolism. Overall, there has been surprisingly little recent work on effects of metals on respiration and metabolism of marine fishes.

3.3.2 Organics – Oil

Petroleum hydrocarbons (WSF of crude oil and No. 2 fuel oil) increased “coughing rates” of pink salmon (*Oncorhynchus gorbuscha*) fry (Rice et al. 1977). This is a response in which fish flex their gills to expel unwanted irritants. Rates increased in proportion to oil concentration and remained above normal over 72 h of exposure, although the fish had begun to depurate the aromatics by 24 h of exposure. The increased metabolic rate may be detrimental in the long term. Sole (*Solea solea*) were exposed to oil conditions found during the weeks following the *Erika* oil spill (December 1999, off the coast of France). Davoodie and Claireaux (2007) measured basal and active metabolic rates, calculated aerobic metabolic scope, and compared the ability of control and exposed sole to cope with reduced oxygen. While basal

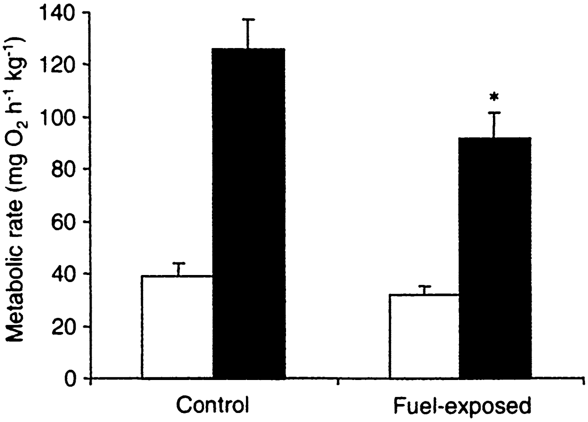


Fig. 3.7 Standard and active metabolic rates in control 5-day fuel-exposed fish. For a given group, the difference between standard and active metabolic rates is the aerobic metabolic scope (Reprinted from Davoodie and Claireaux 2007: 931, courtesy of Elsevier Publishing Co)

metabolic rate was not altered, active metabolic rate and therefore metabolic scope, was reduced in exposed fish (Fig. 3.7). The ability to deal with reduced oxygen was impaired, as indicated by a 65 % increase in the critical oxygen level.

Pyrene alone was found to impair metabolic scope and scope for activity. Juvenile common gobies *Pomatoschistus microps*, were exposed under acute conditions – 0.125, 0.25, 0.5 or 1 mg l^{-1} pyrene for 96 h. Swimming performance was significantly decreased at all concentrations tested (Oliviera et al. 2012). Changes in enzymes suggested changes in pathways of energy production. Exposure to naphthalene (2 mg l^{-1}) caused a typical stress response: elevation of plasma cortisol and an increase in glucose in striped mullet (*Mugil cephalus*) (Thomas and Neff 1985). Responses of golden grey mullet, *Liza aurata,* to a chemically dispersed oil, a single dispersant, a mechanically dispersed oil simulating natural dispersion of oil, a water soluble fraction of oil simulating an undispersed and untreated oil slick, and control seawater were evaluated (Milinkovich et al. 2012). Biliary PAH metabolites showed that incorporation of PAH compounds increased when the oil was dispersed either mechanically or chemically. However, the aerobic metabolic scope and the critical swimming speed of exposed fish were not altered, perhaps because of the relatively brief (48 h) exposure.

3.3.3 Hypoxia

Juvenile turbot *Scophthalmus maximus* were fed to satiation at reduced O_2 concentrations of 3.5, 5.0 mg l^{-1} and at 7.2 mg l^{-1} (normoxia). Both feed intake and growth were significantly lower under hypoxia. Oxygen consumption of feeding fish was significantly higher under normoxia, but following 7 days of feed deprivation oxygen consumption was similar under normoxia and hypoxia (Pichavant et al. 2000).

O'Connor et al. (2011) examined responses of three populations of sticklebacks to acute and chronic hypoxia (DO 2.2 mg l^{-1}). Fish from the population least likely to experience hypoxia in their normal habitat had the greatest response to low oxygen, demonstrating anaerobic glycolysis after only 2 h of hypoxia. However, there was no measurable effect of a more prolonged period (7 days) of hypoxia on any of the fish, suggesting that they had acclimated to this low DO over that time.

The epaulette shark (*Hemiscyllium ocellatum*) and grey carpet shark (*Chiloscyllium punctatum*) are commonly found in periodically hypoxic environments. Chapman et al. (2011) measured ventilation rates during an anoxic challenge at 23 °C and during 1.5 h of anoxia followed by 2 h of re-oxygenation at 23 and 25 °C. During the anoxic challenge, epaulette sharks started ventilatory depression significantly earlier at 25 °C. During re-oxygenation, those at 23 °C did not increase their ventilation rates, but those in 25 °C did. Grey carpet sharks had no ventilatory depression during anoxia, but had elevated ventilation rates during re-oxygenation. Anoxia tolerance of both species was temperature-dependent. Temperature was also an important factor for summer flounder, *Paralichthys dentatus,* responses to hypoxia. Fish were subject to progressive hypoxia at acclimation temperature (22 °C) and after an acute temperature increase (to 30 °C). Mean critical oxygen levels (the oxygen levels below which fish could not maintain aerobic metabolism) increased from 27 % saturation (2.0 mg O_2 l^{-1}) at 22 °C to 39 % saturation (2.4 mg O_2 l^{-1}) at 30 °C (Capossela et al. 2012). Gill ventilation and oxygen extraction changed immediately with the onset of hypoxia at both temperatures. The fractional increase in gill ventilation was much larger at 22 °C than at 30 °C, but the decrease in oxygen extraction was similar at both temperatures, and smaller than the changes in gill ventilation. Bradycardia was not observed until 20 and 30 % oxygen saturation at 22 and 30 °C, respectively, which was below critical oxygen levels. The increase in the critical oxygen level at 30 °C suggests a lower tolerance to hypoxia after an acute increase in temperature.

One would predict that the glycolytic pathway would be enhanced in fish at low DO. The specific activities of glycolytic enzymes in liver and skeletal muscle were determined in *F. heteroclitus* prior to the onset of low DO treatments (1 mg l^{-1} for severe hypoxia, 3 mg l^{-1} for moderate hypoxia), and at intervals thereafter (Abbaraju and Rees 2012). Significant effects of low DO were seen on three liver enzymes, PGI, PFK, and PGK, whose specific activities were highest in fish in severe hypoxia, especially after 14 days. In skeletal muscle, only one glycolytic enzyme, ALD, was affected, being significantly lower in fish in severe hypoxia than in those at moderate hypoxia at 14 days. These observations suggest that mechanisms causing these alterations are enzyme- and tissue- specific, rather than applying uniformly to all enzymes in the glycolytic pathway. Cooper et al. (2002) exposed spot (*Leiostomus xanthurus)* to various oxygen tensions (10 % saturation, 0.8 mg/l; 25 %, 2.0 mg/l; 50 %, 4.0 mg/l; 100 %, 8.0 mg/l). After 12 h of exposure, tissue samples were analyzed for citrate synthase (CS) and lactate dehydrogenase

(LDH). There was a significant increase in LDH activity, an indicator of anaerobic metabolism, in the 10 % treatment in gills, while there was no significant change in citrate synthase activity, an indicator of aerobic metabolism. Van Ginnekin et al. (1995) found that with increasing hypoxia, metabolic parameters started to change in the following order: O_2 consumption decreased, PCr (phosphocreatine) decreased, intracellular pH decreased, free ADP concentration increased, ATP decreased. PCr levels fell with the PO_2. After each increment, the PCr reached a stable value while, in some cases, recovery was observed, which could be explained because the balance between anaerobic and aerobic metabolism fluctuates during hypoxia due to changes in the activity of the fish. In all species studied, anaerobic glycolysis was activated, but in contrast to anoxia, metabolic suppression did not occur.

Some fishes can resort to aquatic surface respiration (ASR) in hypoxic water. Intertidal gobies of various genera ventilate their gills with surface water when the DO declines to 2.1 mg l^{-1}. Some of the species hold an air bubble in their buccal chamber, which is frequently exchanged. The bubble provides a source of oxygen and also lifts up the head for easier ASR (Gee and Gee 1991).

3.3.4 Contaminants of Emerging Concern

Handy et al. (2011) reviewed effects of nanoparticles (NPs) on fish, and found that sublethal effects have been reported at concentrations of about 100 μg–1 mg l^{-1}. Exposure to NPs can alter ventilation, mucus secretion, and gill pathology, which may or may not lead to hematological disturbances. The internal target organs (liver, spleen and hematopoietic system, kidney, gut and brain) exhibit oxidative stress and pathology.

Griffit et al. (2012) found that low levels of silver NPs induced adverse effects in juvenile and adult sheepshead minnows (*Cyprinodon variegatus*). Chronic exposure produced thickening of gill epithelia and altered gene expression profiles.

3.3.5 Polluted Environments

F. heteroclitus from a degraded prey-impoverished marsh (PIM) had elevated total metabolic costs compared with fish from reference sites, but also had higher food consumption than reference populations, suggesting that increased food consumption could offset elevated metabolic costs (Goto and Wallace 2010). Only age-2+ females of the PIM had significantly reduced growth. Most PIM fish, however, had lower growth conversion efficiency, suggesting energetic costs of living in degraded habitats.

Juvenile sea bass (*Dicentrarchus labrax*) and turbot (*Scophtalmus maximus*) were caged in areas with differential pollution for 38 days. Physiological performance and metabolism was affected. Fish had higher growth rates, RNA:DNA ratio, growth and condition index in the least contaminated station. Lipid storage index based on the ratio of triacylglycerols to sterols (TAG:ST), was highest in the least contaminated station for both species (Kerambrun et al. 2012).

3.4 Other Taxa

3.4.1 Metals

The polychaete *Neanthes virens* from a clean site had decreased oxygen consumption after they had accumulated 113 ppm Ag. However, worms from Ag-contaminated sites did not show effects of additional Ag exposure (Pereira and Kanungo 1981).

Alutoin et al. (2001) investigated physiological responses of the coral *Porites lutea* to reduced salinity (20 psu) and 10 and 30 μg l^{-1} Cu. No significant changes in respiration were seen, but primary production by the zooxanthellae was reduced in lower salinity, 30 μg l^{-1} Cu, and the combination of the two (Fig. 3.8).

Complicated interactions of Cu and temperature with zooxanthellar production rate were noted in the coral, *Porites cylindrica* after 24 h exposure to 11 μg Cu l^{-1} and increased temperature, separately and in combination (Nyström et al. 2001).

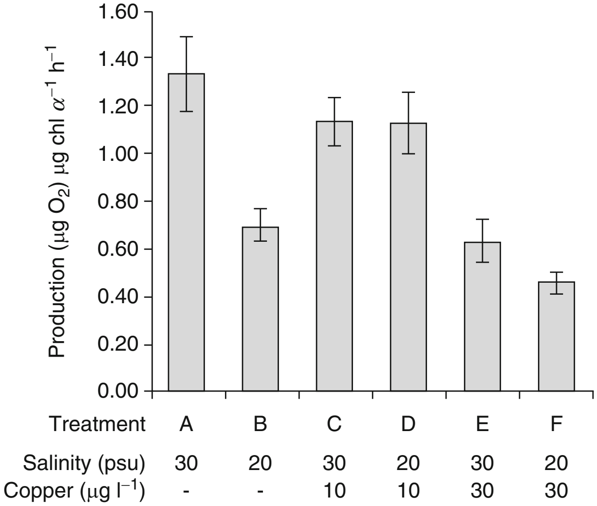


Fig. 3.8 Net primary production rate per chlorophyll a for the six treatments (mean ± SE). Treatment *A* is significantly higher production than *B*, *E*, and *F*, and treatment *C* is significantly higher than *E* and *F*. Treatment *D* is significantly higher than *B*, *E*, and *F* (Reprinted from Alutoin et al. 2001: 295, courtesy of Elsevier Publishing Co)

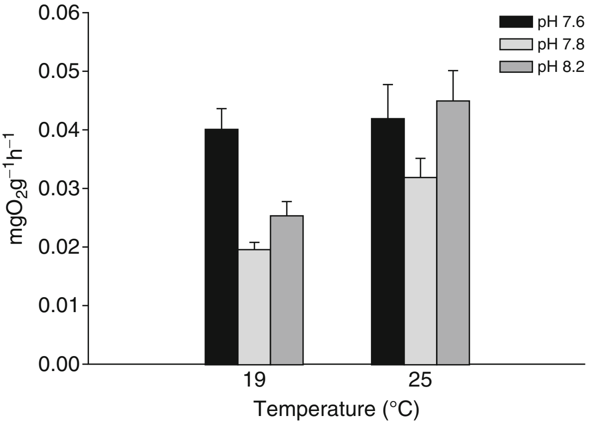


Fig. 3.9 Mean oxygen uptake of *Ophionereis schayeri* acclimated to 19 and 25 °C exposed to pH of 7.6, 7.8, and 8.2. Error bars are ± SE (Reprinted from Christensen et al. 2011: 34, courtesy of Elsevier Publishing Co)

Elevated temperature and the combination of heat and Cu reduced production rate, but after exposure to elevated temperature, corals had higher production after a 5-day recovery period. The combination of the two stressors showed no additive or synergistic effects. Cu alone had no effect on zooxanthellar production rate, but corals that were pre-exposed to increased temperature and again exposed to Cu after 5 days had reduced production.

3.4.2 Ocean Acidification

Edmunds (2012) exposed the massive coral *Porites* sp. to pCO_2 of 76.6 Pa, which had no effects, but 87.2 Pa pCO_2 reduced respiration 36 %, as well as maximum photochemical efficiency. Biomass, calcification, and energy expenditure of calcification were not affected. These results do not support the hypothesis that high pCO_2 reduces coral calcification through increased metabolic costs, but suggest that high $\mathrm{pCO_2}$ causes metabolic depression and photochemical impairment similar to that of bleaching. Response of the brittle star *Ophionereis schayeri* to elevated temperature and lowered pH was studied by Christensen et al. (2011) with animals acclimated to 19 and 25 °C. Temperature had the predicted effect on oxygen uptake, with the Q_{10} being 2.6 at normal pH of 8.2. It was predicted that low pH (7.4–7.8) would be stressful and cause decreased respiration. A pH of 7.8 caused lethargy, which may have caused reduced oxygen uptake. This pH is near the lower range of pH experienced in the field, and short term metabolic depression may be a routine response. However, exposure to pH 7.6 and 7.4 caused a significant increase in oxygen uptake (Fig. 3.9) and copious secretion of mucus, a stress response.

However, when the seastar *Parvulastra exigua* was exposed to intermediate pH (7.8) combined with pulses of warming occasionally experienced in nature (6 °C above ambient), the effect of temperature on metabolism was diminished compared to ambient temperature (McElroy et al. 2012). The results show that the metabolic response is resilient to current levels of stress, but may become vulnerable to the interactive effects of ocean warming and acidification at levels expected in the near-future.

While most studies of ocean acidification on corals focus on calcification, changes in gene expression, respiration, photosynthesis and symbiosis of the coral, *Acropora millepora*, were noted before effects on calcification. Under high CO_2 corals lost over half their *Symbiodinium*, and decreased both photosynthesis and respiration (Kaniewska et al. 2012). Changes in gene expression indicated metabolic suppression, an increase in oxidative stress, apoptosis, and symbiont loss.

3.4.3 Hypoxia

Major differences in response to low DO are seen among Echinoderms. The sea urchin *Stronglylocentrotus nudus* and seastar *Patiria pectinifera* are apparent oxyregulators at levels over 3.0 and 0.66 ml O_2 1^{-1}, respectively. Levels of lactic acid in tissues of the sea urchin are 3–4 times higher than in seastar. The ATP in ambulacral tissues of animals decreases in response to low DO. With decrease in DO to 2.5 ml O_2 1^{-1}, ATP in sea urchin gonads decreased by 33 %, while in seastar it increased eight-fold, and seastars spawned at these low levels of oxygen (Ryabushko et al. 1980).

Benthic polychaetes tend to be resistant to low DO, a factor frequently encountered in the soft sediment habitats where they are dominant species. At 10 and 20 °C and 5 ppt salinity, *Marenzelleria viridis* gradually reduced its metabolic activity with declining DO, whereas *Hediste diversicolor* maintained its metabolic activity. The metabolism of both species stayed fully aerobic down to a pO_2 of 2 kPa (one atmosphere is 101.325 kPa [kilo Pascals]). An additional stress of 0.5 ppt salinity at 20 °C led to a decrease in oxygen consumption in *H. diversicolor* below a pO_2 of 10 kPa, while metabolic heat dissipation remained constant. *M. viridis*, however, further reduced both metabolic heat dissipation and oxygen consumption. The metabolic rates of both species under anoxia were similar, about 20 % of the normoxic rate (Fritzsche and Oertzen 1995). The benthic lugworm, *Arenicola marina*, ventilated intermittently, irrespective of ambient pO_2 and sulphide concentration. The ventilation rate was 28.5 ml h^{-1} g^{-1} wet mass during normoxia, but increased to 175 % of this value during moderate hypoxia, during which time aerobic energy metabolism was maintained. Below a pO_2 of 6.2 kPa, *A. marina* reduced the ventilated volume to 54 % of the normoxic value and became anaerobic (Wohlgemuth et al. 2000).

3.4.4 Polluted Environments

Bioenergetics of the polychaete, *Nephtys incisa*, were studied by Johns and Gutjahr-Gobell (1985), after exposure to dredged material from contaminated Black Rock Harbor, Connecticut, U.S.A. Exposed worms had reduced respiration rate, higher maintenance costs, reduced excretion, and reduced growth efficiency. They reduced burrowing activity, possibly to avoid contact with contaminated sediments, and thus ingested less sediment (from which they obtain their food) reducing their energy intake. *Nereis diversicolor* from the moderately contaminated Loire Estuary had impaired amylase activity, reduced feeding, lower energy reserves (glycogen and lipids), and reduced length and weight compared to those from a reference site (Tankoua et al. 2012).

3.5 Conclusions

A pollutant can alter respiration by altering metabolic enzymes or indirectly by injury to gill tissue thus interfering with gaseous exchange. Studies examining physiological parameters and respiratory enzymes are much more informative than those that measure only oxygen consumption rates. A general response of reduced respiration may help to conserve energy for a stressed organism that has reduced its food intake. A number of studies, however, have found increased respiration in response to some contaminants, especially oil and component hydrocarbons. This can lead to energy deficits if it is accompanied by reduced feeding, which is common.

References

Abbaraju NB, Rees BB (2012) Effects of dissolved oxygen on glycolytic enzyme specific activities in liver and skeletal muscle of *Fundulus heteroclitus*. Fish Physiol Biochem 38:615–624

Albentosa M, Viñas L, Besada V, Franco A, González-Quijano A (2012) First measurements of the scope for growth (SFG) in mussels from a large scale survey in the North-Atlantic Spanish coast. Sci Total Environ 435–436:430–445

Alutoin S, Boberg J, Nyström M, Tedengren M (2001) Effects of the multiple stressors copper and reduced salinity on the metabolism of the hermatypic coral *Porites lutea*. Mar Environ Res 52:289–299

Anderson SJ, Taylor AC, Atkinson RJA (1994) Anaerobic metabolism during anoxia in the burrowing shrimp *Calocaris macandreae* Bell (Crustacea: Thalassinidea). Comp Biochem Physiol A 108:515–522

Ansari ZA, Farshchi P, Faniband M (2010) Naphthalene induced activities on growth, respiratory metabolism, and biochemical composition in juvenile *Metapenaeus affinis* (H. Milne Edwards, 1837). Indian J Mar Sci 39:285–289

Astall CM, Taylor AC, Atkinson RJA (1997) Behavioural and physiological Implications of a burrow-dwelling lifestyle for two species of Upogebiid mud-shrimp (Crustacea: Thalassinidea). Estuar Coast Shelf Sci 44:155–168

Barbieri E (2007) Use of oxygen consumption and ammonium excretion to evaluate the sublethal toxicity of cadmium and zinc on *Litopenaeus schmitti* (Burkenroad, 1936, Crustacea). Water Environ Res 79:641–646

Beniash E, Ivanina A, Lieb NS, Kurochkin I, Sokolova IM (2010) Elevated level of carbon dioxide affects metabolism and shell formation in oysters *Crassostrea virginica*. Mar Ecol Prog Ser 419:95–108

Bridges CR, Brand AR (1980) The effect of hypoxia on oxygen consumption and blood lactate levels of some marine crustaceans. Comp Biochem Physiol A 65A:399–409

Brown B, Newell R (1972) The effect of copper and zinc on the metabolism of the mussel, *Mytilus edulis*. Mar Biol 16:108–118

Calabrese A, Thurberg FP, Dawson MA (1977) Effects of cadmium, mercury and silver on marine animals. Mar Fish Rev 39:1–11

Cantelmo AC, Conklin PJ, Fox FR, Rao KR (1978) Effects of sodium pentachlorophenate and 2,4-dinitrophenol on respiration in crustaceans. In: Rao KR (ed) Pentachlorophenol: chemistry, pharmacology and environmental toxicology. Plenum Press, New York, pp 251–263

Capossela KM, Brill RW, Fabrizio MC, Bushnell PG (2012) Metabolic and cardiorespiratory responses of summer flounder *Paralichthys dentatus* to hypoxia at two temperatures. J Fish Biol 81:1043–1058

Capuzzo JM, Lancaster BA (1981) Physiological effects of South Louisiana crude oil on larvae of the American lobster, *Homarus americanus*. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Biological monitoring of marine pollutants. Academic, New York, pp 405–423

Chapman CA, Harahush BK, Renshaw GM (2011) The physiological tolerance of the grey carpet shark (*Chiloscyllium punctatum*) and the epaulette shark (*Hemiscyllium ocellatum*) to anoxic exposure at three seasonal temperatures. Fish Physiol Biochem 37:387–399

Cheung SG, Cheung RY (1995) Effects of heavy metals on oxygen consumption and ammonia excretion in green-lipped mussels (*Perna viridis*). Mar Pollut Bull 31:381–386

Christensen AB, Nguyen HD, Byrne M (2011) Thermotolerance and the effects of hypercapnia on the metabolic rate of the ophiuroid *Ophionereis schayeri*: inferences for survivorship in a changing ocean. J Exp Mar Biol Ecol 403:31–38

Ciacci C, Barmo C, Gallo G, Maisano M, Cappello T, D'Agata A, Leonzio C, Mauceri A, Fasulo S, Canesi L (2012) Effects of sublethal, environmentally relevant concentrations of hexavalent chromium in the gills of *Mytilus galloprovincialis*. Aquat Toxicol 120–121:109–118

Cooper RU, Clough LM, Farwell MA, West TL (2002) Hypoxia-induced metabolic and antioxidant enzymatic activities in the estuarine fish *Leiostomus xanthurus*. J Exp Mar Biol Ecol 279:1–20

Cortez FS, Pereira CD, Santos AR, Cesar A, Choueri RB, Martini G, Bohrer-Morel MB (2012) Biological effects of environmentally relevant concentrations of the pharmaceutical Triclosan in the marine mussel *Perna perna* (Linnaeus, 1758). Environ Pollut 168:145–150

Davoodi F, Claireaux G (2007) Effects of exposure to petroleum hydrocarbons upon the metabolism of the common sole *Solea solea*. Mar Pollut Bull 54:928–934

Dickinson GH, Ivanina AV, Matoo OB, Pörtner HO, Lannig G, Bock C, Beniash E, Sokolova IM (2012) Interactive effects of salinity and elevated CO_2 levels on juvenile eastern oysters, *Crassostrea virginica*. J Exp Biol 215:29–43

Dissanayake A, Ishimatsu A (2011) Synergistic effects of elevated CO_2 and temperature on the metabolic scope and activity in a shallow-water coastal decapod (*Metapenaeus joyneri*; Crustacea: Penaeidae). ICES J Mar Sci 68:1147–1154

Dissanayake A, Galloway TS, Jones MB (2008) Physiological responses of juvenile and adult shore crabs *Carcinus maenas* (Crustacea: Decapoda) to pyrene exposure. Mar Environ Res 66:445–450

Edmunds P (2012) Effect of pCO2 on the growth, respiration, and photophysiology of massive *Porites* spp. in Moorea, French Polynesia. Mar Biol 159:2149–2160

Edwards RR (1978) Effects of water-soluble oil fractions on metabolism, growth and carbon budget of the shrimp *Crangon crangon*. Mar Biol 46:259–265

Elfwing T, Tedengren M (2002) Effects of copper on the metabolism of three species of tropical oysters, *Saccostrea cucullata, Crassostrea lugubris* and *C. belcheri*. Aquaculture 204:157–166

Engel DW, Fowler B (1979) Copper and cadmium induced changes in the metabolism and structure of molluscan gill tissue. In: Vernberg WB, Thurberg FP, Calabrese A, Vernberg FJ (eds) Marine pollution: functional responses. Academic, New York, pp 239–256

Fasulo S et al (2012) Metabolomic investigation of *Mytilus galloprovincialis* (Lamarck 1819) caged in aquatic environments. Ecotoxicol Environ Saf 84:139–146

Fernández-Reiriz MJ, Range P, Álvarez-Salgado XA, Labarta U (2011) Physiological energetics of juvenile clams *Ruditapes decussatus* in a high CO_2 coastal ocean. Mar Ecol Prog Ser 433:97–105

Forbes VE, Depledge M (1992) Cadmium effects on the carbon and energy balance of mud snails. Mar Biol 113:263–269

Fritzsche D, Oertzen JA (1995) Metabolic responses to changing environmental conditions in the brackish water polychaetes *Marenzelleria viridis* and *Hediste diversicolor*. Mar Biol 121:693–699

Gaudy R, Guérin J-P, Kerambrun P (1991) Sublethal effects of cadmium on respiratory metabolism, nutrition, excretion and hydrolase activity in *Leptomysis lingvura* (Crustacea: Mysidacea). Mar Biol 109:493–501

Gee JH, Gee P (1991) Reactions of Gobioid fishes to hypoxia: buoyancy and aquatic surface respiration. Copeia 1991(1):17–28

Gharaei A, Ghaffari M, Keyvanshokooh S, Akrami R (2011) Changes in metabolic enzymes, cortisol and glucose concentrations of Beluga (*Huso huso*) exposed to dietary methylmercury. Fish Physiol Biochem 37:485–493

Goto D, Wallace WD (2010) Bioenergetic responses of a benthic forage fish (*Fundulus heteroclitus*) to habitat degradation and altered prey community in polluted salt marshes. Can J Fish Aquat Sci 67:1566–1584

Griffit RJ, Brown-Peterson NJ, Savin DA, Manning CS, Boube I, Ryan RA, Brouwer M (2012) Effects of chronic nanoparticulate silver exposure to adult and juvenile sheepshead minnows (*Cyprinodon variegatus*). Environ Toxicol Chem 31:160–167

Hagerman L, Søndergaard T, Weile K, Hosie D, Uglow RF (1990) Aspects of blood physiology and ammonia excretion in *Nephrops norvegicus* under hypoxia. Comp Biochem Physiol A 97:51–55

Hammer KM, Kristiansen E, Zachariassen KE (2011) Physiological effects of hypercapnia in the deep-sea bivalve *Acesta excavata* (Fabricius, 1779) (Bivalvia; Limidae). Mar Environ Res 72:135–142

Handy RD, Al-Bairuty G, Al-Jubory A, Ramsden CS, Boyle D, Shaw BJ, Henry TB (2011) Effects of manufactured nanomaterials on fishes: a target organ and body systems physiology approach. J Fish Biol 79:821–853

Hutcheson M, Miller DC, White AQ (1985) Respiratory and behavioral responses of the grass shrimp *Palaemonetes pugio* to cadmium and reduced dissolved oxygen. Mar Biol 88:59–66

Ivanina A, Sokolov EP, Sokolova IM (2010) Effects of cadmium on anaerobic energy metabolism and mRNA expression during air exposure and recovery of an intertidal mollusk *Crassostrea virginica*. Aquat Toxicol 99:330–342

Ivanina AV, Froelich B, Williams T, Sokolov EP, Oliver JD, Sokolova IM (2011) Interactive effects of cadmium and hypoxia on metabolic responses and bacterial loads of eastern oysters *Crassostrea virginica* Gmelin. Chemosphere 82(3):377–389

Johns DM, Gutjahr-Gobell R (1985) The impact of dredged material on the bioenergetics of the polychaete *Nephtys incise*. In: Vernberg FJ, Thurberg FP, Calabrese A, Vernberg WB (eds) Marine pollution and physiology: recent advances. University of South Carolina Press, Columbia, pp 327–350

Kaniewska P, Campbell PR, Kline DI, Rodriguez-Lanett M, Rodriguez-Lanetty M, Miller DJ, Dove S, Hoegh-Guldber O (2012) Major cellular and physiological impacts of ocean acidification on a reef building coral. PLoS ONE 7(4):art. e34659

Kerambrun E, Henry F, Courcot L, Gevaert F, Amara R (2012) Biological responses of caged juvenile sea bass (*Dicentrarchus labrax*) and turbot (*Scophtalmus maximus*) in a polluted harbour. Ecol Indic 19:161–171

Kozuki Y, Yamanaka R, Matsushige M, Saitoh A, Otani S, Ishida T (2013) The after-effects of hypoxia exposure on the clam *Ruditapes philippinarum* in Omaehama beach, Japan. Estuar Coast Shelf Sci 116:50–56

Laughlin R, Linden O (1983) Oil pollution and Baltic mysids: acute and chronic effects of the water soluble fractions of light fuel oil on the mysid shrimp *Neomysis integer*. Mar Ecol Prog Ser 12:29–41

Leung KM, Taylor AC, Furness RW (2000) Temperature-dependent physiological responses of the dogwhelk *Nucella lapillus* to cadmium exposure. J Mar Biol Assoc UK 80:647–660

Li W, Gao K (2012) A marine secondary producer respires and feeds more in a high CO_2 ocean. Mar Pollut Bull 64:699–703

Maas AE, Wishner KF, Seibel BA (2012) The metabolic response of pteropods to acidification reflects natural CO_2-exposure in oxygen minimum zones. Biogeosciences 9(747–757):2012

Manachini B, Arizza V, Rinaldi A, Montalto V, Sarà G (2013) Eco-physiological response of two marine bivalves to acute exposition to commercial Bt-based pesticide. Mar Environ Res 83:29–37

Manno C, Morata N, Primicerio R (2012) *Limacina retroversa*'s response to combined effects of ocean acidification and sea water freshening. Estuar Coast Shelf Sci 113:163–171

Marshall DJ, McQuaid CD (1993) Effects of hypoxia and hyposalinity on the heart beat of the intertidal limpets *Patella granvlaris* (Prosobranchia) and *Siphonaria capensis* (Pulmonata). Comp Biochem Physiol A 106:65–68

McElroy DJ, Nguyen HD, Byrne M (2012) Respiratory response of the intertidal seastar *Parvulastra exigua* to contemporary and near-future pulses of warming and hypercapnia. J Exp Mar Biol Ecol 416–417:1–7

McKenney CL (1985) Associations between physiological alterations and population changes in an estuarine mysid during chronic exposure to a pesticide. In: Vernberg FJ, Thurberg FP, Calabrese A, Vernberg WB (eds) Marine pollution and physiology: recent advances. University of South Carolina Press, Columbia, pp 397–418

McKenney CL, Matthews E (1990) Alterations in the energy metabolism of an estuarine mysid (*Mysidopsis bahia*) as indicators of stress from chronic pesticide exposure. Mar Environ Res 30:1–19

McKenney CL, Weber DE, Celestial DM, MacGregor MA (1998) Altered growth and metabolism of an estuarine shrimp (*Palaemonetes pugio*) during and after metamorphosis onto fenvalerate-laden sediment. Arch Environ Contam Toxicol 35:464–471

Melatunan S, Calosi P, Rundle S, Moody AJ, Widdicombe S (2011) Exposure to elevated temperature and pCO2 reduces respiratory rate and energy status in the periwinkle *Littorina littorea*. Physiol Biochem Zool 84:583–594

Milinkovich T, Lucas J, Le Floch S, Thomas-Guyon H, Lefrançois C (2012) Effect of dispersed crude oil exposure upon the aerobic metabolic scope in juvenile golden grey mullet (*Liza aurata*). Mar Pollut Bull 64:865–871

Navarro JM, Torres R, Acuña K, Duarte C, Manriquez PH, Lardies M, Lagos NA, Vargas C, Aguilera V (2013) Impact of medium-term exposure to elevated pCO_2 levels on the physiological energetics of the mussel *Mytilus chilensis*. Chemosphere 90:1242–1248

Nilin J, Pestana JL, Ferreira NG, Loureiro S, Costa-Lotufo L, Soares A (2012) Physiological responses of the European cockle *Cerastoderma edule* (Bivalvia: Cardidae) as indicators of coastal lagoon pollution. Sci Total Environ 435–436:44–52

Nonnotte L, Boitel R, Truchot JP (1993) Waterborne copper causes gill damage and hemolymph hypoxia in the shore crab *Carcinus maenas*. Can J Zool 71:1569–1576

Nyström M, Nordemar I, Tedengren M (2001) Simultaneous and sequential stress from increased temperature and copper on the metabolism of the hermatypic coral, *Porites cylindrica*. Mar Biol 138:1225–1231

O'Connor EA, Pottinger TG, Sneddon LU (2011) The effects of acute and chronic hypoxia on cortisol, glucose and lactate concentrations in different populations of three-spined stickleback. Fish Physiol Biochem 37:461–469

Oliviera M, Gravato C, Guilhermino L (2012) Acute toxic effects of pyrene on *Pomatoschistus microps* (Teleostei, Gobiidae): mortality, biomarkers and swimming performance. Ecol Indic 19:206–214

Pereira JJ, Kanungo K (1981) Effects of silver on respiration and ion and water balance in *Neanthes virens*. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Biological monitoring of marine pollutant. Academic, New York, pp 107–125

Pichavant K et al (2000) Effects of hypoxia on growth and metabolism of juvenile turbot. Aquaculture 108:103–114

Rice SD, Thomas RE, Short JW (1977) The effect of petroleum hydrocarbons on breathing and coughing rates and hydrocarbon uptake-depuration in pink salmon fry. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Physiological responses of Marine Biota to pollutants. Academic, New York, pp 259–277

Ryabushko VI, Zhuchikhina AA, Lutsik NV (1980) Effects of environmental oxygen concentrations on the level of metabolism of some echinoderms from the Sea of Japan. Comp Biochem Physiol B 67:171–174

Seibel BA, Maas AE, Dierssen HM (2012) Energetic plasticity underlies a variable response to ocean acidification in the pteropod, *Limacina helicina antarctica*. PLoS ONE 7(4):art.e30464

Smith RL, Hargreaves BR (1985) Respiratory rate in the mysid *Neomysis americana*: effects of naphthalene, temperature and other factors. In: Vernberg FJ, Thurberg FP, Calabrese A, Vernberg WB (eds) Marine pollution and physiology: recent advances. University of South Carolina Press, Columbia, pp 477–503

Spicer JI, Weber RE (1991) Respiratory impairment in crustaceans and molluscs due to exposure to heavy metals. Comp Biochem Physiol C 100:339–342

Spicer JI, Weber RE (1992) Respiratory impairment by water-borne copper and zinc in the edible crab C*ancer pagurus* (L.) (Crustacea: Decapoda) during hypoxic exposure. Mar Biol 112:429–435

St-Amand L, Gagnon R, Packard TT, Savenkoff C (1999) Effects of inorganic mercury on the respiration and the swimming activity of shrimp larvae, *Pandalus borealis*. Comp Biochem Physiol C 122:33–43

Stickle WB, Rice SD, Villars C, Metcalfe W (1985) Bioenergetics and survival of the marine mussel, *Mytilus edulis* L., during long-term exposure to the water-soluble fraction of Cook Inlet crude oil. In: Vernberg FJ, Thurberg FP, Calabrese A, Vernberg WB (eds) Marine pollution and physiology: recent advances. University of South Carolina Press, Columbia, pp 427–446

Stickle WB, Kapper MA, Shirley TC, Carls MG, Rice SD (1987) Bioenergetics and tolerance of the pink shrimp (*Pandalus borealis*) during long-term exposure to the water soluble fraction and oiled sediment from Cook Inlet crude oil. In: Vernberg WB, Calabrese A, Thurberg FP, Vernberg FJ (eds) Pollution physiology of estuarine organisms. University of South Carolina Press, Columbia, pp 87–106

Tankoua OF, Buffet PE, Amiard JC, Amiard-Triquet C, Méléder V, Gillet P, Mouneyrac C, Berthet B (2012) Intersite variations of a battery of biomarkers at different levels of biological organisation in the estuarine endobenthic worm *Nereis diversicolor* (Polychaeta, Nereididae). Aquat Toxicol 114–115:96–103

Taylor AC, Spicer JI (1987) Metabolic responses of the prawns *Palaemon elegans* and *P. serratus* (Crustacea: Decapoda) to acute hypoxia and anoxia. Mar Biol 95:521–530

Taylor AC, Spicer JI (1988) Functional significance of a partial-emersion response in the intertidal prawn *Palaemon elegans* (Crustacea: Palaemonidae) during environmental hypoxia. Mar Ecol Prog Ser 44:141–147

Taylor EW, Butler PJ, Sherlock PJ (1973) The respiratory and cardiovascular changes associated with the emersion response of *Carcinus maenas* (L.) during environmental hypoxia, at three different temperatures. J Comp Physiol A 86:95–115

Thomas P, Neff JM (1985) Plasma corticosteroid and glucose responses to pollutants in striped mullet: different effects of naphthalene, benzo[a]pyrene and cadmium exposure. In: Vernberg FJ, Thurberg FP, Calabrese A, Vernberg WB (eds) Marine pollution and physiology: recent advances. University of South Carolina Press, Columbia, pp 63–82

Thurberg FP, Calabrese A, Dawson MA (1974) Effects of silver on oxygen consumption of bivalves at various salinities. In: Vernberg FJ, Vernberg WB (eds) Pollution and physiology of marine organisms. Academic, New York, pp 67–78

Thurberg FP, Calabrese A, Gould E, Grieg RA, Dawson MA, Tucker RK (1977) Response of the lobster *Homarus americanus*, to sublethal levels of cadmium and mercury. In: Vernberg FJ, Thurberg FP, Calabrese A, Vernberg WB (eds) Physiological responses of Marine Biota to pollutants. Academic, New York, pp 185–197

Van Ginneken V, van den Thillart G, Addink A, Erkelens C (1995) Fish muscle energy metabolism measured during hypoxia and recovery: an in vivo 31P-NMR study. Am J Physiol Regul Integr Comp Physiol 268:R1178–R1187

Vandermeulen JH, Hanrahan J, Hemsworth T (1980) Respiratory changes and stability of haemocyanin—O_2 binding capacity in the crab *Cancer irroratus* exposed to Kuwait crude oil in sea water. Mar Environ Res 3:161–170

Vernberg WB, DeCoursey PJ, O'Hara J (1974) Multiple environmental factor effects on physiology and behavior of the fiddler crab *Uca pugilator*. In: Vernberg FJ, Vernberg WB (eds) Pollution and physiology of marine organisms. Academic, New York, pp 381–425

Vernberg FJ, Guram MS, Savory MA (1978) Metabolic response to thermal changes of the adult fiddler crab *Uca pugilator* and the effect of PCBs. Mar Biol 48:135–141

Verslycke T, Roast SD, Widdows J, Jones MB, Janssen CR (2004) Cellular energy allocation and scope for growth in the estuarine mysid *Neomysis integer* (Crustacea: Mysidacea) following chlorpyrifos exposure: a method comparison. J Exp Mar Biol Ecol 306:1–16

Viant MR, Walton JH, Tjeerdema RS (2001) Comparative sublethal actions of 3-Trifluoromethyl-4-nitrophenol in marine molluscs as measured by *in Vivo*$^{\mathbf{31}}$P NMR. Pestic Biochem Physiol 71:40–47

Vijayavel K, Balasubramanian MP (2006) Changes in oxygen consumption and respiratory enzymes as stress indicators in an estuarine edible crab *Scylla serrata* exposed to naphthalene. Chemosphere 63:1523–1531

Vosloo D, Sara J, Vosloo A (2012) Acute responses of brown mussel (*Perna perna*) exposed to sub-lethal copper levels: integration of physiological and cellular responses. Aquat Toxicol 106–107:1–8

Wang SY, Stickle WB (1987) Bioenergetics, growth and molting of the blue crab, *Callinectes sapidus*, exposed to the water-soluble fraction of Couth Louisiana crude oil. In: Vernberg WB, Calabrese A, Thurberg FP, Vernberg FJ (eds) Pollution physiology of estuarine organisms. University of South Carolina Press, Columbia, pp 107–126

Wang Y, Hu M, Wong WH, Shin P, Cheung SG (2011) The combined effects of oxygen availability and salinity on physiological responses and scope for growth in the green-lipped mussel *Perna viridis*. Mar Pollut Bull 63:255–261

Widdows J, Page DS (1993) Effects of tributyltin and dibutyltin on the physiological energetics of the mussel, *Mytilus edulis*. Mar Environ Res 35:233–249

Wohlgemuth SE, Taylor AC, Grieshaber MK (2000) Ventilatory and metabolic responses to hypoxia and sulphide in the lugworm *Arenicola marina* (L.). J Exp Biol 203:3177–3188

Wu H, Zhang X, Wang Q, Lianzhen Li, Ji C, Liu X, Zhao J, Yin X (2013) A metabolomic investigation on arsenic-induced toxicological effects in the clam *Ruditapes philippinarum* under different salinities. Ecotoxicol Environ Saf. http://dx.doi.org/10.1016/j.ecoenv.2012.02.022

Yadetie F, Karlson OA, Lanzén A, Berg K, Olsvik P, Hogstrand C, Goksøyr A (2013) Global transcriptome analysis of Atlantic cod (*Gadus morhua*) liver after in vivo methylmercury exposure suggests effects on energy metabolism pathways. Aquat Toxicol 126:314–325

Zhang L, Liu X, You L, Zhou D, Wu H, Li L, Zhao J, Feng J, Yu J (2011) Metabolic responses in gills of Manila clam *Ruditapes philippinarum* exposed to copper using NMR-based metabolomics. Mar Environ Res 72:33–39

Chapter 4
Osmoregulation and Excretion

Abstract Maintaining a constant internal chemical environment (homeostasis) is a critical physiological activity that is particularly important in certain taxa living in fluctuating environments such as estuaries and in those that migrate between fresh- and salt water. Stress, such as exposure to contaminants, typically causes a disruption of this activity, which involves primarily the gills. The ability to ionoregulate or to osmoregulate (mostly Na and Cl regulation) following exposure to stress decreased in most of the species studied with most of the contaminants. Concomitant with osmoregulation is the necessity to excrete ammonia, a waste product of protein metabolism; this activity may be either enhanced or depressed by contaminants and is affected by salinity and whether the animals are fed or not. Increased excretion rates reflect increased reliance on protein metabolism.

Keywords Ammonia • Carbonic anhydrase • Gills • Ions balance • Salinity • Na-K-ATPase

4.1 Osmoregulation

Many marine invertebrates tend to be isotonic in relation to seawater, i.e., they are as salty as the water outside. These animals, called osmoconformers, do not have to regulate ion levels, but are usually restricted to narrow ranges of salinity. Other aquatic organisms maintain internal homeostasis, and keep a constant internal salt environment. Osmoregulation is the process by which this is done. Osmoregulators must actively regulate ions to maintain their body fluids at ionic concentrations different from the surrounding water. In a hyperosmotic environment like the ocean, they take in water to offset loss. They drink seawater and subsequently excrete the excess salt from their gills. This has an energy cost, the magnitude of which depends on a number of factors: how different the animal's osmolarity is from the surrounding water, how easily water moves across the animal's surface, and

J.S. Weis, *Physiological, Developmental and Behavioral Effects of Marine Pollution*,
DOI 10.1007/978-94-007-6949-6_4,

how much membrane-transport is needed to pump solutes across the membrane. In freshwater there is a tendency to take in water, so they excrete copious dilute urine to compensate. The ability to maintain osmotic concentrations in body fluids regardless of the salt concentration of the environment is particularly important for animals living in estuaries with changing salinity. If the animal moves to a lower salinity part of an estuary, it is in a hypoosmotic environment, where it must retain salts and excrete water. These animals may be exposed to both salinity and pollution stress. The literature suggests that stress often disrupts ionic regulation – mostly of Na and Cl – and therefore osmotic regulation. The ability to ionoregulate or to osmoregulate following exposure to stress decreases in most pollution studies.

Two enzymes play a major role in osmoregulation: sodium, potassium ATP-ase (Na^+–K^+-ATPase) and carbonic anhydrase (CA). Na^+–K^+-ATPase, in intestines and gills maintains gradients needed for salt movements and is related to Na^+ and Cl^- exchanges across tissues. CA is involved in the hydration of CO_2 to produce H^+ and $HCO_3{}^-$, and plays a role in osmoregulation as well as gas exchange and acid–base balance (Lionetto et al. 2000). It should be noted that impaired osmoregulation, like decreased feeding, could result in altered uptake of contaminants, which could then modify toxic effects.

4.1.1 Crustaceans

Exposure to pollutants and other environmental stressors often results in a decrease in Na^+ and Cl^- regulation and/or of osmoregulatory capacity (OC: defined as the difference between the osmotic pressure of the hemolymph and the external medium at any given salinity). The loss of osmoregulatory and ionoregulatory capacity can be caused by altered structure of the gills and/or excretory organs, and changes in activity of the enzyme Na^+, K^+-ATPase, ionic fluxes and surface permeability. Measurement of OC variations was proposed by Lignot et al. (2000) as a way to monitor the physiological condition and effects of stressors. Responses can vary with the molt cycle, but very few studies have taken that into consideration.

Metals

Many trace metals are more toxic in lower salinity water, in part because more of the metal is in free ion form and therefore more bioavailable, but also because of physiological responses of organisms. Metal uptake is reduced as salinity approaches the isosmotic point of a species because of reduced activity of ion exchange pumps. Effects of metals on estuarine animals were reviewed by Monserrat et al. (2007). A key mechanism of acute metal toxicity in many organisms has been reported to be osmoregulatory impairment associated with gill Na^+–K^+-ATPase inhibition.

Cu, Ag, Cd, Pb, Zn, and Hg have all been found to impair osmoregulation via inhibition of Na^+–K^+-ATPase in a variety of freshwater, brackish and marine animals (Péquex et al. 1996; Bianchini and di Castilho 1999).

Inhibitory effects of many metals – Ag (0.05–0.5 μM), Cd (0.05–0.5 μM or 1.25 mg l^{-1}), Cu (0.05–0.5 μM) and Zn (2–6 μM) have been also reported on carbonic anhydrase in euryhaline crabs (Vitale et al. 1999; Skaggs and Henry 2002).

Copper

Exposure to copper (1 mg l^{-1}) induces disturbances in ion homeostasis and acid–base balance in green crabs *Carcinus maenas* (Boitel and Truchot 1990). At sublethal Cu levels, hemolymph concentrations of Na^+ and Cl^- decrease when exposure is in low salinity, but not in full-strength seawater. A metabolic acidosis develops in both media, but in full-strength sea water the crabs can recover. Exposure of *C. maenas* to 1 mg l^{-1} Cu altered hemolymph osmolality and ion balance (Bjerregaard and Vislie 1986); disruption of Na^+-K^+-ATPase was considered the cause of the disturbance. Hansen et al. (1992a) found that exposure to 10 mg l^{-1} Cu for 1 week reduced this enzyme by 50–60 %, resulting in a major reduction in hemolymph Na^+ concentration. However, osmoregulation was less sensitive to Cu than respiration (Hebel et al. 1999). In this species, the anterior gills (numbers 1–6) are primarily respiratory in function, while the posterior gills (numbers 7–9) play an osmoregulatory role. Following exposure to Cu, gill damage (epithelial hyperplasia and necrosis) initially occurred at 100 μg Cu l^{-1}, but only in the respiratory gills. No damage was seen in osmoregulatory gills at levels up to 300 μg Cu l^{-1}. In an investigation of Cu (0.78 μM) effects on blue crabs (*Callinectes sapidus)* at low (2 psu) and high (30 psu) salinity, Martins et al. (2011) found that crabs that had been acclimated to dilute seawater showed inhibition of expression of mRNA of the genes for Na^+/K^+-ATPase and the $Na^+/K^+/2Cl^-$ co-transporter, but that Na^+/K^+-ATPase activity itself was not affected, indicating that the gene transcription is down-regulated before significant inhibition of enzyme activity occurs. Authors felt that this suggests that there may be a compensatory response of this enzyme after short-term exposure to environmentally relevant Cu concentrations. No effects were seen at high salinity, possibly because of lower bioavailability of toxic Cu (free ion). Bambang et al. (1995) found that concentrations of 500 (“low”), 1,000 (“medium”) and 1,500 (“high”) μg Cu l^{-1} altered both hypo- and hyper-osmoregulation in larvae of the shrimp *Penaeus japonicus*. Hypo-osmoregulation was reduced after 4 days at the low Cu concentration and was suppressed in the medium and high concentrations. Hyper-osmoregulatory capacity was significantly reduced after 4 days exposure to low and medium concentrations (Fig. 4.1). Only the shrimp from low and medium Cu recovered their hypo-osmoregulatory ability after 7 days in clean seawater. Tolerance to Cu increased when nauplii became juveniles. It should be mentioned that many other investigators would not consider 500 μg Cu l^{-1} to be a “low” concentration.

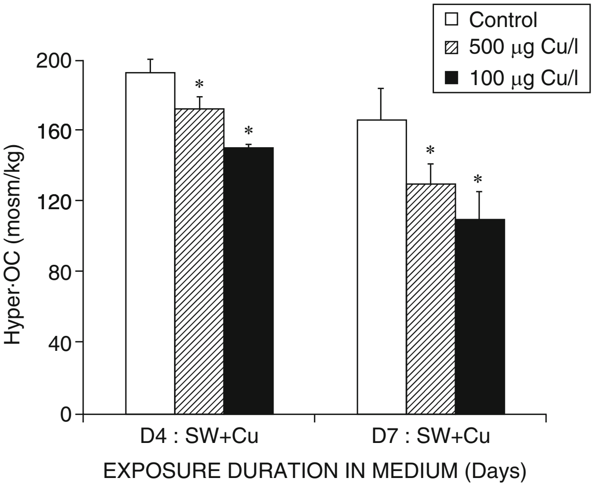


Fig. 4.1 Hyper-osmoregulatory capacity in juvenile *Penaeus japonicus* after 4 (D4) and 7 (D7) days exposure to Cu in dilute seawater (SW). * = significantly different from controls ($P < 0.05$) (Reprinted from Bambang et al. 1995: 132, courtesy Elsevier Publishing Co)

Mercury

Hypothesizing that mercury interferes with osmoregulation and that its effects are greater in lower salinity waters, Pequeux et al. (1996) examined its effects on three crab species that have different degrees of osmoregulatory ability: the strong regulator *Eriocheir sinensis*, the weak regulator *Carcinus maenas* (both euryhaline), and the stenohaline osmoconformer *Cancer pagurus*. They found synergistic effects between salinity and $HgCl_2$ (0.1 mg l^{-1}) toxicity in the two euryhaline hyperregulators in dilute media (*E. sinensis* and *C. maenas*). In *E. sinensis,* Na^+ and Cl^- permeability of the gill epithelium was affected, as well as Na^+ and Cl^- active transport processes. They showed that Hg drastically disturbs the Na^+/K^+ pump and the Cl^- channels in the posterior gills.

Lead

The crab *Hepatus pudibundus* is an osmoconformer, while *Callinectes ornatus* is an estuarine weak hyper-osmoregulator in dilute seawater. Amado et al. (2012) noted that osmoconformers are expected to have greater cell volume regulation, while gill cells of an osmoregulators are expected to have greater levels of ion transporters. They investigated the influence of lead nitrate (10 µM) on the ability of isolated gill cells from both species to regulate their volume under isosmotic and hyposmotic conditions. Pb was associated with hyposmotic shock; *C. ornatus* cells lost more volume in hyposmotic conditions, while *H. pudibundus* cells had volume regulation, as predicted. They concluded that osmoregulators can be more susceptible to metals than osmoconformers in low salinity, because of poorer volume regulation and expected higher uptake of Pb^{2+} via pathways that operate in absorption of salts, such as Na^+/K^+-ATPase.

Zn

Zn at 0.05 mg l^{-1} reduced oxygen uptake and gill Na^+ K^+ ATPase in the estuarine crab *Chasmagnathus granulata* (Bianchini and di Castilho 1999). Zn also impaired osmoregulation by affecting hemolymph osmolality and sodium levels in amphipods (*Gammarus duebeni*). However, amphipods collected from a contaminated site were less sensitive to these effects than those from a reference site (Johnson and Jones 1990). Furthermore, this effect was observed only at low salinity (10 psu), and only at very high zinc concentrations $\geq$ 500 μg Zn 1^{-1}.

Cd

Cd had minor effects on osmoregulation in the mysid, *Mysidopsis bahia*, at 3.62 μg l Cd^{-1} at salinity of 12 psu. Effects were reduced by calcium and increased salinity (DeLisle and Roberts 1994). Bambang et al. (1994) investigated effects of Cd on different life stages of the shrimp *Penaeus japonicus*. In juvenile shrimp, 2,000 μg Cd 1^{-1} significantly reduced hypo- and hyper-OC in a dose- and time-dependent response. Surviving shrimp recovered their hypo-osmoregulatory capacity after 6 days in cadmium-free seawater.

Tributyltin

Tolerance to TBT in shrimp (*Penaeus japonicus)* increased with development from larvae to juveniles. In shrimp kept in seawater or dilute seawater, acute TBT exposures (0.88 μg l^{-1} for nauplii to 708 μg l^{-1} for juveniles) decreased the osmoregulatory capacity (Lignot et al. 1998). Effects were time- and dose-dependent, and were more severe in pre-molt animals (Fig. 4.2). Histopathological effects in gills increased with the TBT concentration and were considered the cause of impaired osmoregulation. However, the ability to osmoregulate recovered after shrimp were kept in TBT-free water for 48–120 h.

Organic Chemicals

Pesticides and PCBs

Neufield and Pritchard (1979a) found that gill Na^+, K^+-ATPase was inhibited by both *in vitro* and *in vivo* exposure to DDT (1 μg l^{-1}) in the rock crab *Cancer*

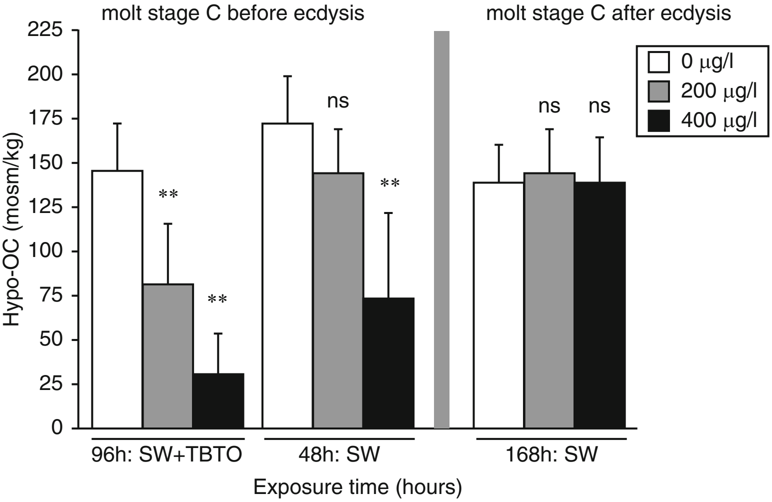


Fig. 4.2 Hypo-osmoregulatory capacity of *P. japonicus* after 96 h exposure to 200 and 400 ug l-1 of TBTO and after 48 and 168 h in seawater free of TBTO at 25 °C. Error bars = SD, *vertical line* marks ecdysis (Reprinted from Lignot et al. 1998: 288, courtesy Elsevier Publishing Co)

irroratus. However, in *Callinectes sapidus,* the *in vivo* response was transient and disappeared after return to clean water (Neufield and Pritchard 1979b). The authors felt it was likely that induction of new Na^+, K^+-ATPase in response to osmoregulatory stress protected the crabs from osmotic failure in response to DDT exposure.

Sublethal levels of lindane altered ionic and osmoregulatory ability of the mud crab *Eurypanopeus depressus*. Chloride ion regulation was disrupted at concentrations as low as 0.70 $\mu g\ l^{-1}$, while hemolymph osmotic concentration was reduced at 1.45 $\mu g\ l^{-1}$ (Shirley and McKenney 1987). In juvenile *Penaeus japonicus* in seawater or diluted seawater, the insecticide fenitrothion decreased osmoregulatory capacity at low $\mu g\ l^{-1}$ concentrations (Lignot et al. 1997). The effect was time- and dose-dependent. In seawater, shrimp could recover in less than 48 h in clean water. In dilute sea water, however, recovery by 48 h was possible only after exposure to the lowest tested concentration.

Silvestre et al. (2002) investigated effects of Atrazine (1 mg l^{-1}) *in vitro* in contact with the basolateral membrane of gills of the mitten crab, *Erocheir sinensis*, a species that migrates between fresh and salt water. Atrazine increased the transepithelial potential difference (TEP) in isolated perfused posterior gills. No TEP modification by the herbicide was detected *in vivo*, however, indicating to the authors that molecular mechanisms located on the basolateral membrane are likely to be the only ones affected or that the cuticular barrier prevents the chemical from penetrating into the gill in the whole animal. Hemolymph osmolarity, Na^+ and Cl^- concentrations of crabs in freshwater with atrazine (1 mg l^{-1}) for 14 days were not significantly changed.

The PCB mixture Aroclor 1254 at 7.5 or 29 μg l^{-1} did not alter hemolymph chloride and osmotic concentrations or chloride-exchange kinetics in adult grass shrimp *P. pugio* (Roesijadi et al. 1976), but it did disrupt hemolymph chloride regulation in juveniles, and was associated with mortalities not seen in adults.

Both positive and negative effects of pesticides were observed by Galindo-Reyes et al. (2000). Shrimp, *Litopenaeus vannamei* were exposed to DDT, Lindane, Chlordane, Lorsban, Gusathion, Folidol, Diazinon or Tamaron. Increases in osmoregulation were observed in shrimp exposed to Folidol, Diazinon and Gusathion, whereas decreases were seen with DDT, Lindane and Lorsban. Authors concluded that different pesticides can cause different alterations in physiological and biochemical functions.

Oil/Hydrocarbons

Palaemon adspersus, a hyper- and hypo-osmoregulating shallow-water shrimp, was exposed to 20, 70, 100 and 200 μg l^{-1} WSF of North Sea crude oil (Baden 1982). The ability to maintain hyper-osmolality decreased after 1, 2, and 3 weeks exposures to 200, 70, and 100 μg l^{-1}, but no effect was observed in 20 μg l^{-1}. Shore crabs, *Carcinus maenas*, were tested for osmoregulatory responses to various toxicants (Bamber and DePledge 1997). While exposure to Cu was clearly detrimental, crabs exposed to high concentrations of B[a]P showed no significant impairment of osmoregulatory ability. Authors concluded that acute B[a]P exposure poses little immediate threat to processes controlling osmoregulation in adult shore crabs.

Hypoxia

Osmoregulatory capacity (OC) was used to study the effects of hypoxia in the shrimp *Penaeus vannamei*. Since OC varied with molt stages with reduced OC before and after ecdysis, only shrimp at intermolt stages C-D_0 were used. Hyper-OC and hypo-OC, respectively, in low salinity and in seawater, were both depressed after 1–2 days exposure to low oxygen tension of 4–8 kPa. OC recovered fully after 24 h in O_2 saturated water (Charmantier et al. 1994). In *Carcinus maenas* at both 13.5 and 30.0 psu salinity, blood pH increased during hypoxia at 40 torr due to an elevation of scaphognathite beating rate. However at 20 torr in both salinities the increase in blood pH was greater than at 40 torr even though the scaphognathite beat rate was the same (Johnson and Uglow 1987). At both salinities blood lactate increased with decreasing pO_2, the changes being larger at 13.5 psu than 30.0 psu for a given pO_2. Blood Na^+ did not change at any pO_2/salinity combination, but in 13.5 psu blood Cl^- decreased during hypoxia.

The brackish water isopod *Saduria entomon* from the Gulf of Gdañsk, Poland appeared to be quite resistant to hypoxia. While hypoxia (15 % saturation) had no significant effect on osmoregulation, anoxia (<1 % saturation) decreased the osmotic concentration of hemolymph. After 6 h at salinity of 3 psu there was a significant difference between anoxic isopods and controls, and after 96 h of anoxia osmotic concentrations decreased at all salinities except 25 psu. A tendency was observed for hemolymph Na to decrease in anoxia, which was significant at 7.3 psu after 96 h but not statistically significant at the other salinities (Dobrzycka-Krahel and Szaniawska 2007).

Hypoxia can interact with other stresses such as metals and salinity. While blood Na^{+} and Cl^{-1} levels in *Crangon crangon* and *Carcinus maenas* were not significantly affected during metal Cu/Zn (0.2 5mg l^{-1}) or hypoxic ($p_{w}O_2 = 40$ torr), exposure at both 13.5 and 27.0 psu decreased blood ion levels were seen in exposures to metal plus hypoxia at low salinity. In *C. maenas* blood Ca^{2+} regulation was not affected by metal or hypoxic exposure individually, but combinations resulted in salinity-dependent increases in blood Ca^{2+} levels (Johnson 1988). Hypoxia also interacts with ammonia. Shrimp (*Litopenaeus stylirostris*) treated separately with ammonia or hypoxia had low mortality; most shrimp that died were in early post molt (stage A) in ammonia, while hypoxia affected mainly late premolt animals (stage D_2). A synergistic effect of ammonia and hypoxia affected mostly shrimp in late premolt stage D_2. The common physiological response was a reduced OC and an increase in Ca^{2+}. Plasma lactate levels increased in shrimp in hypoxia and hypoxia plus ammonia (Mugnier et al. 2008). Total protein concentration was reduced in ammonia and combined treatments, and was more pronounced in late premolt stage than in intermolt shrimp. The combination of ammonia plus hypoxia led to a stronger response than ammonia alone and/or hypoxia alone.

Polluted Sites

Animals from polluted sites have been studied for degree of response to contaminants. The mangrove crabs *Ucides cordatus* and *Callinectes danae* were sampled from “polluted” mangrove areas and from a reference site (Harris and Santos 2000). Individuals of both species from the polluted site showed greater capacities for regulating blood osmotic concentrations at low salinity (9 psu). However, *U. cordatus* showed reduced hypo-regulatory ability in 34 psu. *C. danae* from the polluted site showed significantly higher Na^{+}/K^{+}-ATPase levels in posterior gills compared to crabs from the reference site. These differences may reflect adaptive changes following long-term exposure to contamination.

Climate Change/Acidification

Hermit crabs, *Pagurus bernhardus,* at low pH (6.8) showed osmoregulatory changes (de la Haye et al. 2012). Analysis of their hemolymph revealed a greater concentration of chloride ions (Cl^{-}) in reduced pH, suggesting iono-regulatory disruption.

The iono-regulating prawns *Palaemon elegans* and *P. serratus*, could compensate for a $p\text{CO}_2$ of 0.30 kPa over a 30 day period (Dissanayake et al. 2010). However, ion regulation was maintained at the expense of acid–base balance. The crabs *Necora puber* and *Cancer magister*, which are relatively poor iono-regulators, could compensate for acid–base disturbances in 24 h after exposure to 0.10–0.20 kPa CO_2 (Pane and Barry 2007; Spicer et al. 2007). Compensation was via elevation in hemolymph $[\text{HCO}_3^-]$. Continued exposure to the same $p\text{CO}_2$ had a negative effect on *N. puber*, as bicarbonate buffering started to fail after 16 days when $[\text{HCO}_3^-]$ reached 27 mmol l^{-1} (Spicer et al. 2007). However, Small et al. (2010) found that hemolymph $[\text{HCO}_3^-]$ was much lower after 30 days at the same $p\text{CO}_2$.

4.1.2 Mollusks

Most mollusks are iso-osmotic, having the same level of osmotic pressure as their environment. However, those inhabiting estuaries may be able to osmoregulate, but impacts of pollution on osmoregulation in mollusks have not been extensively studied. Dillon and Anderson (1979) found that low-level mercury contamination has minimal effects on the ability of the clam *Rangia cuneata* to adapt to reduced salinity. They found a transitory “lag” effect in which mercury-exposed clams were somewhat slower in adjusting osmotically active blood constituents in reduced salinity. In response to a salinity decrease, the hemolymph of *Rangia cuneata* exhibits an initial rapid decrease in inorganic ions followed by additional osmotic adjustments. Liu et al. (2011) investigated effects of Hg on metabolomics in gills of three varieties of Manila clam (*Ruditapes philippinarum*). In all three mercury-exposed (White, Liangdao Red and Zebra) varieties of clams, increased glycine and decreased taurine and homarine were found, which were interpreted as disturbances in osmotic regulation.

Bivalve mollusks are considered to be likely to be vulnerable to ocean acidification because they are poor iono-regulators and show little ability to buffer the acidifying effects of elevated CO_2 in their body compartments (Fabry et al. 2008; Doney et al. 2009).

4.1.3 Fishes

In a review, Bonga and Lock (1991) concluded that fishes respond to many toxicants because they affect the gills by increasing the permeability of the gill epithelium to water and ions, and by inhibiting ion exchange in the chloride cells. They stated that many stressors can affect osmoregulation indirectly via effects of catecholamines on gills. Since many toxicants evoke a stress response, it may be difficult to distinguish specific toxic effects on gills from non-specific stress responses.

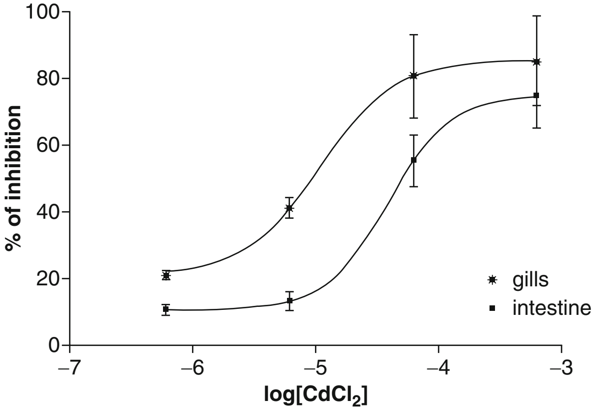


Fig. 4.3 Dose–response curves of the effect of Cd on carbonic anhydrase activity in eel intestinal and gill homogenates. Values = M ± SE of four experiments. Ordinate: percentage of enzymatic activity inhibition (Reprinted from Lionetto et al. 2000: 564, courtesy of Elsevier Publishing Co)

Metals

Kim et al. (2012) investigated effects of Hg on flounder *Paralichthys olivaceus*. Fish were injected twice intraperitoneally with mercuric chloride (2, 4, or 8 mg Hg/kg BW). Very low levels of calcium and chloride, and reduced osmolality, were observed at 8 mg Hg/kg BW. The sheepshead minnow, *Cyprinodon variegatus,* is a euryhaline fish that lives in estuaries and coastal marshes that have a wide range of salinities. Many of these areas also have elevated contaminants, creating the possibility of metals interfering with the uptake of ions for osmoregulation. To determine whether the effect of Cu on osmoregulation depends on the osmotic conditions that fish were living in, fish were acclimated for 14 days to 2.5, 10.5 or 18.5 psu salinity and then exposed to free cupric ion (14.6 μM Cu^{2+}) for 6 h (Adeyemi et al. 2012). Plasma Na, plasma Cl, wet/dry weight ratio, transepithelial potential difference (TEPD) and branchial Na^+/K^+-ATPase activity were determined before and after Cu exposure. Fish acclimated to low salinity (2.5) had lower TEPD and plasma Na and higher gill Na^+/K^+-ATPase activity compared to fish at higher salinities. Cu caused a significant decrease in plasma Na and Na^+/K^+-ATPase activity, and an increase in wet/dry weight ratio, but only in fish in low salinity. No significant changes in plasma Cl were detected. The results show that effects of Cu on osmoregulation depend on the fish's previous salinity regime, and tend to be more severe at lower salinity.

Osmoregulation is particularly important for species such as eels that migrate between freshwater and the ocean. Cd exposure affected CA in the intestine and gills of the eel, *Anguilla anguilla*, with the gill enzyme being more sensitive than that of the intestines (Fig. 4.3) (Lionetto et al. 2000). Lorin-Nebel et al. (2013)

studied single and combined effects of Cu plus infestation with the nematode *Anguillicoloides crassus* in *A. anguilla* after a salinity challenge from nearly isotonic (18 psu) to hypo- (5 psu) and hypertonic (29 psu) conditions. In 18 psu, blood osmolality remained constant over the 6 weeks with Cu^{2+} and *Anguillicoloides crassus*. In fish exposed to hypertonic 29 psu for 2 weeks, no significant change in blood osmolality, Na^+/K^+-ATPase (NKA) activity, or Na^+ and Cl^- concentrations was seen. After 2 weeks at 5 psu however, a significant decrease in blood osmolality was seen in fish with the parasite and Cu^{2+}. Gill NKA activity was lower in eels in the combined stress. Thus, the low salinity disturbed osmoregulation in eels exposed to Cu and the parasite. Again, low salinity again produced more severe effects than high salinity.

Juvenile salmon migrate from freshwater to the ocean. To simulate a situation in which juvenile coho salmon, *Oncorhynchus kisutch*, are exposed to Cr in freshwater (FW) before migrating to seawater (SW), effects of FW exposure to Cr on salinity tolerance and serum osmolality in SW were investigated (Sugatt 1980). SW survival decreased in salmon exposed to 0.23 mg Cr l^{-1} for 4 weeks or to 0.5 mg Cr l^{-1} for 2 weeks and transferred to 20 or 30 psu salinity, respectively. Serum osmolality after 2 weeks of FW exposure to 0.5 mg Cr l^{-1} then transfer to 20 or 30 psu SW, was elevated in Cr-exposed fish shortly after transfer but not 1 week after transfer. The author suggested that Cr acted non-specifically on osmoregulatory epithelia to cause dehydration in SW, and that salinity tolerance and serum osmolality are sensitive responses.

Some non-migratory species are able to live in both freshwater and seawater. Effects of arsenic (As_2O_3) on plasma osmolarity, Na and K concentrations, gill Na−K-ATPase, and the ultrastructure of gill chloride cells were compared between seawater tilapia (*Oreochromis mossambicus*) and freshwater tilapia (Hwang and Tsai 1993). No significant effect was found on plasma ion concentrations and osmolarity, enzyme activity, or chloride cells in freshwater tilapia after 96 h exposure to concentrations up to 70 mg l^{-1} arsenic. However, 96 h exposure to 15 mg l^{-1} produced an increase in plasma osmolarity and activity of gill Na^+/K^+-ATPase, as well as better development of the chloride cell tubules in the seawater tilapia. This is an unusual case of effects being greater in higher salinity, but these effects are not deleterious. Authors felt that the activation of gill Na^+K^+-ATPase and chloride cells in seawater tilapia may indicate an adaptation in the osmoregulatory mechanism to enhance gill secretion of ions or arsenic.

Spiny dogfish (*Squalus acanthias*) were exposed to 20 and 100 μM Pb, which accumulated primarily in gills and skin (Eyckmans et al. 2013). A disturbance in acid–base status was noted after 1 day. The increase in pH was temporary at 20 μM, but persisted at 100 μM. After 2 days, plasma Na and Cl concentrations were reduced at 100 μM Pb, and urea excretion rates increased. Pb impaired Na^+/K^+-ATPase activity in gills, but not in the rectal gland. The authors concluded that spiny dogfish experienced relatively low ion-osmoregulatory and respiratory distress when exposed to lead, especially when compared to other metals.

Organics

Pesticides and PCBs

Miller and Kinter (1977) found that DDT inhibited Na^+/K^+-ATPase in intestinal tissue of *F. heteroclitus*, with significant effects after 24 h exposure to 0.75 mg l^{-1}, which the authors thought was related to the inhibition of amino acid transport (discussed in digestion chapter). Plasma Na increased with concentration and time of exposure. Both DDT and PCBs inhibited osmoregulation in killifish and eels (*Anguilla rostrata*) by inhibiting Na^+/K^+-ATPase (Kinter et al. 1972).

Effects of the antifoulant Mexel®432 (0.5, 1 and 2 mg l^{-1}) on osmoregulation (osmolality and Na^+/K^+-ATPase activity) in juvenile flatfish *Solea senegalensis* were investigated by Lopez-Galindo et al. (2010) Gill histopathology and alterations due to oxidative stress were also examined. They noted significant changes in osmoregulatory parameters, histological lesions in gills, and decreased branchial Na^+/K^+-ATPase activity in exposed fish.

Osmoregulation is critical in smolting salmonids that have to migrate from freshwater to the ocean. Exposure of Atlantic salmon smolts to 0.5 μg l^{-1}atrazine in freshwater resulted in a significant decrease in gill Na^+K^+ ATPase activity but, surprisingly, not at the higher 5.0 μg l^{-1} concentration (Moore et al. 2007). On transfer to saltwater there was no significant effect on gill Na^+K^+ ATPase activity. Jørgensen et al. (2004) studied effects of PCB exposure on smoltification and subsequent seawater performance in hatchery-reared, anadromous Arctic charr (*Salvelinus alpinus*). The fish were placed for two summer months in seawater, then orally dosed with 0 (Control, C), 1 (Low Dose, LD) or 100 mg Aroclor 1,254 kg^{-1} body mass (High Dose, HD) in November. They were then kept in fresh water without being fed to mimic overwintering in fresh water until they smolted the following June. They were then transferred to seawater and fed to mimic their summer feeding residence in seawater followed by a period without food in freshwater from August until maturation in October. HD charr had reduced plasma growth hormone, insulin-like growth factor-1, and thyroxin and triiodothyronine during smoltification. These hormonal alterations correlated with impaired hyposmoregulatory ability as well as reduced growth rate and survival after transfer to seawater.

PAHs

Goanvec et al. (2011) exposed juvenile turbot, *Scophthalmus maximus* to the WSF of a heavy fuel oil (about 320 ng l^{-1}) for 5 days and then placed them for 30 days in clean seawater for recovery. Osmolality, $[Na^+]$ and $[Cl^-]$ rapidly increased and then slowly decreased back to normal levels during the recovery period. Branchial histology showed decreased numbers of mucocytes and chloride cells. The authors suggested that the osmotic imbalance was due to structural alteration of the gills, i.e., the reduction of ionocyte numbers. Boese et al. (1982) investigated effects of exposure to various concentrations of petroleum refinery wastewater on gill ATPase, plasma protein, plasma osmolarity, and hematocrit of the staghorn sculpin, *Leptocottus armatus*. The extent of the reduction in Na^+, K^+-ATPase activity resulting from the exposure to the two refineries' wastewaters was considered to be related to the specific chemical composition of the two wastewaters.

To investigate chronic effects of a Brazilian oil spill on a resident fish, the Brazilian silverside *Atherinella brasiliensis* was sampled from 2 affected sites and an unaffected reference area 1, 4, and 7 months after a spill (Souza-Bastos and Freire 2011). Four months after the spill, fish from the oil spill site had increased plasma osmolality (<525 mOsm/kg H_2O, or 70 % above reference fish) and chloride (<214 mM or 51 % above reference fish). Affected fish also showed branchial CA inhibition (56 % of the values in reference fish). Thus, osmoregulation was still affected in these fish months after the oil spill, and was considered a sensitive tool for evaluating chronic effects of oil spills.

Contaminants of Emerging Concern

Estrogenic substances such as 17β-estradiol (E2) and 4-nonylphenol (NP) can affect osmoregulation in Atlantic salmon (*Salmo salar)*. Compared to controls, plasma chloride concentrations of E2-treated fish were decreased 5.5 mM in freshwater (FW) and increased 10.5 mM in saltwater (SW). There was no effect of NP or E2 on gill Na^+/K^+-ATPase activity in FW smolts, but E2 treatment in SW reduced gill Na^+/K^+-ATPase activity and altered the number and size of ionocytes (Lerner et al. 2012). The study indicates that E2 reduces SW tolerance, which may be part of its normal function for reproduction and movement into FW. Farmen et al. (2012) showed that the gills of Atlantic salmon accumulated Ag from silver nanoparticles, which caused increases in plasma glucose and gill gene expression of heat shock protein. A concentration-dependant inhibition of Na^+/K ATPase expression indicated impaired osmoregulation at >20 $\mu g\ l^{-1}$ of Ag-NP. The Ag-NP suspension caused acute necrosis of gill lamellae at 100 $\mu g\ l^{-1}$.

Larvae of many fishes are planktonic and live near the surface of the ocean where they can be exposed to UV radiation. The effects of increased UVB radiation on osmoregulation of *Dicentrarchus labrax* larvae, which drift high in the water, were investigated by Sucré et al. (2012). In young larvae, osmoregulation depends on ion transporting cells, ionocytes, on the skin of the trunk and yolk sac. A loss of osmoregulatory ability occurred in larvae after 2 days of low (50 $\mu W\ cm^{-2}$: 4 h l/20 h D) and medium (80 $\mu W\ cm^{-2}$: 4 h l/20 h D) UVB exposure. Compared to control larvae in the dark, a significant increase in blood osmolality and high mortalities were seen in larvae exposed to UVB for 2 days or more. Decreased ionocytes and mucus cells were noted after 2 days, which were considered responsible for impaired osmoregulation.

Hypoxia

Atlantic cod (*Gadus morhua* L.) acclimated to salinities 28–7 psu were exposed to mild (8.0 kPa) or severe (4.0 kPa) hypoxia for 6 h (Claireaux and Dutil 1992). During mild hypoxia, a strong hyperventilatory response was noted, causing a respiratory alkalosis. Plasma Cl^- and pyruvate both increased. In more severe hypoxia, the ventilatory response was the same, and a weak metabolic acidosis was superimposed. Both Na^+ and Cl^- concentrations increased, and metabolic disturbances were noted: plasma lactate, pyruvate and glucose concentrations increased, indicative of glycolysis. Salinity affected the degree of the responses: the amplitude decreased with decreasing salinity – opposite of the usual effect of low salinity.

Peterson (1990) investigated plasma osmolality, plasma chloride ion concentration, and survival of two mangrove species, sheepshead minnow, *Cyprinodon variegatus* and sailfin molly, *Poecilia latipinna* under normoxic (150 mmHg) and hypoxic (40 mmHg) conditions (30 °C and 30 psu salinity). Reductions in oxygen consumption occurred in both species in hypoxia. Plasma osmolality increased in sheepshead minnows in hypoxia but plasma chloride did not change in either species. There was no mortality in either species during the 24 h tests. Results suggest tolerance of hypoxia in both species. *P. latipinna* was observed to use aquatic surface respiration (ASR) in hypoxic conditions.

Acidification

Osmoregulation is critical in species that migrate between freshwater and the ocean. Atlantic salmon, *Salmo salar*, smolts of both wild and hatchery origin were held for 5–16 days in ambient (pH 6.35), limed (pH 6.72), or acidified (pH 5.47) freshwater (Magee et al. 2001). Osmoregulation was assessed by measuring Na^+/K^+ ATPase, hematocrit, and blood Cl concentration in freshwater and after 24-h in seawater. Both hatchery and wild smolts exposed to acidic water had ionoregulatory stress in both fresh and seawater; stress was so severe that there were mortalities of some treated wild smolts in seawater.

Polluted Sites

DeDomenico et al. (2011) investigated impacts of contaminated sediments from a petrochemical region on gills of seabass, *Dicentrarchus labrax*. Sublethal endpoints were assessed such as histopathological lesions and molecules involved in homeostasis. Exposed fish showed a reduction of gill cell membrane permeability, which could result in altered osmotic balance and gas exchange, although these processes were not directly measured.

4.1.4 Other Taxa

Metals

Effects of silver on ion and water balance were studied in the polychaete *Neanthes virens*. Worms from a clean site showed increased K^+ and decreased Ca^{2+} in their coelomic fluid after accumulating 88 mg l^{-1} Ag, but worms from a Ag-contaminated site in Long Island Sound showed effects only on Ca^{2+} (Pereira and Kanungo 1981). All worms developed edema and a curled posture, but these effects were more severe in the worms from the reference site, indicating that chronic exposure had led to some Ag tolerance.

Ocean Acidification

Echinoderms are considered likely to be vulnerable to acidification because they are poor iono-regulators that have low capability to buffer elevated CO_2 in their body (Dupont et al. 2010). Spicer et al. (2011) investigated the effect of a 5 day exposure to CO_2-acidified sea water on the extracellular acid–base balance of the sea urchin *Strongylocentrotus dröebachiensis* and found respiratory acidosis which increased with decreasing pH. This was similar to another sea urchin, *Psammechinus miliaris*. However, at the lowest pH (6.78) there was a metabolic component to the acidosis (correlated with increased l-lactate) in *S. dröebachiensis* but not *P. miliaris*. The acidosis was accompanied by an increase Ca in coelomic fluid.

4.2 Excretion

Excretion, ridding the body of waste products, is primarily a consequence of protein breakdown; when proteins are converted to carbohydrates to provide energy, the amino group is removed and becomes a waste product that must be eliminated. The amino group is oxidized to form ammonia, which is toxic and highly soluble in water. If the organism has a sufficient source of water, ammonia can simply be diluted and excreted into the water. Ammonia can diffuse passively out of gills, but to be excreted via kidneys much water is required to dissolve and flush the toxic ammonia. Animals that have enzymes to convert ammonia to urea or uric acid can produce urine that is more concentrated and thus conserve water. This is particularly important in terrestrial animals, but detoxification of ammonia to urea has also been noted in some aquatic organisms including elasmobranch and teleost fishes. Excretion is related to osmoregulation since marine osmoregulators, which tend to lose water to the environment, compensate by drinking sea water, eliminating the excess salt via salt secreting organs (e.g. chloride cells in fish gills), and excreting highly concentrated urine. Freshwater species, which tend to absorb water from the environment, drink little and excrete large amounts of dilute urine. High concentrations of ammonia are produced in aquaculture operations where population density of animals is high, and ammonia can have toxic effects on a wide variety of organisms. There is an extensive body of literature on the toxicity of ammonia to both invertebrates and fishes, which will not be covered here.

While there has been considerable research into the excretion of contaminants and their by-products, there has not been as extensive a research effort into the physiological effects of contaminants on the process of excretion itself. Within the limited literature, there have been reports of both increases and decreases in ammonia excretion in different species, sometimes in response to the same toxicants. Many other factors such as salinity and feeding play a role in the process.

4.2.1 Crustacea

Ammonia excretion in crustaceans may show a relation to the molt cycle. At the early premolt (stage D_0) in *Crangon crangon* excretion is higher than levels at intermolt; at late premolt (stage D_2) excretion decreases to a minimum (Regnault 1979). After ecdysis, the excretion rate is about twice that of intermolt shrimp. Results of contaminant exposures show both increases and decreases in ammonia excretion; some of the variation in effects may be due to investigators not taking the molt cycle into consideration when planning studies.

Metals

Gaudy et al. (1991) reported that 0.05 mg l^{-1} Cd reduced ammonia excretion rate in *Leptomysis.* These mysids had reduced ability to utilize food (see chapter on Digestion); the reduced excretion could be a result of low food intake. After exposure to Cd or Zn (1 mg l^{-1}), ammonium excretion in the white shrimp, *Litopenaeus vannamei,* was higher than controls (Fig. 4.4) (Wu and Chen 2004) – the opposite effect from that observed by Gaudy et al. (1991). Wu and Chen suggested that elevated ammonium excretion was related to decreased osmotic pressure of shrimp blood. Barbieri (2007) found similar results in *Litopenaeus schmitti*: 0.18–0.98 mg l^{-1} Cd and 0.31–1.64 mg l^{-1} Zn increased ammonium excretion. Barbieri et al. (2005) found that while Hg (0.045 mg l^{-1}) reduced oxygen consumption, it increased ammonia excretion in larvae of *Farfantepenaeus brasiliensis*. Barbieri and Paes (2011) studied effects of salinity on the toxicity of Cd in *F. paulensis.* Oxygen consumption and ammonium excretion were measured in shrimp at three salinities (36, 20 and 5), at temperature of 20 °C. At 5 psu salinity Cd reduced oxygen consumption. Elevated ammonium excretion was seen at all salinities, but was greatest at 5 psu salinity. In contrast, postlarvae of *Penaeus indicus* decreased their ammonia excretion when exposed to increasing concentrations of Pb up to 7 mg l^{-1} (Chinni et al. 2002).

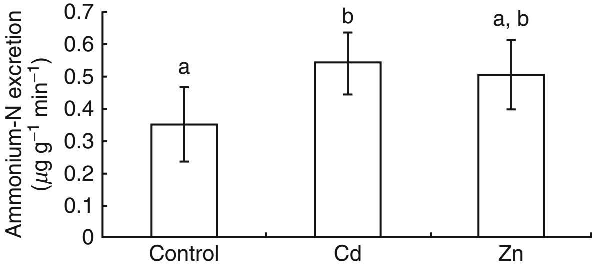


Fig. 4.4 Ammonium (M ± SE) excreted by control *L. vannamei* and individuals exposed to 3 mg l^{-1} Cd and 3 mg l^{-1} Zn for 24 h. Means with different letters significantly differ ($P < 0.05$) (Reprinted from Wu and Chen 2004: 1594, courtesy of Elsevier Publishing Co)

Mysids (*Praunus flexuosus*) responded differently depending on the season (Garnacho et al. 2001). Metabolic responses to copper (0, 5, 25, 75 and 200 μg l^{-1}) were measured in winter and summer at 24 h, 96 h and 10 days of exposure. Control metabolic rates were different between seasons. Respiration and ammonia excretion were 2.5–6 times lower in winter and O:N ratios varied from 3.5 to 5 in summer to 8 in winter. Cu exposure decreased the O:N ratio (from 10 to 2–4). The responses to Cu were greater in summer, causing larger changes and being effective at lower concentrations. Alterations of the O:N ratio were a very sensitive indicator. Other investigators generally do not consider the role of season in determining responses to toxicants.

Oxygen consumption and excretion patterns (ammonia and nitrite) of mud crabs *Sesarma quadratum* were studied at two sublethal concentrations of 1/10 (2.8 mg l^{-1}) and 1/3 (9.3 mg l^{-1}) of the LC_{50} of copper chloride, and 1/10 (0.29 mg l^{-1}) and 1/3 (0.98 mg l^{-1}) of the LC_{50} value of chlorine for 1, 7, 14 and 21 days (Valarmathi and Azariah 2002). The rates of oxygen consumption and excretion both decreased and were negatively correlated to the concentrations of the toxicants.

Organic Contaminants

Laughlin and Linden (1983) found that exposure of the Baltic mysid, *Neomysis integer*, to WSF of oil at concentrations between 200 and 1,000 pg 1^{-1} produced decreases in ammonia excretion that were strongly influenced by temperature, with the greatest effect at the highest temperature tested.

Mysidopsis bahia were exposed throughout their life cycle to the defoliant DEF (McKenney et al. 1991). After 5-day exposure to ≥0.085 μg l^{-1} DEF, mysids excreted less ammonia. O:N ratios of unexposed mysids decreased as juveniles matured, and DEF exposure resulted in higher O:N ratios. Higher O:N ratios during maturation of exposed mysids suggests greater reliance on energy-rich lipids to support metabolic demands.

Hypoxia

Norway lobsters (*Nephrops norvegicus*) were exposed to hypoxic conditions (Hagerman et al. 1990). Blood ammonia levels decreased over time in both normoxia and hypoxia, indicating a decreasing metabolic rate over the experimental period. In short term experiments, blood ammonia decreased in hypoxia and ammonia excretion was negatively related to DO. Responses to hypoxia can be affected by feeding. In *Carcinus maenas*, ammonia excretion decreased in hypoxia (60 and 35 % saturation): a 40–45 % decrease was seen in fed crabs at 35 % saturation and in 3 day-unfed crabs at both hypoxic levels (Regnault and Aldrich 1988). In crabs that had been unfed for 6 days, the effect of hypoxia was confounded by the effect of starvation. Oxygen consumption rate was directly related to the external O_2 tension, regardless of the crab's nutritional state. A strong relationship was noted between ammonia excretion and oxygen consumption rates in fed crabs in hypoxia, but not in starved crabs. In *Cancer pagurus*, Regnault (1993) found a 50 and 60 % decrease in ammonia excretion at a $Pw0_2$ of 40 Torr and 15 Torr, respectively, in regularly fed crabs. This decrease was seen within the 1st hour. In 2-week starved crabs, the ammonia excretion rate in normoxia was reduced by 40 % due to starvation. A further decrease took place at both hypoxic levels, but the hypoxia effect was secondary to the primary starvation effect.

Effects of hypoxia can also interact with salinity. Rosas et al. (1999) exposed juvenile shrimp (*Penaeus setiferus*) to DO of 2, 3, 4 and 5.8 mg l^{-1} at two salinities (15 and 35 psu) for 60 days. Ammonia excretion was significantly greater in 15 than in 35 psu, and in 15 psu excretion decreased with DO; in unfed animals it diminished in direct proportion to the DO, while fed shrimp could regulate ammonia excretion at DO between 5.8 and 4 mg l^{-1}. In 35 psu salinity, excretion increased in fed animals at 2 and 3 mg l^{-1} DO. In low salinity the shrimp maintained proteins as their energy source at all DO levels, while in high salinity they changed their energy source from lipids-proteins to proteins when at low DO.

Acidification

The Chinese mitten crab, *Eriocheir sinensis*, migrates between fresh and salt water, and thus can be exposed to a wide variety of pHs, both acidic and alkaline. Yu et al. (2008) exposed mature female crabs to pH 4.5, 6.0, 7.5, 9.0 and 10.5 for 24 h, and measured ammonia excretion rate and hemolymph nitrogen content. They found no significant difference in ammonia excretion at pH between 4.5 and 9.0, but inhibitory effects were seen at the alkaline pH of 10.5. The hemolymph pH was relatively constant from pH 4.5–9.0, but increased at pH 10.5. Hemolymph urea and urate were also affected by pH; urea and urate increased between pH 7.5 and pH 10.5. The results indicated that exposure to low pH did not affect the crabs' excretory physiology, but that high alkaline pH produced a decrease in ammonia excretion, and elevated the hemolymph ammonia, urea, urate and total free amino acids.

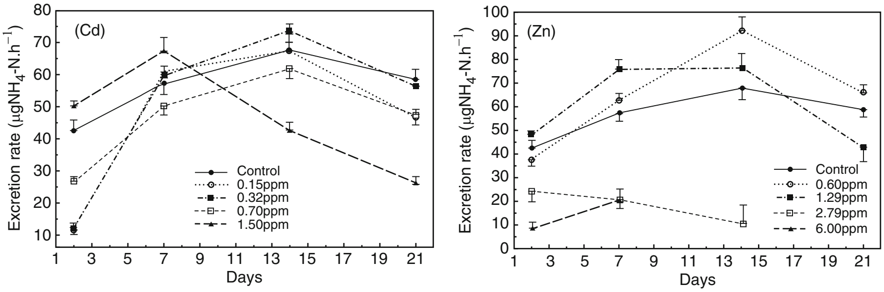


Fig. 4.5 Mean ammonia excretion rates (± SE) of *P. viridis* exposed to various concentrations of Cd or Zn at 24 °C (Reprinted from Cheung and Cheung 1995: 383, courtesy Elsevier Publishing Co)

4.2.2 Mollusks

Metals

Mussels, *Perna viridis*, exposed to Cd (0.15, 0.32, 0.70 and 1.50 mg l^{-1}) and Zn (0.60, 1.29, 2.79 and 6.00 mg l^{-1}) generally exhibited both reduced oxygen consumption and ammonia excretion (Fig. 4.5) (Cheung and Cheung 1995). Cheung and Wong (1998) exposed the prosobranch gastropod, *Babylonia lutosa*, to copper (0.02, 0.06 and 0.20 mg l^{-1}) for 23 days. The oxygen consumption rate and the ammonia excretion rate decreased with increasing concentrations of Cu and increased time, while the O:N ratio remained low.

Perna perna were exposed to 12.5, 25.0, 37.5 and 50.0 μg l^{-1} Cu for 24 h. In contrast to *P. viridis* in the previous study, mucus secretion, nitrogen excretion, and oxygen consumption rates all increased at 25 and 50 μg l^{-1} copper. *Perna perna* changed its substrate utilization in favor of protein metabolism, thus increasing excretion of ammonia (Vosloo et al. 2012). Sze and Lee (2000) investigated effects of Cu exposure on growth and physiological responses of the green mussel *P. viridis* by exposing mussels to 50 μg l^{-1} Cu for 3 months at 17 and 25 °C. While growth and condition index were reduced by Cu exposure, along with clearance rates, feces production, assimilation efficiency, and oxygen consumption, ammonia excretion increased with chronic Cu exposure.

Effects can also be seen in larval stages. D-shaped larvae of *P. perna* were exposed to 0.47 mg l^{-1} $ZnSO_4$, which reduced both oxygen consumption and ammonia excretion. The O:N ratios were low, indicating that larvae were metabolizing mostly protein (Radlvic et al. 2007).

Organics

Pesticides

Effects of two pesticides, methamidophos and omethoate, at 1, 10, 50, 100, and 200 μg l^{-1} for 96 h. on oxygen consumption, ammonia excretion, and filtration rate were studied in *Mytilus edulis* (Zhen et al. 2010). While oxygen consumption increased in all concentrations of methamidophos and in the lower concentrations of omethoate from 6 to 24 h, after 24 h, it decreased significantly. Ammonia excretion rates were elevated after 36 h of exposure to methamidophos, but lower than controls in omethoate. The O:N ratios increased initially when mussels were exposed to the pesticides and then decreased. At 100 and 200 μg l^{-1}, the O:N ratio was below 30 after 72 h. Fuhrer et al. (2012) examined effects of the organophosphate pesticide chlorpyrifos on the activity of acetylcholinesterase and on the O:N ratio in the mussel *Aulacomya aterwas.* Chlorpyrifos concentrations between 0.2 and 1.61 μg l^{-1} inhibited AChE activity, and concentrations between 0.8 and 1.61 μg l^{-1} stimulated ammonia excretion and decreased the O:N ratio, reflecting increased reliance on protein metabolism. El Shenawy et al. (2003) investigated the toxicity of a herbicide (48 % glyphosate, Roundup®) and an insecticide (50 % chlorpyrifos-methyl, Reldan®) to the clam *Ruditapes decussatus*. After determining LC_{50}, effects of ½ LC_{50} of Roundup® (1.1 μl l^{-1}) and Reldan® (0.6 μl l^{-1}) were ascertained. Reldan® reduced valve activity more than Roundup®, and the decreased respiration rate was associated with decreased ammonia excretion. Reduced respiration and excretion accounted for the decline in metabolism and excretion with increased time of exposure.

PAHs

Stickle et al. (1984) exposed the carnivorous snail *Thais lima* to the water soluble fraction (WSF) of Cook Inlet crude oil for 28 days. Predation rate on *M. edulis* declined linearly with increasing aromatic hydrocarbon concentrations. Percentages of total energy expenditure were dominated by respiration (87 %), while ammonia excretion was responsible for only 9 % and did not vary with dose or time. O:N ratios were also unaffected by concentration or time and indicated that *T. lima* derived most of its energy from protein catabolism.

Axiak and George (1987) studied physiological responses of the bivalve *Venus verrucosa* to low levels of WAF of Kuwait crude oil (100 μg l^{-1}) for 145 days. Clams decreased their feeding rates and food absorption efficiencies, while increasing oxygen consumption and ammonia excretion. The integration of these responses into the scope for growth, as well as the O:N ratio, showed that exposure reduced the energy available for somatic growth and reproduction and enhanced protein catabolism.

Oysters (*C. gigas*) exposed to 50 μg l^{-1} PAHs initially maintained their metabolic rate, but when exposure was prolonged, they decreased their energy intake while increasing catabolism to satisfy energy demands. When exposed to 200 μg l^{-1}, they reduced energy intake and increased their excretion rate, which can facilitate elimination of toxic chemicals (Jeong and Cho 2007). Kim et al. (2007) also investigated effects of PAH on *C. gigas*, by exposing the oysters to 0, 50, 100 and 200 μg l^{-1} for 7 days with a 3-day acclimation period. Filtration and respiration rates increased at 50 μg l^{-1} PAH and decreased at higher concentrations. Ammonia excretion increased with increasing PAH levels, with a significant elevation at 200 μg l^{-1}. Overall, oysters exposed to 50 μg l^{-1} had scope for growth (SFG) similar to controls, but those at to 200 μg l^{-1} PAH had negative SFG values.

Effects can also be seen in larval stages of mollusks. D-shaped larvae of *P. perna* were exposed to 5.69 μL.L^{-1} benzene, which reduced their ammonia excretion. The O:N ratios were low, indicating that the larvae were metabolizing mostly protein (Radlvic et al. 2007).

Acidification

Juvenile *M. galloprovincialis* under conditions of −0.3 and −0.6 pH units had increased absorption efficiency and ammonium excretion. Overall these mussels had increased scope for growth and tissue dry weight, suggesting that this species is relatively tolerant to acidification (Fernandez-Reiriz et al. 2012).

Polluted Environment

Bakke (1988) measured ingestion, food absorption efficiency, ammonia excretion, respiration and their integration into scope for growth in periwinkles *Littorina littorea* from 4 field populations from Norway, and 4 groups exposed in mesocosms to different concentrations of oil and copper for 4 months. Measurements did not vary among the different mesocosm treatments. The author concluded using physiological energetics of L. *littorea* in pollution monitoring was premature, and recommended method development and a better understanding of the factors regulating the processes.

Grant and Thorpe (1991) exposed soft-shell clams (*Mya arenaria*) to a flow-through suspension of intertidal sediment (100–200^{-1}) with ambient filtered seawater control. Clams were given microalgae as food. Clams decreased their oxygen consumption and increased ammonia excretion in the turbidity treatment. O:N ratios fell in exposed clams, suggesting that they were utilizing body protein reserves to meet nutritional needs. While control animals grew over the study period, treated clams did not, suggesting that *M. arenaria* copes with turbidity by reducing its ventilation rate, which gives them tolerance to intermittent turbidity at the expense of nutrition.

4.2.3 Fishes

The excretion of ammonia is critical because ammonia is toxic and ammonia itself can be considered a pollutant with deleterious effects on biota. A body of literature on ammonia toxicity suggests that during ammonia exposures, estuarine fish may be most at risk as larvae or juveniles, at elevated temperatures, low salinity, and low pH (reviewed by Eddy 2005), conditions that can promote ammonia uptake and retention. Fish are more susceptible to ammonia toxicity if they are not feeding, are stressed and if they are active. Some fishes convert the ammonia they produce to urea for excretion. Relatively few studies of the effects of environmental contaminants on excretion in marine fishes have been performed.

Metals

Zimmer et al. (2012) exposed euryhaline guppies (*Poecilia vivipara*) to 20 µg Cu l^{-1} in 0 and 25 psu salinity for 96 h. In both salinities, Cu initially inhibited ammonia excretion, which recovered by the end of the 96 h exposure. The activities of Na^+/K^+-ATPase and carbonic anhydrase (CA) were studied in the gills at 12 and 96 h. At both salinities, CA activity was inhibited after 12 h, which the authors felt was the first *in vivo* evidence of Cu-induced inhibition of CA in fish. The inhibition and subsequent recovery of CA were correlated with the inhibition and recovery of ammonia excretion, so CA inhibition may be a mechanism of inhibition of ammonia excretion. No effects were seen on Na^+/K^+-ATPase. Since many metal contaminants are known to damage gill epithelia and/or kidney, ammonia excretion could certainly be affected.

Organics

The Florida pomapano, *Trachinotus carolinus* was exposed to naphthalene to investigate physiological effects after acute (50 min and 24 h) and chronic (12 days) exposures (Dos Santos et al. 2006). The 96h-LC_{50} at 24 °C was 2.83 mg l^{-1} of naphthalene. After acute exposures, fish had a tendency to increase oxygen consumption, but after chronic exposures, they decreased oxygen consumption at the highest concentration, suggesting a narcotic effect. Ammonia excretion was reduced significantly in all the exposed organisms, and the O:N ratio indicated a tendency to use lipids to supply metabolic demands.

Contaminants of Emerging Concern

The gulf toadfish, *Opsanus beta*, was implanted intraperitoneally with various concentrations of the selective serotonin reuptake inhibitor, fluoxetine (0, 25, 50, 75 and 100 µg g^{-1}). Fluoxetine concentrations of 25 and 50 µg g^{-1} were sublethal and were used in subsequent experiments (Morando et al. 2009). Fish treated with 25 or 50 µg g^{-1} had significantly higher circulating levels of 5-HT than controls, suggesting that 5-HT sensitive physiological processes could be affected. However, only the fish treated with 25 µg g^{-1} fluoxetine (the lowest dose) showed a significant increase in urea excretion. A similar increase was not seen in fish treated with the higher concentration of fluoxetine, probably because of their high circulating levels of cortisol, which inhibits urea excretion in toadfish. This may also be in keeping with effects of endocrine disruptors which do not follow typical dose–response relationships.

Hypoxia

Juvenile turbot (*Scophthalmus maximus*) were fed to satiation at O_2 concentrations of 3.5, or 5.0 mg l^{-1} (hypoxia) and 7.2 mg l^{-1} (normoxia). Food intake and growth were both significantly lower under hypoxia than normoxia (Pichavant et al. 2000). During the first 2 weeks of the experiment, food intake of hypoxic fish was reduced by half. Nitrogen excretion and oxygen consumption of feeding fish were significantly reduced under hypoxia. Reduced N excretion was probably related to the lower intake of food.

Ocean Acidification

Ip and Chew (2010) in a review article stated that it is likely that ocean acidification will not be a problem for ammonia-excreting organisms because lowering the pH facilitates ammonia excretion. Conversely, they said, elevated pH could be a significant problem, but this does not occur in seawater. However, since low pH-induces long-term increases in ammonia excretion and protein catabolism in many species, prolonged exposure to reduced pH could have deleterious effects on energetics and growth.

4.2.4 Other Taxa

Metals

Oxygen consumption and ammonia excretion of the sipunculid worm *Phascolosoma esculenta* exposed to Cd (0.45, 0.96, 2.04, and 4.46 mg l^{-1}) or Zn (1.09, 2.34, 4.96, and 10.91 mg l^{-1}) was measured over 21 days. Oxygen consumption decreased from day 1 to day 6, but at low Cd (0.45 and 0.96 mg l^{-1}), oxygen consumption increased (Chen et al. 2009). Oxygen consumption decreased significantly with time in Zn-exposed individuals. Changes occurred in ammonia excretion rates and O:N ratios. Although low O:N ratios (<30) were seen in most of the treatments, no predictable correlation was found between metal concentration and O:N ratio.

Acidification

Sea urchins (*Strongylocentrotus droebachiensis*) from the Kattegat region are normally exposed to periods of low pH. Urchins exposed to moderately (102–

145 Pa, 1,007–1,431 μatm) and highly (284–385 Pa, 2,800–3,800 μatm) elevated seawater $p\text{CO}_2$ for 45 days showed a shift in energy budgets, leading to reduced growth (Stumpp et al. 2012). Exposed animals had increased ammonia excretion and decreased O:N ratios, suggesting that protein metabolism was enhanced in order to maintain ion homeostasis. The acid–base status indicated that the sea urchins could fully or partially compensate for pH changes by slow accumulation of bicarbonate. Sea urchins in the high $p\text{CO}_2$ treatment fell into two distinct groups: 29 % of them had food in their digestive system and maintained partially compensated pH, while the other 71 % had an empty digestive system and a severe metabolic acidosis. This suggested to the authors that some of the urchins from the Kattegat might be pre-adapted to high $p\text{CO}_2$ because of the natural variability in $p\text{CO}_2$ in their habitat. Seawater $p\text{CO}_2$ values of >200 Pa, which are expected to occur in this century during seasonal hypoxia, might be tolerated for a few weeks.

4.3 Conclusions

While osmoregulation is typically depressed by a wide range of contaminants, excretion can be either depressed or enhanced, depending on a number of factors including the organism, the contaminant, the length of time of exposure, the salinity, and food intake. Increased ammonia excretion is a result of increased protein catabolism, which is a response to some contaminants, while decreased excretion often follows decreased food intake, which is a very common response to stresses.

References

Adeyemi JA, Deaton LE, Pesacreta TC, Klerks PL (2012) Effects of copper on osmoregulation in sheepshead minnow, *Cyprinodon variegatus* acclimated to different salinities. Aquat Toxicol 109:111–117

Amado E, Freire CA, Grassi MT, Souza MM (2012) Lead hampers gill cell volume regulation in marine crabs: stronger effect in a weak osmoregulator than in an osmoconformer. Aquat Toxicol 106–107:95–103

Axiak V, George J (1987) Bioenergetic responses of the marine bivalve *Venus verrucosa* on long-term exposure to petroleum hydrocarbons. Mar Environ Res 23:33–47

Baden SP (1982) Impaired osmoregulation in the shrimp *Palaemon adspersus* exposed to crude oil extract. Mar Pollut Bull 13:208–210

Bakke T (1988) Physiological energetics of *Littorina littorea* under combined pollutant stress in field and mesocosm studies. Mar Ecol Prog Ser 46:123–128

Bambang Y, Charmantier G, Thuet P, Trilles J-P (1994) Effect of cadmium on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* (Crustacea: Decapoda). Mar Biol 123:443–450

Bambang Y, Thuet P, Charmantier-Daures M, Trilles J-P, Charmantier G (1995) Effect of copper on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* (Crustacea, Decapoda). Aquat Toxicol 33:125–139

Bamber SD, DePledge M (1997) Responses of shore crabs to physiological challenges following exposure to selected environmental contaminants. Aquat Toxicol 40:79–92

Barbieri E (2007) Use of oxygen consumption and ammonium excretion to evaluate the sublethal toxicity of cadmium and zinc on *Litopenaeus schmitti* (Burkenroad, 1936, Crustacea). Water Environ Res 79:641–646

Barbieri E, Paes ET (2011) The use of oxygen consumption and ammonium excretion to evaluate the toxicity of cadmium on *Farfantepenaeus paulensis* with respect to salinity. Chemosphere 84:9–16

Barbieri E, Passos EA, Garcia CA (2005) Use of metabolism to evaluate the sublethal toxicity of mercury on *Farfantepenaeus brasiliensis* larvae (Latreille 1817). J Shellfish Res 24:1229–1233

Bianchini A, di Castilho PC (1999) Effects of zinc exposure on oxygen consumption and gill $Na^+ K^+$ ATPase in the estuarine crab *Chasmagnathus granulata* Dana 1851 (Decapoda Grapsidae). Bull Environ Contam Toxicol 62:63–69

Bjerregaard P, Vislie T (1986) Effect of copper on ion- and osmoregulation in the shore crab *Carcinus maenas*. Mar Biol 91:69–76

Boese BL, Johnson VG, Chapman DE, Ridlington JW (1982) Effects of petroleum refinery wastewater exposure on gill ATPase and selected blood parameters in the pacific staghorn sculpin (*Leptocottus armatus*). Comp Biochem Physiol C 71:63–67

Boitel F, Truchot JP (1990) Comparative study of the effects of copper on haemolymph ion concentrations and acid–base balance in shore crabs *Carcinus maenas* acclimated to full-strength or dilute seawater. Comp Biochem Physiol C 95:307–312

Bonga W, Lock RA (1991) Toxicants and osmoregulation in fish. Neth J Zool 42:478–493

Charmantier G, Soyez C, Aquacop C (1994) Effect of molt stage and hypoxia on osmoregulatory capacity in the peneid shrimp *Penaeus vannamei*. Ecology 178:233–246

Chen X, Lu CY, Ye Y (2009) Effects of Cd and Zn on oxygen consumption and ammonia excretion in sipuncula (*Phascolosoma esculenta*). Ecotoxicol Environ Saf 72:507–515

Cheung SG, Cheung RY (1995) Effects of heavy metals on oxygen consumption and ammonia excretion in green-lipped mussels (*Perna viridis*). Mar Pollut Bull 31:381–386

Cheung SG, Wong LS (1998) Physiological responses of the subtidal prosobranch, *Babylonia lutosa* (Gastropoda: Buccinidae), to copper. Sci Total Environ 214:185–191

Chinni S, Khan RN, Yallapragada PR (2002) Acute toxicity of lead on tolerance, oxygen consumption, ammonia-N excretion, and metal accumulation in *Penaeus indicus* postlarvae. Ecotoxicol Environ Saf 51:79–84

Claireaux G, Dutil JD (1992) Physiological response of the Atlantic Cod (*Gadus morhua*) to hypoxia and various environmental salinities. J Exp Biol 163:97–118

De la Haye KL, Spicer JI, Widdicombe S, Briffa M (2012) Reduced pH sea water disrupts chemo-responsive behaviour in an intertidal crustacean. J Exp Mar Biol Ecol 412:134–140

De Lisle PF, Roberts MH Jr (1994) The effect of salinity on cadmium toxicity in the estuarine mysid, *Mysidopsis bahia*: roles of osmoregulation and calcium. Mar Environ Res 37:47–62

DeDomenico E, Mauceri A, Giordano D, Maisano M, Gioffrè G, Natalotto A, D'Agata A, Ferrante M, Brundo MV (2011) Effects of *"in vivo"* exposure to toxic sediments on juveniles of sea bass (*Dicentrarchus labrax)*. Aquat Toxicol 105:688–697

Dillon T, Anderson JW (1979) Ionic and osmotic adjustments of the clam *Rangia cuneata* gray in response to reduced salinity and mercury exposure. Comp Biochem Physiol A 62:519–526

Dissanayake A, Clough R, Spicer JI, Jones MB (2010) Effects of hypercapnia on acid–base balance and osmo-/ionoregulation in prawns (Decapod: Palaemonidae). Aquat Biol 11:27–36

Dobrzycka-Krahel A, Szaniawska A (2007) The effect of hypoxia and anoxia on osmotic and ionic regulation in the brackish water isopod *Saduria entomon* (Linnaeus) from the Gulf of Gdansk (Southern Baltic). J Shellfish Res 26:147–152

Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: the other CO2 problem. Ann Rev Mar Sci 1:169–192

Dos Santos TA, Ngan PV, Passos MJ, Gomes V (2006) Effects of naphthalene on metabolic rate and ammonia excretion of juvenile Florida pompano, *Trachinotus carolinus*. J Exp Mar Biol Ecol 335:82–90

Dupont S, Ortega-Martinez O, Thorndyke M (2010) Impact of near-future ocean acidification on echinoderms. Ecotoxicology 19:449–462

Eddy BF (2005) Ammonia in estuaries and effects on fish. J Fish Biol 67:1495–1513

El Shenawy NS, Abdel-Nabi IM, Moawad TI, Taha IA (2003) Physiological and behavioural responses of *Ruditapes decussatus* to roundup and reldan. Egypt J Biol 5:108–119

Eyckmans M, Lardon I, Wood CM, De Boeck G (2013) Physiological effects of waterborne lead exposure in spiny dogfish (*Squalus acanthias*). Aquat Toxicol 126:373–381

Fabry VJ, Seibel BA, Feely RA, Orr JC (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. ICES J Mar Sci 65:414–432

Farmen E, Mikkelsen HN, Evensen Ø, Einset J, Heier LS, Rosseland BO, Salbu B, Tollefsen KE, Oughton DH (2012) Acute and sub-lethal effects in juvenile Atlantic salmon exposed to low µg/L concentrations of Ag nanoparticles. Aquat Toxicol 108:78–84

Fernandez-Reiriz M, Range P, Alvarex-Salgado X, Espinosa J, Labarta U (2012) Tolerance of juvenile *Mytilus galloprovincialis* to experimental seawater acidification. Mar Ecol Prog Ser 454:65–74

Führer E, Rudolph A, Espinoza C, Díaz R, Gajardo M, Camaño N (2012) Integrated use of biomarkers (O:N Ratio and Acetylcholinesterase inhibition) on *Aulacomya ater* (Molina, 1782) (Bivalvia: Mytilidae) as a criteria for effects of organophosphate pesticide exposition. J Toxicol. doi:10.1155/2012/951568

Galindo-Reyes JG, Dalla Venezia L, Lazcano-Alvarez G, Rivas-Mendoza H (2000) Enzymatic and osmoregulative alterations in white shrimp *Litopenaeus vannamei* exposed to pesticides. Chemosphere 40:233–237

Garnacho E, Peck LS, Tyler PA (2001) Effects of copper exposure on the metabolism of the mysid *Praunus flexuosus*. J Exp Mar Biol Ecol 265:181–201

Gaudy R, Guérin J-P, Kerambrun P (1991) Sublethal effects of cadmium on respiratory metabolism, nutrition, excretion and hydrolase activity in *Leptomysis lingvura* (Crustacea: Mysidacea). Mar Biol 109:493–501

Goanvec C, Poirier E, Le-Floch S, Theron M (2011) Branchial structure and hydromineral equilibrium in juvenile turbot (S*cophthalmus maximus*) exposed to heavy fuel oil. Fish Physiol Biochem 37:363–371

Grant J, Thorpe B (1991) Effects of suspended sediment on growth, respiration, and excretion of the soft-shell clam (*Mya arenaria*). Can J Fish Aquat Sci 48:1285–1292

Hagerman L, Søndergaard T, Weile K, Hosie D, Uglow RF (1990) Aspects of blood physiology and ammonia excretion in *Nephrops norvegicus* under hypoxia. Comp Biochem Physiol A 97:51–55

Hansen JI, Mustafa T, Depledge M (1992) Mechanisms of copper toxicity in the shore crab *Carcinus maenas*. I. Effects on Na-K-ATPase activity, haemolymph electrolyte concentrations and tissue water contents. Mar Biol 114:253–257

Harris RR, Santos MC (2000) Heavy metal contamination and physiological variability in the Brazilian mangrove crabs *Ucides cordatus* and *Callinectes danae* (Crustacea: Decapoda). Mar Biol 137:691–703

Hebel DK, Jones MB, Moate RM, Depledge MH (1999) Differing sensitivities of respiratory and osmoregulatory gill tissue of *Carcinus maenas* (Crustacea: Decapoda) to water-borne copper. Mar Biol 133:675–681

Hwang PP, Tsai N (1993) Effects of arsenic on osmoregulation in the tilapia *Oreochromis mossambicus* reared in seawater. Mar Biol 117:551–558

Ip YK, Chew SF (2010) Ammonia production, excretion, toxicity, and defense in fish: a review. Front Physiol 1:134. doi:10.3389/fphys.2010.00134

Jeong WG, Cho SM (2007) Long-term effect of polycyclic aromatic hydrocarbon on physiological metabolisms of the Pacific oyster, *Crassostrea gigas*. Aquaculture 265:343–350

Johnson I (1988) The effects of combinations of heavy metals, hypoxia and salinity on ion regulation in *Crangon crangon* (L.) and *Carcinus maenas* (L.). Comp Biochem Physiol C 91:459–463

Johnson IT, Jones MB (1990) Effect of zinc on osmoregulation of *Gammarus duebeni* (Crustacea: Amphipoda) from the estuary and sewage treatment works at Looe, Cornwall. Ophelia 31:187–196

Johnson I, Uglow RF (1987) The effects of hypoxia on ion regulation and acid–base balance in *Carcinus maenas* (L.). Comp Biochem Physiol A 86:261–267

Jørgensen EH, Aas-Hansen Ø, Maule AG, Strand JE, Vijayan MM (2004) PCB impairs smoltification and seawater performance in anadromous Arctic charr (*Salvelinus alpinus*). Comp Biochem Physiol C 138:203–212

Kim MC, Cho S-M, Jeong W-G (2007) Short-term physiological response of the Pacific oyster, *Crassostrea gigas*, on exposure to varying levels of polycyclic aromatic hydrocarbon. Aquacult Res 38:1612–1618

Kim JH, Lee J-S, Kang J-C (2012) Effect of inorganic mercury on hematological and antioxidant parameters on olive flounder *Paralichthys olivaceus*. Fish Aquat Sci 15:215–220

Kinter WB, Merkens LS, Janicki RH, Guarino AM (1972) Studies on the mechanism of toxicity of DDT and polychlorinated biphenyls: disruption of osmoregulation in marine fish. Environ Health Perspect 1:169–173

Laughlin R, Linden O (1983) Oil pollution and Baltic mysids: acute and chronic effects of the water soluble fractions of light fuel oil on the mysid shrimp *Neomysis integer*. Mar Ecol Prog Ser 12:29–41

Lerner DT, Sheridan MA, McCormick SD (2012) Estrogenic compounds decrease growth hormone receptor abundance and alter osmoregulation in Atlantic salmon. Gen Comp Endocrinol. http://dx.doi.org/10.1016/j.ygcen.2012.08.001

Lignot JH, Trilles J-P, Charmantier G (1997) Effect of an organophosphorus insecticide, fenitrothion, on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* (Crustacea: Decapoda). Mar Biol 128:307–316

Lignot JH, Pannier F, Trilles J-P, Charmantier G (1998) Effects of tributyltin oxide on survival and osmoregulation of the shrimp *Penaeus japonicus* (Crustacea, Decapoda). Aquat Toxicol 41:277–299

Lignot JH, Spanings-Pierrot C, Charmantier G (2000) Osmoregulatory capacity as a tool in monitoring the physiological condition and the effect of stress in crustaceans. Aquaculture 191:209–245

Lionetto MG, Gioradano ME, Villela S, Schettino T (2000) Inhibition of eel enzymatic activities by cadmium. Aquat Toxicol 48:561–571

Liu X, Zhang L, You L, Yu J, Zhao J, Li L, Wang Q, Li F, Li C, Liu D, Wu H (2011) Differential toxicological effects induced by mercury in gills from three pedigrees of Manila clam *Ruditapes philippinarum* by NMR-based metabolomics. Ecotoxicology 20:177–186

Lopez-Galindo C, Vargas-Chacoff L, Nebot E, Casanueva JF, Rubio D, Solé M, Mancera JM (2010) Sublethal effects of the organic antifoulant Mexel®432 on osmoregulation and xenobiotic detoxification in the flatfish *Solea senegalensis*. Chemosphere 79:78–85

Lorin-Nebel C, Felten V, Blondeau-Bidet E, Grousset E, Amilhat E, Simon G, Biagianti S, Charmantier G (2013) Individual and combined effects of copper and parasitism on osmoregulation in the European eel *Anguilla anguilla*. Aquat Toxicol 130–131:41–50

Magee JA, Haines TA, Kocik JF, Beland KF, McCormick SD (2001) Effects of acidity and aluminum on the physiology and migratory behavior of Atlantic salmon smolts in Maine, USA. Water Air Soil Pollut 130:881–886

Martins CG, Almeida DV, Marins LF, Bianchini A (2011) mRNA expression and activity of ion-transporting proteins in gills of the blue crab *Callinectes sapidus*: effects of waterborne copper. Environ Toxicol Chem 30:206–211

McKenney CL, Hamaker TL, Matthews E (1991) Changes in the physiological performance and energy metabolism of an estuarine mysid (Mysidopsis bahia) exposed in the laboratory through a complete life cycle to the defoliant DEF. Aquat Toxicol 19:123–135

Miller DS, Kinter WB (1977) DDT inhibits nutrient absorption and osmoregulatory function in Fundulus heteroclitus. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Physiological responses of Marine Biota to pollutants. Academic, New York, pp 63–74

Monserrat JM, Martínez PE, Geracitano LA, Amado LL, Martins CM, Pinho GL, Chaves IS, Ferreira-Cravo M, Ventura-Lima J, Bianchini A (2007) Pollution biomarkers in estuarine animals: critical review and new perspectives. Comp Biochem Physiol C: Toxicol Pharmacol 146:221–234

Moore A, Lower N, Mayer I, Greenwood L (2007) The impact of a pesticide on migratory activity and olfactory function in Atlantic salmon (*Salmo salar* L.) smolts. Aquaculture 273:350–359

Morando MB, Medeiros LR, McDonald MD (2009) Fluoxetine treatment affects nitrogen waste excretion and osmoregulation in a marine teleost fish. Aquat Toxicol 95:164–171

Mugnier C, Zipper E, Goarant C, Lemonnier H (2008) Combined effect of exposure to ammonia and hypoxia on the blue shrimp *Litopenaeus stylirostris* survival and physiological response in relation to molt stage. Aquaculture 274:398–407

Neufeld GJ, Pritchard JB (1979a) Osmoregulation and gill Na, K-ATPase in the rock crab, *Cancer irroratus*: response to DDT. Comp Biochem Physiol C 62:165–172

Neufeld GJ, Pritchard JB (1979b) An assessment of DDT toxicity on osmoregulation and gill Na, K-ATPase activity in the blue crab. In: Marking L and Kimerle R (eds) Aquatic toxicology. ASTM STP 667. American Society for Testing and Materials, Philadelphia, pp 23–34

Pane EF, Barry JP (2007) Extracellular acid–base regulation during short-term hypercapnia is effective in a shallow water crab, but ineffective in a deep-sea crab. Mar Ecol Prog Ser 334:1–9

Pereira JJ, Kanungo K (1981) Effects of silver on respiration and ion and water balance in *Neanthes virens*. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Biological monitoring of marine pollutants. Academic, New York, pp 107–125

Péquex A, Bianchini A, Gilles R (1996) Mercury and osmoregulation in the euryhaline crab, *Eriocheir sinensis*. Comp Biochem Physiol C 113:149–155

Peterson MS (1990) Hypoxia-induced physiological changes in two mangrove swamp fishes: sheepshead minnow, *Cyprinodon variegatus* Lacepede and sailfin molly, *Poecilia latipinna* (Lesueur). Comp Biochem Physiol A 97:17–21

Pichavant K et al (2000) Effects of hypoxia on growth and metabolism of juvenile turbot. Aquaculture 108:103–114

Radlvic J, Lemos D, Moreira GS (2007) Effect of zinc and benzene on respiration and excretion of mussel larvae (*Perna perna*) (Linnaeus, 1758) (Mollusca; Bivalvia). Braz J Biol 67:111–115

Regnault M (1979) Ammonia excretion of the sand-shrimp *Crangon crangon* (L.) during the moult cycle. J Comp Physiol 133:199–204

Regnault M (1993) Effect of a severe hypoxia on some aspects of nitrogen metabolism in the crab *Cancer pagurus*. Mar Behav Physiol 22:131–140

Regnault M, Aldrich JC (1988) Short-term effect of hypoxia on ammonia excretion and respiration rates in the crab C*arcinus maenas*. Mar Behav Physiol 13:257–271

Roesijadi G, Anderson JW, Petrocelli SR, Giam CS (1976) Osmoregulation of the grass shrimp *Palaemonetes pugio* exposed to polychlorinated biphenyls (PCBs). I. Effect on chloride and osmotic concentrations and chloride-and water-exchange kinetics. Mar Biol 38:343–355

Rosas C, Martinez E, Gaxiola G, Brito R, Sánchez A, Soto LA (1999) The effect of dissolved oxygen and salinity on oxygen consumption, ammonia excretion and osmotic pressure of *Penaeus setiferus* (Linnaeus) juveniles. J Exp Mar Biol Ecol 234:41–57

Shirley MA, McKenney CL (1987) Influence of lindane on survival and osmoregulatory/metabolic responses of the larvae and adults of the estuarine crab *Eurypanopeus depressus*. In: Vernberg WB, Calabrese A, Thurberg FP, Vernberg FJ (eds) Pollution physiology of estuarine organisms. University of South Carolina Press, Columbia, pp 275–297

Silvestre F, Trausch G, Spanò L, Devos P (2002) Effects of atrazine on osmoregulation in the Chinese mitten crab, *Eriocheir sinensis*. Comp Biochem Physiol C 132:385–390

Skaggs HS, Henry RP (2002) Inhibition of carbonic anhydrase in the gills of two euryhaline crabs, *Callinectes sapidus* and *Carcinus maenas*, by heavy metals. Comp Biochem Physiol C 133:605–612

Small D, Calosi P, White D, Spicer JI, Widdicombe S (2010) Impact of medium-term exposure to CO2 enriched seawater on the physiological functions of the velvet swimming crab *Necora puber*. Aquat Biol 10:11–21

Souza-Bastos LR, Freire CA (2011) Osmoregulation of the resident estuarine fish *Atherinella brasiliensis* was still affected by an oil spill (Vicuña tanker, Paranaguá Bay, Brazil), 7 months after the accident. Sci Total Environ 409:1229–1234

Spicer JI, Raffo A, Widdicombe S (2007) Influence of CO2-related seawater acidification on extracellular acid–base balance in the velvet swimming crab *Necora puber*. Mar Biol 151:1117–1125

Spicer JI, Widdicombe S, Needham HR, Berge JA (2011) Impact of CO_2-acidified seawater on the extracellular acid–base balance of the northern sea urchin *Strongylocentrotus dröebachiensis*. J Exp Mar Biol Ecol 407:19–25

Stickle WB, Rice SD, Moles A (1984) Bioenergetics and survival of the marine snail *Thais lima* during long-term oil exposure. Mar Biol 80:281–289

Stumpp M, Trübenbach K, Brennecke D, Hu MY, Melzner F (2012) Resource allocation and extracellular acid–base status in the sea urchin *Strongylocentrotus droebachiensis* in response to CO_2 induced seawater acidification. Aquat Toxicol 110–111:194–207

Sucré E, Vidussi F, Mostajir B, Charmantier G, Lorin-Nebel C (2012) Impact of ultraviolet-B radiation on planktonic fish larvae: alteration of the osmoregulatory function. Aquat Toxicol 109:194–201

Sugatt RH (1980) Effects of sublethal sodium dichromate exposure in freshwater on the salinity tolerance and serum osmolality of juvenile coho salmon, *Oncorhynchus kisutch*, in seawater. Arch Environ Contam Toxicol 9:41–52

Sze PW, Lee SY (2000) Effects of chronic copper exposure on the green mussel *Perna viridis*. Mar Biol 137:379–392

Valarmathi S, Azariah J (2002) Impact of two sublethal concentrations of copper chloride and chlorine on the excretory products of crab *Sesarma quadratum* (Fabricius). Turk J Zool 26(2002):357–361

Vitale AM, Monserrat JM, Castilho PC, Rodríguez EM (1999) Inhibitory effects of cadmium on carbonic anhydrase activity and ionic regulation of the estuarine crab *Chasmagnathus granulate* (Decapoda, Grapsidae). Comp Biochem Physiol C 122:121–129

Vosloo D, Sara J, Vosloo A (2012) Acute responses of brown mussel (*Perna perna*) exposed to sub-lethal copper levels: integration of physiological and cellular responses. Aquat Toxicol 106–107:1–8

Wu JP, Chen H-C (2004) Effects of cadmium and zinc on oxygen consumption, ammonium excretion, and osmoregulation of white shrimp (*Litopenaeus vannamei*). Chemosphere 57:1591–1598

Yu M, Wang S-C, Lu Y (2008) Ammonia excretion and haemolymph nitrogenous contents of mitten crab (*Eriocheir sinensis*) at different environmental pH. Acta Hydrobiol Sinica 32:62–67

Zhen Y, Aili J, Changhai W (2010) Oxygen consumption, ammonia excretion, and filtration rate of the marine bivalve *Mytilus edulis* exposed to methamidophos and omethoate. Mar Freshw Behav Physiol 43:243–255

Zimmer AM, Barcarolli IF, Wood CM, Bianchini A (2012) Waterborne copper exposure inhibits ammonia excretion and branchial carbonic anhydrase activity in euryhaline guppies acclimated to both fresh water and sea water. Aquat Toxicol 122–123:172–180

Part II
Reproduction and Development

Chapter 5
Reproduction

Abstract Reproduction is obviously a very important endpoint, since impaired reproduction can have rapid repercussions at the population level. Life-cycle characteristics of different organisms are a major factor in determining their vulnerability to particular contaminants. There are many ways in which reproduction can be affected, but one of particular concern is by very low levels of some environmental chemicals that can interfere with the endocrine system, termed endocrine disruption. Contaminants can also directly affect gametogenesis, mating, and fertilization. These various stages of the reproductive process are clearly connected to one another.

Keywords Endocrine • Fertilization • Gametogenesis • Mating • Vitellogenin

This chapter and the three to follow cover life cycle functions; separate chapters cover reproduction, embryonic development, larval development, and subsequent developmental processes. The present chapter is subdivided into sections dealing with Endocrine Effects, Gametogenesis and Fecundity, and Mating and Fertilization. All these processes are continuous, and the subdivisions between sections (and chapters) are not totally distinct from each other. It is clear that effects at one stage can produce subsequent effects at later stages.

5.1 Endocrine Effects

The endocrine system regulates all biological processes in the body, including metabolism, development of the nervous system, and the growth and function of the reproductive system. The female ovaries, male testes, and pituitary, thyroid, and adrenal glands are major constituents of the vertebrate endocrine system. These glands produce hormones that circulate in the blood and interact with cells by binding to special proteins called receptors, which are specific for each

J.S. Weis, *Physiological, Developmental and Behavioral Effects of Marine Pollution*,
DOI 10.1007/978-94-007-6949-6_5,

hormone. When enough binding sites are occupied, a message is passed on to the target cell nucleus stimulating genes that result in physiological changes regulating metabolism, development, growth, reproduction etc.

For the past 20 or so years, there has been considerable attention devoted to effects of very low levels of contaminants on the endocrine system; chemicals having such effects (and there are many) have been called “endocrine disruptors.” Some of the concern is because hormones have effects at extremely low concentrations in the body, and endocrine-disrupting chemicals similarly can produce effects at levels far below those that had previously been considered safe. Since hormones are already present in the body in biologically active concentrations, additional exposure to relatively small amounts of hormonally active substances can disrupt the proper functioning of the endocrine system. In some cases, these effects disappear at higher concentrations, being an exception to general dose–response relationships in toxicology. Another cause for concern is that exposures during early life stages (e.g. embryos) can produce delayed effects that become apparent only when the organism is mature and theoretically ready to reproduce.

The bulk of research on endocrine disruption has focused on sex steroids. The two main classes of sex steroids are androgens and estrogens, which include testosterone and estradiol. In general, androgens are considered “male sex hormones” since they have masculinizing effects, while estrogens are considered “female sex hormones” although all types are present in each sex, but at different levels. Some chemicals mimic a natural hormone, fooling the body into over-responding to the stimulus, or responding at inappropriate times. Other endocrine-disrupting chemicals block the effects of a hormone from certain receptors, stimulate or inhibit the endocrine system causing overproduction or underproduction of hormones. Environmental chemicals can be estrogen mimics, anti-estrogens, androgen mimics, and anti-androgens. Endocrine disrupting chemicals may resemble sex steroids structurally. These chemical properties allow them to bind to hormone-specific receptors on the cells of target organs. This binding may result in activating the cell inappropriately or blocking natural hormones from binding. Among xenoestrogens are widely used industrial compounds, such as PCBs, Bisphenol-A, and phthalates. They can cause an estrogen-like response at the wrong time or in the wrong amounts in both sexes, and can enhance female traits in males (feminize). Hormone blockers include drugs like tamoxifen, a specific antiestrogen used to treat breast cancers that need estrogen to grow. DDE, a breakdown product DDT, blocks androgen receptors so testosterone cannot bind.

5.1.1 Crustaceans

In crustaceans ecdysteroids and terpenoids play major roles in regulating development, growth, maturation, and reproduction. Laboratory studies have demonstrated the susceptibility of crustaceans to endocrine disruptors, and studies have shown endocrine disruption in field populations, though the causal link between abnormalities and particular environmental chemicals is often lacking. Indicators of reproductive endocrine disruption (e.g., intersexuality) allow investigation of the degree to which endocrine disruptors are affecting populations (LeBlanc 2007), yet there are few studies that examine gonadal development, intersex or other direct reproductive endocrine effects. Ecdysteroids regulate aspects of embryo development, molting, and reproduction, so chemicals that interfere with ecdysteroid signaling can produce effects. Chemicals with anti-ecdysteroidal activity can function as ecdysteroid synthesis inhibitors or ecdysteroid receptor antagonists. Such chemicals include many of the classic estrogen receptor agonists (e.g. bisphenol a, DDT metabolites, nonylphenol). Many of these endpoints are appropriate to chapters on embryo and larval development, but will be described here. Alterations in molt frequency can indicate disruption of ecdysteroid signaling, but effects of chemicals on molting will be discussed in the chapter that covers growth (Chap. 8).

Pesticides and Contaminants of Emerging Concern

Methyl farnesoate is a major terpenoid hormone of crustaceans. Many laboratory studies have been performed with insect growth- regulating insecticides (McKenney 2005) which function as methyl farnesoate mimics. Metamorphic success of decapods is among the most sensitive endpoints affected by insect growth regulators. Delayed metamorphosis or metamorphic abnormalities caused by exposure to insect growth regulators have been reported in shrimp, crabs, and lobsters (Christiansen et al. 1977a, b; McKenney and Celestial 1993; Celestial and McKenney 1994; Cripe et al. 2003). These will be discussed in greater detail in Chap. 7, on larval development. Estrogens themselves (17b-estradiol, diethylstilbestrol, 17a-ethinyl estradiol, estrone) can elicit effects on crustaceans including altered gonadal development in amphipods (Segner et al. 2003), and reduced vitellin levels in mysids (Ghekiere et al. 2006).

Metals

There has been very little work on endocrine-disrupting effects of metals on crustacean reproduction. Tributyltin exposure of hermit crabs, *Clibanarius vittatus* females caused disorganization and atrophy of the ovaries, which would impair reproduction (Sant'Anna et al. 2012). This chemical has profound endocrine disrupting effects on females of many groups of organisms, described below for mollusks and fishes.

Polluted Environment

There have been reports of intersex individuals of many species, but they have not been generally correlated with particular pollutants. Copepods *Paramphiascella* *hyperborean, Stenhelia gibba,* and *Halectinosoma* sp. collected near sewage outfalls had elevated incidence of intersex (Moore and Stevenson 1991, 1994). This similar effect in different species in the same vicinity suggests an environmental cause, but no clear relationship could be established between sewage effluent and incidence of intersex (Moore and Stevenson 1994).

5.1.2 Mollusks

Metals

TBT

One of the earliest reported examples of endocrine disruption found in the field was a condition called *imposex* in female gastropods exposed to very low levels of tributyltin (TBT), a commonly used constituent of antifouling paints on vessels until the 1980s, when it was restricted or banned in many countries. Affected female snails developed male structures including a penis and vas deferens, which could block the passage of eggs down the oviduct preventing egg deposition and causing reproductive failure. Many dogwhelk, *Nucella lapillus,* populations had females in which vas deferens overgrew the female opening, rendering the female sterile (Gibbs and Bryan 1986); this was associated with population declines around Southwest England (Bryan et al. 1986). Population recovery occurred only after restrictions on the use of TBT came into effect. While some species recovered quickly after TBT use was restricted, in *Nassarius reticulatus*, tissue levels of TBT in polluted sites dropped by 5–10 times between 1987 and 1993, but the rate of imposex declined very slowly; this was attributed to longevity of the snails and limited recruitment of less-affected females (Bryan et al. 1993). Even in 2011, however, Qiu et al. found that imposex remained severe in *Thais clavigera* from Victoria Harbour and other sites with extensive shipping activities. Imposex severity (measured by relative penis length in females) was correlated with tissue concentrations of TBT. High levels of imposex were also found in *Thais biserialis*, *T. brevidentata*, and *T. kiosquiformis* in many coastal sites in Ecuador (Castro et al. 2012). Butyltin compounds (TBT, dibutylin -DBT, and monobutylin -MBT) were found in sediments. TBT does degrade over time, but not as rapidly as was originally thought. Although BT degradation suggested an older input of TBT, the high imposex levels suggest that restrictions on TBT were still not effective in Ecuador.

Abidli et al. (2011) studied testosterone and estradiol in two gastropods, *Hexaplex trunculus* and *Bolinus brandaris*, to clarify the impact of TBT on free and esterified steroids. Two months exposure to 50 ng l^{-1} induced imposex. Testosterone and estradiol were present in free and esterified forms in the digestive gland-gonad complex. In female *B. brandaris*, 50 ng TBT l^{-1} elevated free testosterone and decreased the esterified form. However, in female *H. trunculus*, TBT elevated both the free and esterified form of testosterone.

5.1.3 Fishes

Fishes have considerable sexual plasticity. Although the sexes are usually separate, there are some functional hermaphrodites (e.g., some Serranidae and Sparidae). In some species, individuals can change functional sex in response to social and environmental cues. There are examples of both protandrous (male first) and protogynous (female first) groups, e.g., bluehead wrasses and clownfish, respectively, but increasing numbers of species are being found to have this ability. It has become clear that this natural hormonal balance may be disturbed by chemicals with hormone-like activities. Fish are particularly vulnerable to potential endocrine-disrupting chemicals (EDCs) in surface waters.

The egg protein, vitellogenin (VTG), is produced in the female's liver and transported to the ovaries, where it is added to egg yolk prior to ovulation. Synthesis of this protein is regulated by estrogens and thus can serve as a marker of exposure to environmental estrogens. VTG induction in male fish (which have very low endogenous estrogens, but whose livers nevertheless are able to synthesize VTG in response to exogenous ones) is an effect of estrogenic contamination; numerous studies have measured this biomarker in field populations. VTG induction in males is an excellent biomarker of exposure to estrogens acting via hepatic estrogen receptors. Other commonly observed responses are intersex individuals and altered sex ratios. Biochemical changes in sex hormones or enzymes involved in their synthesis are also frequently studied. Numerous bioassays have been developed using steroid hormones and VTG as end points (MacLatchy et al. 2003).

An analogous biomarker to VTG that is sensitive to androgens has been developed in the three-spine stickleback (*Gasterosteus aculeatus*) (Katsiadaki et al. 2002). This is the protein *spiggin*, which is produced in the male kidney and secreted for use as a glue during nest construction. Spiggin is produced in females only after exposure to exogenous androgens.

Metals

Thomas (1989) examined effects of Cd on the reproductive endocrine function in female Atlantic croaker (*Micropogonias undulatus*) Fish were exposed to 1 mg l^{-1} Cd for 40 days during the period of ovarian recrudescence. Exposure accelerated ovarian growth and elevated plasma estradiol concentrations, suggesting a stimulation of vitellogenesis. He also observed an increase in the spontaneous secretion of gonadotropin (GTH) from pituitaries of Cd-exposed fish *in vitro*. Depew et al. (2012) reviewed effects of dietary mercury on fish and found that adverse effects on behavior had a wide range of effective dietary concentrations, but generally occurred above 0.5 $\mu g\ g^{-1}$ wet weight. In contrast, effects on reproduction (generally endocrine effects) occurred at dietary concentrations that were much lower ($<0.2\ \mu g\ g^{-1}$ wet wt). Tributyltin (TBT) produces masculinization in fish, reminiscent of effects in invertebrates. Genetically female Japanese flounder

(*Paralichthys olivaceus*) were fed an artificial diet containing tributyltin oxide (TBTO) at concentrations of 0.1 and 1.0 μg/g diet from 35 to 100 days after hatching, which includes the sex differentiation period (Shimasaki et al. 2003). The ratio of sex-reversed males increased to 25.7 % in flounder fed the 0.1 μg/g diet and to 31.1 % in those fed the 1.0 μg/g diet compared with the control (2.2 %). TBT's breakdown product, dibutyltin (DBT), also can act as a masculinizing agent in fish (McGuiness et al. 2012). Zhang et al. (2013) investigated effects of TBT on ovarian lipid accumulation and testosterone esterification in rockfish (*Sebastiscus marmoratus*). After exposure to TBT (0, 1, 10 or 100 ng l^{-1} as Sn) for 48 days, there was delay in oogenesis, a decrease of neutral lipid droplets in the ooplasm of ovaries. Exposure also induced an increase of interstitial ectopic lipid accumulation and total lipids in ovaries. A decrease of serum T3 and T4 (triiodothyronine and thyroxine) concentrations (at 10 and 100 ng l^{-1}) was a possible cause for the lipid responses. In addition, the percentage of testosterone in esterified form was decreased in the ovaries by TBT, which might be a mechanism by which free testosterone levels increased. The accumulation of ectopic lipids and increase of free testosterone in ovaries could impact ovarian functions and oocyte development.

Organics

Chlorinated Chemicals

PCBs and dioxins have frequently been associated with endocrine disruption. Thomas (1989) examined effects of the PCB mixture Aroclor 1254 on the reproductive endocrine function in female Atlantic croaker (*M. undulatus*). Fish were fed PCBs in the diet (0.5 mg/100 g body wt/day) during the period of ovarian recrudescence, which impaired ovarian growth and decreased plasma estradiol. Pituitaries from treated fish decreased their spontaneous secretion of gonadotropin *in vitro.* Loomis and Thomas (1999) identified an estrogen receptor in the testis in Atlantic croaker. Xenoestrogens, including DDT, chlordecone (Kepone), nonylphenol, and PCBs, bound to this receptor with relatively low binding affinities, 10^{-3} to 10^{-5} that of estradiol. Khan and Thomas (1992, 1997, 2001, 2006) accumulated evidence of the involvement of PCBs in disruption of the serotoninergic systems in fish brains that regulate reproductive hormones. In male *M. undulatus*, exposure to Aroclor 1254 during gonadal recrudescence caused a significant decline in 5-HT (serotonin) and DA (dopamine) and an increase in their metabolites (Khan and Thomas 1997). The reduction in 5-HT led to an inhibition of luteinizing hormone (LH) secretion and an absence of gonadal growth, since 5-HT stimulates LH secretion in this species (Khan and Thomas 1992, 1997; Khan et al. 2001).

Dioxins, furans and dioxin-like polychlorinated biphenyls (PCBs) were analyzed in muscle of yellow phase European eel (*Anguilla anguilla*) from 38 sites (Geeraerts et al. 2011). In most sites, eels had levels considered detrimental for reproduction; these chemicals were suggested as factors contributing to the population decline of this species.

Oil and PAHs

Oil has major impacts on fish embryos that may be manifested in adult stages as endocrine disruption. Pink salmon that had been exposed as embryos to *Exxon Valdez* oil and survived to migrate to the ocean, returned from the sea at only half the rate of control fish (Heintz et al. 2000). These returning adults showed reproductive impairment and their embryos had reduced survival. Thus, the second generation was affected by the sublethal exposures their parents had had as embryos and fry (Peterson et al. 2003).

Specific PAHs may act as endocrine disruptors. Dong et al. (2008) hypothesized that altered expression of genes for the P450 enzyme aromatase could be responsible for reproductive dysfunction caused by Benzo(a)Pyrene (BaP). Aromatase is involved in steroid balance by converting androgens into estrogens. CYP19A1 expression decreased after BaP exposure in 3-month-old *Fundulus* immature oocytes, but BaP did not affect its expression in adult oocytes. In embryo brains, BaP significantly decreased CYP19A2, and in adults, CYP19A2 expression was decreased in the pituitary and hypothalamus. The study gives insights into molecular mechanisms of action of BaP.

Following the *ExxonValdez* oil spill in 1989, Sol et al. (2000) studied the effect of oil on reproductive parameters in wild populations of female dolly varden, yellowfin sole, and pollock. Exposure to oil was the highest in the first year and decreased in subsequent years of sampling. A higher proportion of dolly varden sampled in 1989 had depressed plasma estradiol-17β compared to the fish in 1990.

Contaminants of Emerging Concern

Some studies have exposed animals to hormones themselves instead of hormone mimics, often ethynylestradiol (EE_2), since this estrogen (used in birth control pills) is released from sewage treatment plants. In some cases, EE_2 has been used to develop a bioassay for contaminants with estrogenic effects. *F. heteroclitus* exposed to 17α-ethynylestradiol showed decreased plasma reproductive steroid levels, decreased gonadal steroid production, increased plasma VTG, decreased fecundity and impaired fertilization. In exposed males, testosterone production decreased, indicating effects on the steroidogenic pathway. Hepatic transcript levels of estrogen receptor alpha (ERα) and VTG increased in treated males, an estrogenic response (Hogan et al. 2010). Recrudescing *F. heteroclitus* were exposed to EE_2 for 7–15 days (MacLatchy et al. 2003). At high EE_2 (>250 ng l^{-1}), males had depressed androgen synthesis and plasma steroid levels and females had depressed gonadal production and circulating E_2; however, <100 ng l^{-1} EE_2 increased gonadal production and plasma E_2. Male and female plasma VTG responded in a concentration-dependent fashion, with the low effect concentration being 1 ng l^{-1}.

Loomis and Thomas (2000) studied short-term effects of estrogens and xenoestrogens on androgen production by testicular tissue from the Atlantic croaker (*M. undulatus*). Incubation of testicular tissue with estradiol (37 nM to 37 μM) decreased gonadotropin-stimulated 11-ketotestosterone (11-KT) production. The effect was specific for estrogens; progesterone, cortisol, or the synthetic androgen mibolerone did not alter 11-KT production at similar concentrations. Diethylstilbestrol, the antiestrogen ICI 182,780, and several xenoestrogens including Kepone (chlordecone), 4-nonylphenol, and a hydroxylated PCB metabolite also decreased gonadotropin-stimulated 11-KT production. The action of estradiol was rapid (<5 min) and was not blocked by actinomycin D or cycloheximide (inhibitors of transcription and translation, respectively) demonstrating that estrogens (and also probably xenoestrogens) act on the cell surface via a nongenomic mechanism to alter androgen production. However, genes normally induced by estradiol (E_2) in female fish, those for VTG and zona radiata proteins, are inducible in males exposed to estrogenic chemicals. Male sheepshead minnows (*Cyprinodon variegatus*) were exposed to both E_2 and para-nonylphenol (NP), to determine a dose–response (Knoebl et al. 2004). Quantitative real time PCR measured mRNA for the genes. Both E_2 and NP elicited a dose-related increase in all of the mRNAs tested. Hogan et al. (2008) also examined genetic responses as well as hormonal ones. To determine the sensitivity of genes to induction by hormones, male and female three-spine sticklebacks (*G. aculeatus*) were exposed to 1, 10 and 100 ng l^{-1} of methyltestosterone (MT) or estradiol (E2). Spiggin induction in females, and VTG induction in males were both detectable at 10 ng l^{-1} of either hormone. Gonadal steroid hormone production was measured in exposed fish to compare gene expression endpoints to an endpoint of hormonal reproductive alteration. Reduction in testosterone production in ovaries at all three MT exposure concentrations, and ovarian estradiol synthesis at the 100 ng l^{-1} exposure were observed *in vitro* for both hormone exposures.

Pharmaceuticals that are not designed for reproductive functions may also interfere with androgen synthesis. The *in vitro* interference of fibrate (gemfibrozil, clofibrate, clofibric acid), anti-inflammatory (ibuprofen, diclofenac), and anti-depressive (fluoxetine, fluvoxamine) drugs with key enzymes – C17,20-lyase and CYP11β – that are involved in androgen synthesis in gonads of male fish were investigated by Fernandes et al. (2011). Fluvoxamine and fluoxetine were the strongest inhibitors of C17,20-lyase and CYP11β enzymes at concentrations of 321–335 and 244–550 μM, respectively.

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) and the active ingredient of Prozac. Usually detected <1 μg l^{-1}, fluoxetine and its metabolite norfluoxetine bioaccumulate in fish, particularly in the brain. In the Atlantic croaker *Micropogonias undulatus*, serotonin is involved in the neuroendocrine stimulation of reproduction by increasing LH release (Khan and Thomas 1992, 1994).

EE_2 exposure of juveniles can have delayed effects. Maunder et al. (2007) exposed juvenile sticklebacks to 1.75 and 27.7 ng l^{-1} EE_2 for 4 weeks post-hatch and reared them in clean water until they matured. Exposure to the higher concentration caused the occurrence of ovotestis in males, which had less intense nuptial coloration, built fewer nests, in which fewer eggs were deposited. The group exposed to 1.75 ng l^{-1} also built significantly fewer nests than controls.

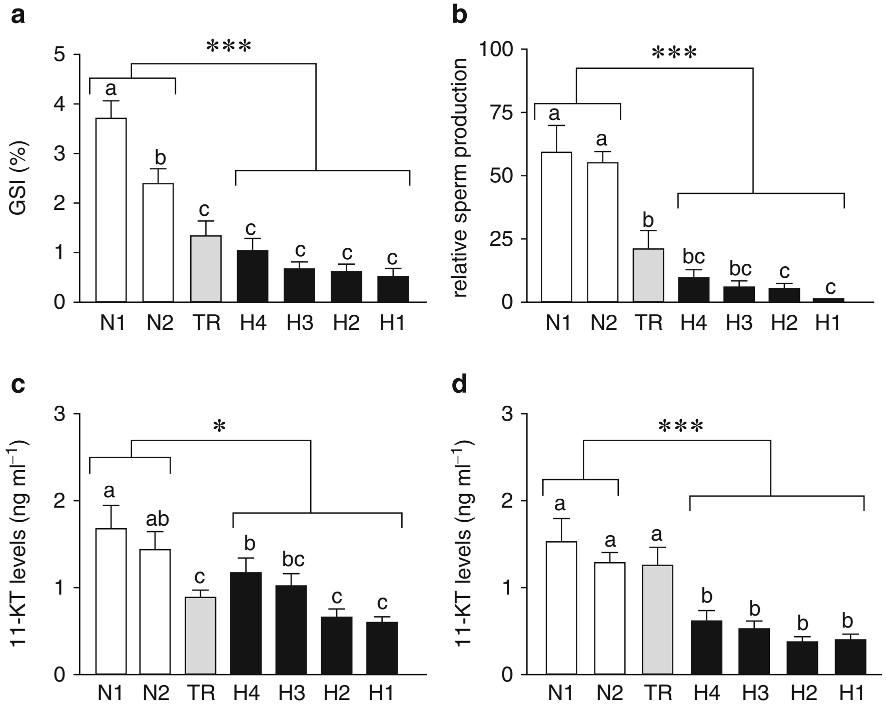


Fig. 5.1 Testicular development and endocrine function in male croaker collected from normoxic (N1, N2) and hypoxic (H 1,2,3,4) sites. (**a**) GSI (testicular growth). (**b**) Relative sperm production. (**c**, **d**) Plasma 11-KT levels. * = significant differences between normoxic and hypoxic sites. *** = highly significant differences between normoxic and hypoxic sites. Individual site differences indicated with different letters ($p < 0.05$) (Reprinted from Thomas et al. 2007: 2696, courtesy The Royal Society)

Hypoxia

It is interesting that exposure to low DO can also trigger reproductive endocrine disruption. Chronic environmental exposure of Atlantic croaker (*M. undulatus*) to hypoxia suppressed ovarian and testicular growth, associated with impairment of reproductive neuroendocrine function and decreases in hypothalamic serotonin (5-HT) and activity of the 5-HT biosynthetic enzyme, tryptophan hydroxylase (Thomas et al. 2007) (Fig. 5.1). Pharmacological restoration of hypothalamic 5-HT levels restored neuroendocrine function, indicating that the serotonergic neuroendocrine pathway is a major site of hypoxia-induced reproductive disruption.

Thomas et al. (2006) studied effects of 10 week exposures to low DO (2.7 and 1.7 mg l^{-1}) on reproductive responses in female *M. undulatus* in the laboratory, and in fish from hypoxic sites. Exposure to moderate hypoxia during ovarian recrudescence, both in the laboratory and field, impaired ovarian growth and decreased production of mature oocytes, associated with decreases in the estrogen signaling pathway for production of VTG. The results indicated that endocrine and morphological biomarkers are sensitive to moderate hypoxia, and are early warning indicators of reproductive failure. Sustained diel exposure to hypoxia was associated with smaller gonadosomatic index (GSI, gonad mass/body mass) and lower sex steroid concentrations in wild Gulf killifish (*F. grandis)* (Cheek et al. 2009). Testes and ovaries were significantly smaller under both moderate (2.61 mg l^{-1}, 0.6 h day^{-1}) and severe (0.93 mg l^{-1}, 3.4 h day^{-1}) diel hypoxia. Male 11-KT concentrations were lower under moderate hypoxia, while both testosterone (T) and 11-KT were significantly reduced under severe diel hypoxia, which may affect reproduction by inhibiting steroidogenic enzymes in the gonad. Reproductive success, growth, and physiological status under longer hypoxic episodes (5 h daily for 30 days) were examined by Cheek (2011). Growth, GSI, steroid hormone levels, and fertilization rate were unaltered by exposure to diel hypoxia, but at sites with diel hypoxia egg production was 50–85 % lower than at sites in the same estuary without daily hypoxia.

Climate Change

Susceptibility of *F. heteroclitus* to EE2 exposure, as indicated by increases in VTG gene expression changed with temperature. Liver *vtg1* mRNA was induced in males exposed to EE2. Males acclimated to 26 °C and exposed to 250 ng l^{-1} EE2 produced 3.5-fold more *vtg1* mRNA than EE2-exposed males acclimated to 10 °C, suggesting that they are more susceptible to EE2 under temperature increases that are expected with warming of coastal waters (Chandra et al. 2012).

Polluted Sites

Animals in polluted estuaries are exposed to complex mixtures of xenobiotics which can alter normal reproduction. Many effects have been reported in flatfish, which spend their lives in close contact with contaminated sediments. Lye et al. (1998) reported VTG induction and testicular abnormalities in male flounder (*Platichthys flesus*) from near a sewage discharge in NE England. More detailed surveys (Matthiessen et al. 2002) showed that VTG induction is widespread in the males of this species. It is worth noting that reduced VTG has occasionally been reported in females (e.g. Casillas et al. 1991) from contaminated sites, which might be attributable to antiestrogen or androgen exposure or to generalized stress, causing lower VTG synthesis, which could reduce normal egg development. Female sole from contaminated sites also have lower estrogen levels and inhibited ovarian development (Johnson et al. 1988). Significant levels of VTG were found in male English sole from several urban sites, with especially high numbers in Elliott Bay, along the Seattle Waterfront (Johnson et al. 2008). At the sites with male VTG production, the timing of spawning appeared altered.

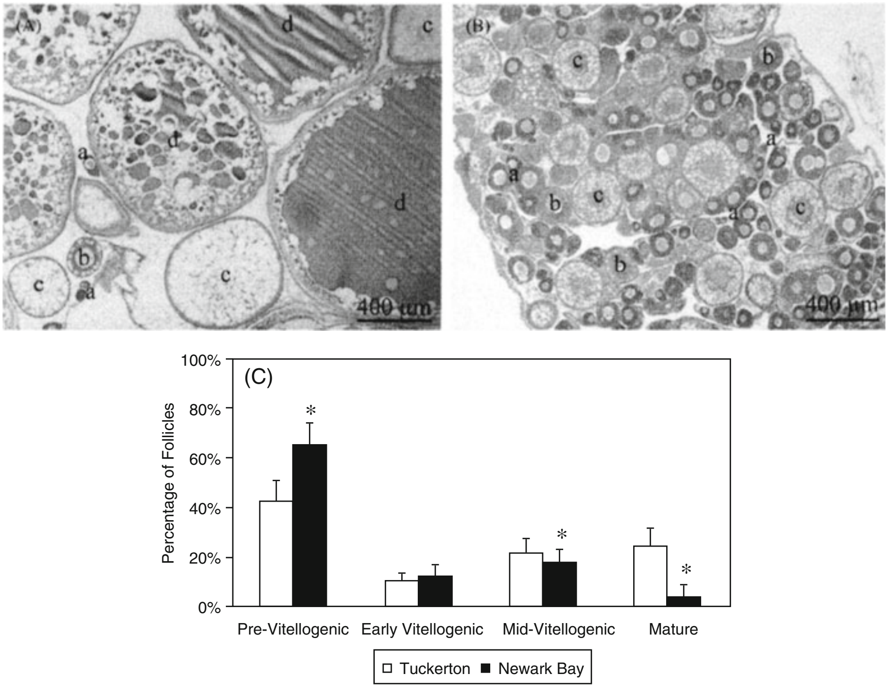


Fig. 5.2 Representative photomicrographs of (**a**) Tuckerton (reference site) and (**b**) Newark Bay ovaries. Previtellogenic (*a*), early vitellogenic (*b*), mid-vitellogenic (*c*) and mature follicles (*d*) are shown. (**c**) Ovarian follicle developmental stages. Mean ± SD. * = significantly different from Tuckerton, $p < 0.05$ (Reprinted from Bugel et al. 2010: 188, courtesy Elsevier Publishing Co)

Experimental exposure to pulp-mill effluent depressed testosterone in *F. heteroclitus* (Dubé and MacLatchy 2000), possibly via action on pituitary GTH secretion. Bugel et al. (2009) found impaired reproductive health in both sexes of *F. heteroclitus* in industrialized Newark Bay (NB), New Jersey. Males had decreased gonad weight, altered testis development and decreased gonadal aromatase mRNA expression; females had decreased gonad weight, inhibited gonadal development, decreased hepatic VTG production, and increased mRNA expression of gonadal aromatase, as well as fewer mature follicles (Fig. 5.2). NB fish also had reduced fecundity and lower hatching success, as well as lower mass and yolk-volume of eggs. Circulating 17β-estradiol levels in NB females were eight-fold lower than females from the reference site (Bugel et al. 2011).

Sewage plant effluent with anti-androgenic activity affected reproductive physiology and behavior of three-spined sticklebacks (*G. aculeatus*) exposed for 21 days. Levels of spiggin and VTG were unaffected, but male reproductive behavior was impaired (Sebire et al. 2011). Males in full strength effluent built fewer nests, and courtship behavior was reduced in 50 and 100 % effluent treatments. This is another example of behavior being more sensitive than the biochemical biomarkers. Wild gudgeon were collected upstream and downstream of urban and pharmaceutical manufacture effluents by Sanchez et al. (2011). Fish downstream of the effluent exhibited endocrine disruption including VTG induction, intersex and male-biased sex-ratio. These effects were associated with reduced population density.

5.1.4 Other Taxa

Polychaetes

Lewis and Watson (2012) have encouraged increased focus on reproductive endpoints in polychaetes. They suggest the following be investigated: (1) reproductive endpoints for the traditional ‘model’ species and those that have different reproductive traits to ensure broad ecological relevance; (2) Nereids and *Arenicola marina* be used to investigate the interaction of pollutants with the endocrine/ environmental control of reproduction; (3) Use of polychaetes to assess male ecotoxicity effects; and (4) Assess emerging pollutants with reproductive endpoints. Long-term exposure to Cu-spiked sediment had deleterious effects on sperm and egg production in *N. virens* (Watson et al. 2013). Differences in the number of normal embryos produced by eggs fertilized with sperm from exposed males showed that sperm were more susceptible to toxicity, although eggs were also affected at higher concentrations.

Marine Mammals

Marine mammals are frequently top carnivores, and as such accumulate very high concentrations of chlorinated chemicals that can be endocrine disruptors. Although experimental studies are rare, there have been many correlative studies of which a few are presented here. Relationships between organochlorine compounds such as PCBs, DDTs, hexachlorobenzene, and oxychlordane, and hormones in Arctic mammals imply that these chemicals pose a threat to endocrine systems of these animals. The most pronounced relationships were reported with the thyroid hormone system, but effects are also seen in sex steroid hormones (Jenssen 2006). Pseudo-hermaphroditism in polar bears is thought to be an effect of EDCs. Over the past several decades, female polar bears at Svalbard, Norway have been reported with both female and male genitals (Wiig et al. 1998). The pseudo-hermaphrodites were genetically females but also had small penises. California sea lions had premature births, associated with accumulation of organochlorines (DeLong et al. 1973). Harbor seals from the Dutch Wadden Sea had low reproductive success and declining population numbers that were attributed to the impact of PCBs. Experimental studies showed that female harbor seals fed fish from the polluted Wadden Sea had a lower reproductive success (50 %) than seals fed less-contaminated fish. Implantation failure was found to be associated with reduced levels of 17β-estradiol (Reijnders 1986) induced by EDCs. Increasing levels of PCBs and DDE in the blubber of Dall's porpoises were found to have a negative association with testosterone in blood, which decreased in a statistically significant way with increasing DDE concentrations (Subramanian et al. 1987). These results collectively suggest that current levels of persistent organochlorines are causing an imbalance in sex hormones and subsequent reproductive abnormalities in marine mammals. Recent reduction of chlorinated organic chemical levels was correlated with improved reproductive status (Roos et al. 2012). In female sea otters, reproduction increased after 1990. In grey seals, pregnancy rate increased 1990–2010 and uterine obstructions ceased after 1993. The frequency of uterine tumors was highest 1980–2000. Organochlorine concentrations decreased at annual rates of between 3.5 and 10.2 %. The estimated mean concentration (mg/kg) for total-PCB decreased from 70 to 8 (otters), and from 110 to 15 (seals). The corresponding concentrations for ΣDDT decreased from 3.4 to 0.2 (otters), and from 192 to 2.8 (seals).

Corals

The scleractinian coral, *Oculina patagonica*, inhabiting contaminated vs. uncontaminated reference sites in the Mediterranean was investigated by Armoza-Zvuloni et al. (2012), who found significantly higher steroid levels in water and coral tissue from contaminated sites, suggesting that corals accumulate steroids from the surrounding waters. Despite their higher steroid levels, corals from the contaminated sites showed reproductive potential comparable to those of the reference sites.

5.2 Gametogenesis and Fecundity

Gametogenesis is the production of gametes. Spermatogenesis, which takes place in the testes, is the process by which male primordial germ cells called spermatogonia undergo meiosis, halving their number of chromosomes. The initial cells in this pathway are primary spermatocytes, which divide into two secondary spermatocytes, each of which divides into two spermatids. Thus, each primary spermatocyte gives rise four spermatids. These undergo development (spermiogenesis) into mature spermatozoa (sperm cells) under the influence of testosterone, by growing a tail (flagellum), and developing a thickened mid-piece, where mitochondria concentrate. Spermiogenesis also involves nuclear condensation, formation of the acrosomal cap from the Golgi apparatus, and removal of unnecessary organelles.

Oogenesis is the comparable process in females, Primary oocytes enlarge and begin to undergo meiosis. The primary oocyte is a very large cell containing many nutrients that will be important for the early embryo. It undergoes the first meiotic division, producing a secondary oocyte and another small cell called the first polar body. During cell division, most of the cytoplasm of the primary oocyte moves to the secondary oocyte. The first polar body may undergo a second meiotic division and its daughter cells degenerate. The secondary oocyte undergoes a second division, producing another polar body and one final ovum. The asymmetric division insures that the ovum retains most of the yolk. The mechanisms of oogenesis vary more between species than for spermatogenesis. For example, in most mammals only a few eggs are produced during an individual's lifetime whereas in other species such as fishes or sea urchins, thousands of eggs can be produced routinely. In the species that produce thousands of eggs, oogonia are stem cells that proliferate throughout the lifespan of the organism. In species that produce fewer eggs, the oogonia divide to form a limited number of egg precursor cells.

As animals approach the mating season, the gonads grow relative to the rest of the body. A measurement of the relative size of gonads is the gonosomatic index (GSI), a reduction of which is a frequently measured response to contaminants.

Observations of endocrine effects lead to the question of whether marine organisms that have experienced disturbances including estrogen alteration, VTG induction, perturbed steroid levels, intersex, etc. have been reproductively compromised, and whether populations are potentially at risk. It is also possible that toxicants can exert direct effects on developing gametes directly, without involving hormones.

5.2.1 Crustaceans

Metals

Hg and Cd, especially when acquired through food, produced decreases in ovarian development, egg production, yolk content of eggs, and hatching rates in the copepods *Acartia hudsonica* and *A. tonsa*. Exposure to dissolved Cd had no effect, but dissolved Hg did affect egg production (Fig. 5.3) (Hook and Fisher (2001). Different exposure routes produced different metal distributions: after water exposures most accumulation was in the exoskeleton, while dietary exposures caused most accumulation in internal organs, which is more likely to produce toxicity. Decreased reproduction was seen at metal concentrations only slightly higher than levels in coastal waters.

Effects of elevated Cu on egg production in the amphipod *Corophium volutator* were studied by Eriksson and Weeks (1994). The amphipods were exposed for 14 days to <0.1, 50 and 100 μg Cu l^{-1} resulting in increased total body Cu and reduced egg production. The effects of TBT on reproduction are not restricted to mollusks. While male hermit crabs *Clibanarius vittatus* exposed to TBT in the laboratory for 9 months showed no effects, exposed females displayed disorganization and atrophy of their ovaries, thus affecting reproduction (Sant'Anna et al. 2012). The amphipod, *Caprella danilevskii*, was exposed to TBT over a generation. Marked delays in growth and molting during the early developmental stages and maturation occurred at 100 and 1,000 ng l^{-1}. Inhibition of maturation and reproduction such as a decrease in the number of juveniles hatched was apparent in 10 and 100 ng l^{-1} (Ohji et al. 2003).

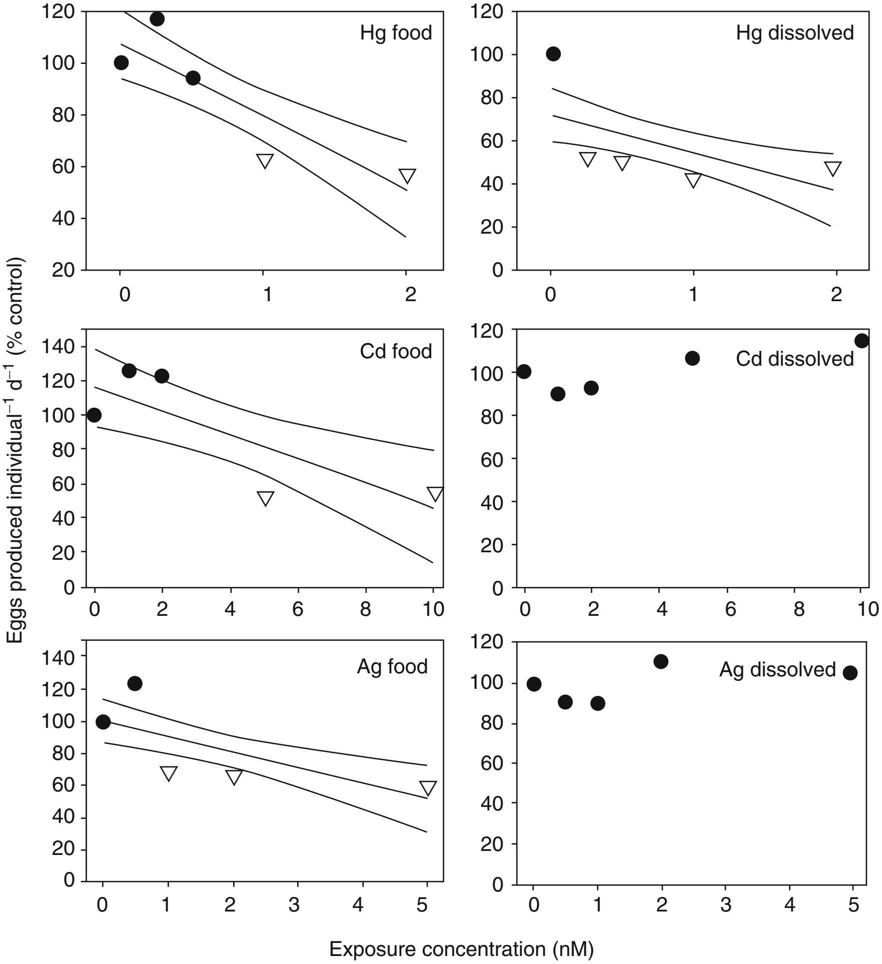


Fig. 5.3 Egg production individual^{-1} day^{-1} as % control in *Acartia tonsa* and *A. hudsonia* following exposure to dietary (*left*) or dissolved (*right*) Ag, Hg, and Cd. Egg production significantly lower than controls when food exposed to 1 nM Hg or 5 nM Cd (*open triangles*). Egg production lower than controls at 0.25 nM dissolved Hg. *All lines* shown are significant ($p < 0.05$) (Reprinted from Hook and Fisher 2001: 1134, courtesy of Springer Publishing Co)

Organics

Male grass shrimp (*P. pugio*) exposed to 63 μg l^{-1} pyrene had delayed molting and time until reproduction, as well as elevated ethoxycoumarin *o*-deethylase (ECOD) activity. Pyrene did not affect females, but their offspring had elevated mortality (Oberdörster et al. 2000). The authors hypothesized that vitellin binds pyrene, making it unavailable to adult females, resulting in maternal transfer of pyrene to the embryos. This would account for the lack of effect on females, and reduced survival of their offspring.

Jensen and Carroll (2010) examined reproduction of *Calanus* spp. exposed to the WSF of crude oil. While egg production in *C. glacialis* exposed to 10.4 and 3.6 μg l^{-1} was unaffected, after eggs were transferred to clean seawater, hatching success was significantly lower in the high dose group. Exposure of *Tigriopus japonicus* to PCB 126 (3,3′,4,4′,5 pentachlorophenol) resulted in increased sensitivity in successive generations of this copepod. While body size was most sensitive, reproduction and intrinsic population growth were reduced at 1 μgl^{-1} in the F1 generation (Guo et al. 2012).

Acidification and Climate Change

Fitzer et al. (2012) determined reproductive response (naupliar production and growth) of the copepod *Tisbe battagliai* over three generations at pH 7.67, 7.82, 7.95, and 8.06. Naupliar production increased at pH 7.95, followed by a decline at pH 7.82, the increase at 7.95 attributed to hormesis. A multi-generational model predicted a gradual decline in naupliar production and growth over the next 100 years. Effects of seawater pH levels (8.2, 7.6 and 6.9) on the reproduction of *Calanus glacialis*, an Arctic copepod, were examined (Weydmann et al. 2012). Low pH did not affect egg production, but pH 6.9 delayed hatching and reduced hatching success. The results indicate that copepods are not very susceptible to acidification. However, studies have been over short periods and have only considered impacts of elevated CO_2. Authors encouraged long-term exposures examining synergistic effects of acidification and warming. Bergey and Weis (2008) observed a much longer breeding season for fiddler crabs (*Uca pugnax*) in New Jersey (US) compared to what had been reported in the 1970s. It was suggested that climate change might have been responsible for the lengthened breeding season.

Hypoxia

Wiklund and Sundelin (2001) investigated effects of hypoxia on reproductive variables in the amphipods *Monoporeia affinis* and *Pontoporeia femorata* including unfertilized/ undeveloped eggs, dead eggs and females carrying a dead brood. Low oxygen (2–6 mg O_2 l^{-1}), resulted in more females carrying dead broods; females exposed to hypoxia had a lower fertility rate than controls. Similarly, the amphipod *Melita longidactyla* was impaired by moderate DO levels (3.5–4.5 mg O_2 l^{-1}), higher than levels considered hypoxic (2.8 mg O_2 l^{-1}). Negative growth and decreases in respiratory energy expenditure were noted after exposure to moderately low DO for 3 weeks. Complete reproductive failure occurred after exposure to 3.5 mg O_2 l^{-1} for 1 month, but no significant effect on percentage copulation, number of broods and offspring or fecundity was seen at 4.5 mg O_2 l^{-1}, indicating that reproductive impairment occurs below 4.5 mg O_2 l^{-1} (Wu and Or 2005). In contrast, Brouwer et al. (2007) found that chronic hypoxia appeared to enhance grass shrimp (*P. pugio*) reproduction. Females exposed to 2.5 mg O_2 l^{-1} had higher fecundity, and a greater percentage produced repeated broods than normoxic shrimp. The hypoxic shrimp took longer to produce their first brood than controls, but starved larvae from hypoxia-exposed mothers lived longer than those from controls. Shrimp exposed to severe hypoxia (1.5 mg O_2 l^{-1}) also had higher fecundity than controls, although embryos from hypoxia-exposed mothers took longer to hatch than control embryos. This species lives in eutrophic estuaries and seems to be quite resilient to hypoxia.

Polluted Sites

Egg membranes of the mole crab *Emerita analoga* near the San Onofre nuclear plant ruptured soon after egg extrusion (Siegel and Wenner 1984). These females had a smaller size at the onset of egg production and a later onset of reproduction than in areas north or south. This may have been due to a failure of overwintering (Wenner 1988). When crabs from the affected area were brought into the laboratory they extruded eggs that developed normally, but their molt rate and molt increment were depressed.

Exposure of copepods, *Tigriopus californicus*, to contaminated sediments from Puget Sound resulted in delays in the period of peak reproduction and reductions in the total number of nauplii produced by each female (Misitano and Schiewe 1990).

5.2.2 Mollusks

Metals

The giant sea scallop, *Placopecten magellanicus* was exposed to Cu or Cd at 20 μg l^{-1} for 7 weeks. In scallops undergoing early gametogenesis, Cd promoted early gamete maturation, while Cu inhibited it (Gould et al. 1985). In scallops with fully differentiated gonads, however, Cu induced gonad regression. Gonads accumulated high levels of the metals. *Mya arenaria* were collected at different sites along the St. Lawrence estuary. Near an active harbor, clams had high levels of TBT and DBT in gonads, along with a lower gonadosomatic index, low progesterone levels and delayed sexual maturation compared to the reference site. Sites with intermediate levels of TBT exhibited intermediate responses of hormones and maturation stages (Siah et al. 2003).

Organics

Dioxin (TCDD)

2,3,7,8-TCDD alters normal development of reproductive organs and early development in bivalve mollusks at 2–20 pg g^{-1} wet weight. In both *C. virginica* and *M. arenaria*, 2,3,7,8-TCDD accumulates in gonads, and in oysters 10 pg g^{-1} caused histopathological lesions by day 14 of gametogenesis in both sexes, resulting in complete inhibition of gonadogenesis. A total body dose of 2 and 10 pg g^{-1} caused abnormal gametogenesis in female and male oysters, respectively, including incomplete oocyte division, inhibition of oocyte growth and maturation, unsynchronized sperm development, and inhibition of spermatogenesis (Wintermyer and Cooper 2007). The sensitivity of gonad maturation is likely due to disruption of cross-talk between steroid, insulin, and metabolic pathways involved in gonad differentiation. Altered gonad development and decreased veliger larval survival can partially explain the lack of self-sustaining bivalve populations in 2,3,7,8-TCDD-contaminated estuaries (Cooper and Wintermyer 2009).

Oil

Mytilus edulis were exposed to dispersed crude oil (0.015–0.25 mg l^{-1}) by Baussant et al. (2011). After 1 month in 0.25 mg l^{-1}, alkali-labile phosphates and the volume and density of atretic oocytes in females were elevated, indicating that oil affected VTG-like proteins and gamete development. Parental oil exposure did not affect subsequent fertilization success, but caused slower development, abnormalities, and reduced larval growth, effects that were enhanced when larvae were raised at 0.25 mg oil l^{-1}.

Pesticides

Akcha et al. (2012) investigated effects of the herbicides glyphosate and diuron on oyster gametes and embryos. Glyphosate had no effect, while diuron significantly affected embryo-larval development from the lowest tested concentration of 0.05 μg l^{-1}, an environmentally realistic concentration. The alkaline comet assay showed diuron had genotoxic effects on sperm at the lowest tested concentration, but did not effect sperm mitochondrial function or acrosomal membrane integrity.

Contaminants of Emerging Concern

Pang et al. (2012) compared effects of CuO nanoparticles with aqueous Cu ($CuCl_2$). They added copper to the sediment as aqueous Cu, nano- (6 nm) and micro- (<5 μm) CuO particles and examined effects on the deposit-feeding snail, *Potamopyrgus antipodarum.* After 8 weeks of exposure to nominal concentrations of 30, 60, 120 and 240 μg Cu g^{-1} dry weight sediment, nano-CuO had greater effects on reproduction than copper added as either micro-CuO or aqueous Cu.

Polluted Sites

Tlili et al. (2011) noted differences in the gametogenic cycle of the clam *Donax trunculus* from polluted and unpolluted sites. The spawning period began in March and was maximum in May at both sites, but the percentage of spawners was higher and the spawning period was shorter at the polluted site. Energy reserves (glycogen, lipids) were lower in clams from the polluted site, suggesting that energy was being shunted to deal with the chemical stress. Scallops *Mizuhopecten yessoensis* from six stations in Peter the Great Bay (Sea of Japan) were studied (Vaschenko et al. 1997). Those from polluted sites had retarded gametogenesis, oocyte resorption, and autolysis of spermatozoa. They had more hermaphrodites, decreased fertilization success, reduced percent of normal larvae, and retardation of larval growth. Clams, *Potamocorbula amurensis* from a silver-contaminated site in San Francisco Bay had a low percentage of reproductive individuals, <60 %. When Ag tissue concentrations decreased, the proportion of reproductive individuals increased to 80–100 % (Brown et al. 2003). There was a negative correlation of tissue Ag and percent of reproductive individuals (Fig. 5.4), and no correlation with other environmental variables.

5.2.3 Fishes

Organics

Pesticides and Industrial Chemicals

Exposure to Kepone or *o,p′*-DDD (100 nM–100 μM) prevented most of the oocytes of *M. undulatus* from completing germinal vesicle breakdown (GVBD); many were arrested at the lipid coalescence or germinal vesicle migration stage after exposure to100 μM (Ghosh and Thomas 1995). In addition, clearing of the ooplasm, oil droplet formation and hydration were incomplete in oocytes that did undergo GVBD. The pesticides inhibited GVBD in a concentration-dependent manner. Exposure to either pesticide for as little as 1 min could block GVBD. Washing the follicle-enclosed oocytes after exposure restored their ability to undergo GVBD.

Paclobutrazol (PBZ), a triazole-containing fungicide, is widely used in agriculture. Li et al. (2012) investigated effects of PBZ at environmentally relevant concentrations on testicular development in male rockfish *Sebastiscus marmoratus.* Exposure to 10, 100 and 1,000 ng l^{-1} for 50 days did not alter the GSI, but reduced the number of mature sperm and late stage spermatocytes in the testes.

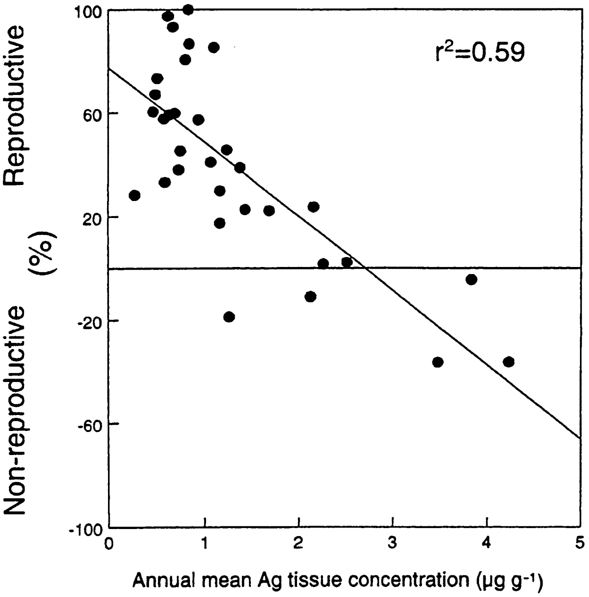


Fig. 5.4 Correlation between annual proportions (%) of reproductive clams (*Potamocorbula anurensis*) with annual mean Ag concentration at four sites. Y-axis = central tendency of the reproduction data: the proportion of clams that were reproductively active (% active + % ripe + % spawned) minus the proportion that were non-reproductively active (% inactive + % spent) (Reprinted from Brown et al. 2003: 110, courtesy Taylor & Francis)

Juvenile Atlantic cod (*Gadus morhua*) were fed different alkyl phenols (APs) (4-*tert*-butylphenol, 4-*n*-pentylphenol, 4-*n*-hexylphenol and 4-*n*-heptylphenol) for 20 weeks during vitellogenesis (Meier et al. 2011). While 60 % of the females and 96 % of the male controls were mature at the end of the experiment, exposure to APs and E_2 had different effects depending on the developmental stage. Juvenile females advanced into maturation, but gonad development was delayed in maturing females and males. The AP-exposed groups (>4 μg/kg body weight) had increased numbers of mature females, suggesting that AP-exposure affects the timing of onset of puberty at extremely low concentrations.

Sea-Nine® 211 (4,5-dichloro-2-*n*-octyl-3(2H)-isothiazolone) is widely used as an antifouling biocide after the banning of TBT. Ito et al. (2013) found testicular toxicity in mummichog *Fundulus heteroclitus*, after 28-days exposure. Although Sea-Nine® 211 did not affect germ cell proliferation, the number of apoptotic spermatocytes was increased in 1.0- and 3.0-μg l^{-1}-exposed groups. The numbers of cysts expressing caspases 2, 3, 6, and 8 (apoptosis-associated proteins) were increased in the 1.0-μg l^{-1} group, and the signal intensity of an anti-apoptotic protein Bcl-xL was reduced in a dose-dependent manner. This suggests that Sea-Nine® 211 induces apoptosis in the testicular germ cells of mummichogs via a caspase-dependent pathway.

Oil/Hydrocarbons

When female winter flounder (*Pseudopleuronectes americanus*) and their developing oocytes were exposed to #2 fuel oil at 100 $\mu g\ l^{-1}$, there was delayed hatching, reduced viable hatch, and increased incidence of malformations. Larvae raised in clean water after exposure only during parental gametogenesis had elevated mortality and slower growth (Kuhnhold et al. 1978).

Produced water discharged from offshore oil industry activities contains toxic substances including PAHs. Reproductive biomarkers were studied by Sundt and Bjorkblom (2011) in prespawning Atlantic cod (*Gadus morhua*) exposed for 12 weeks. Results showed that exposure to sufficiently high levels of PW produced an increase in VTG levels in females, as well as impaired oocyte development and reduced estrogen levels. In males testicular development was altered, showing a rise in amount of spermatogonia and primary spermatocytes and a reduction in mature sperm in the exposed fish compared to control.

Sun et al. (2011) investigated effects of phenanthrene (PHE) at environmentally relevant concentrations on testicular development in *Sebastiscus marmoratus*. After 50 days exposure, the GSI and percentage of sperm produced showed a U-shaped dose response. The levels of gonadotropin releasing hormone, follicle-stimulating hormone, luteinizing hormone mRNA, 17β-estradiol, and γ-glutamyl transpeptidase activity all showed a U-shaped dose responses, which demonstrated the U-shaped effects on spermatogenesis. A U-shaped dose–response curve is well recognized as a hormetic phenomenon. PHE accumulation in the brain also showed an inverse U-shaped increase.

Climate Change

Since rising temperatures are changing the phenology (timing) of reproduction in many taxa, it is not surprising that effects are seen in fishes. Effects were reviewed by van der Kraak and Pankhurst (1997). There have been many more reports since then. Recently, Zucchetta et al. (2012) studied relationships between changes in water temperature and the timing of reproductive investment of the grass goby *Zosterisessor ophiocephalus* in the Venice lagoon. A time series of the monthly GSI was coupled with thermal profiles of water temperatures from 1997 to 2010. Reproductive investment was positively affected by warmer water, in terms of monthly thermal anomalies and cumulative degree days. A predictive model to assess the shift of reproductive peaks in response to thermal fluctuations indicated that in warmer years, the reproductive peak occurred earlier than during colder years.

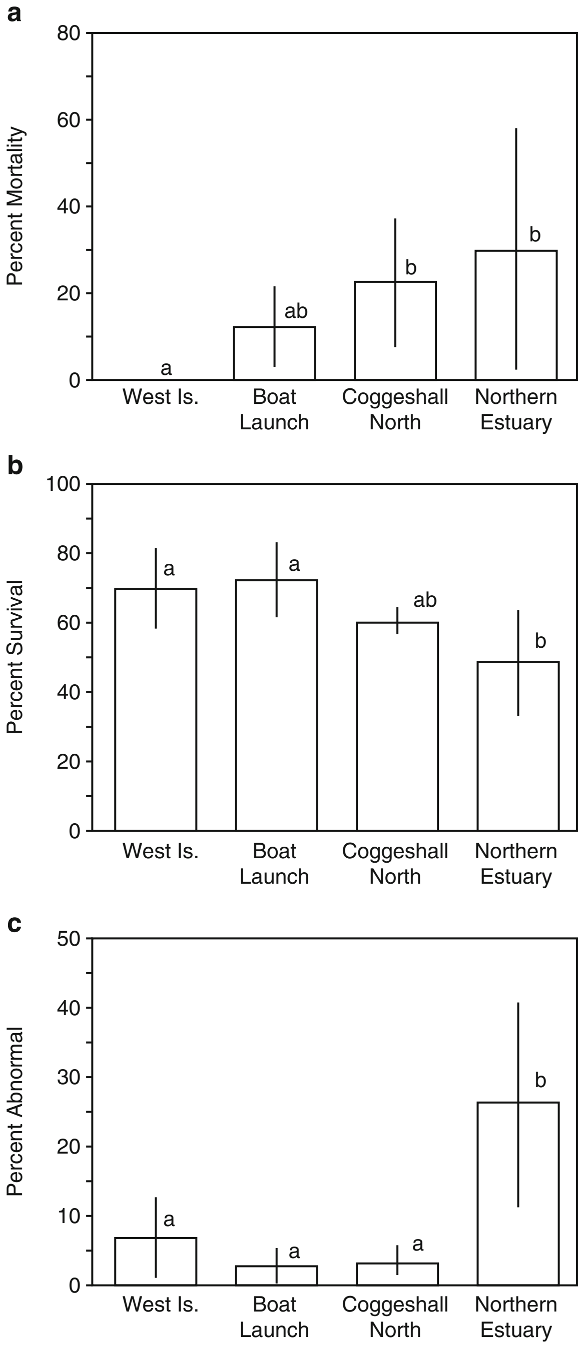
Polluted Sites

A number of field studies have linked reduced hatching success and fry survival to increased levels of lipophilic contaminants in eggs. For example, elevated PCBs in Baltic flounder (*P. flesus*) ovaries were correlated with impaired egg development and fry survival (Westernhagen et al. 1981); a similar study of Baltic herring (*Clupea harengus*) found that ovarian DDE residues of >18 ng g^{-1} wet wt. or PCB residues >120 ng g^{-1} wet wt. were associated with reduced viable hatch (Hansen et al. 1985). High larval mortality and reduced hatching success in Baltic cod (*Gadus morhua*) have also been associated with organochlorines (Petersen et al. 1997). Annual investigations of the health status of female perch (*Perca fluviatilis*) from the Baltic Sea were undertaken by Hansson et al. (2006). Fish were sampled at three coastal sites in Sweden: two in the Baltic Proper and one in the Bothnian Bay. In all, 19 biochemical, physiological and histopathological variables were measured. The most important observation was decreased gonadosomatic index (GSI) in the Baltic proper. The reduced gonad size indicates that unidentified pollutants affect reproduction even in a reference area in the Baltic proper.

Cross and Hose (1988) and Hose et al. (1989) found that a population of white croaker (*Genyonemus lineatus*) from a DDT-contaminated site had early oocyte destruction, preovulatory atresia, lower fecundity and/or spawning inducibility, and lower fertilization success than reference populations. Similarly, flatfish (*P. bilineatus* and *P. vetulus*) from contaminated areas of Puget Sound have precocious sexual maturation, retarded gonadal development, reduced egg weight, and reduced spawning success (Johnson et al. 1998). These fish are contaminated with a variety of chemicals, including aromatic hydrocarbons and PCBs, which are potential causative agents, either as antiestrogens (PAHs) or estrogen-mimics (some PCBs). These English sole have reduced viability of eggs and larvae (Casillas et al. 1991). Starry flounders (*Platichthys stellatus*) from polluted San Francisco Bay had reduced embryo development and hatching success, associated with PCBs in the eggs (Spies et al. 1985). Winter flounder (*P. americanus*) from industrial areas in Boston Harbor had reduced egg size, fertilization success, viable hatch and larval size compared to reference fish (Nelson et al. 1991). *F. heteroclitus* from the PCB-contaminated New Bedford Harbor (MA) produced as many eggs as those from reference sites, but their progeny had reduced survival and increased malformations (Black et al. 1998a) (Fig. 5.5). When adult females were given IP injections of PCBs, egg production was reduced by 77 % at the highest dose (19*u*g PCB per gram of dry liver) (Black et al. 1998b).

Mummichogs near a bleached kraft pulp mill in Canada had delayed gonadal maturation and reduced egg size (Leblanc et al. 1997), but higher fecundity and GSI at their reproductive peak than fish at the other sites. Nesting plainfin midshipman *Porichthys notatus* were collected from areas with low and high contamination on Vancouver Island, British Columbia. Males in contaminated areas had more testicular asymmetry, sperm with shorter heads, and fewer live eggs in their nests (Sopinka et al. 2012).

Fig. 5.5 Survival and reproduction of *F. heteroclitus* collected from New Bedford Harbor and West Island, MA, USA and held under laboratory conditions for 5 weeks. Northern Estuary site is closest to PCB hotspot. (**a**) Adult female mortality (**b**) embryo and larval survival and (**c**) spinal abnormalities in larvae. *Bars* with *different letters* are statistically different $p < 0.05$. Error Bars = SD (Reprinted from Black et al. 1998a: 1411), courtesy John Wiley and Sons



5.2.4 Other Taxa

Corals

Oil

Oil damages the reproductive system of corals resulting in fewer breeding colonies, fewer ovaries per polyp, fewer planula larvae, premature shedding of planulae, abnormal behavior of planulae, and lower growth rates (Loya and Rinkevich 1980). A review of effects of dispersants indicated that they enhanced the damage. Sublethal effects of oil on coral reproduction were evaluated 39 months after a spill in Panama using *Siderastrea siderea* at oiled and unoiled reefs. The number of reproductive colonies and number of gonads per polyp did not differ, but gonads were larger at unoiled than at oiled reefs during spawning periods. Years after the spill, injuries, reduced colony size, and decreased size of gonads at oiled reefs can reduce the number of reproductively viable colonies (Guzmán and Holst 1993).

Effects of the water-accommodated fraction (WAF) of a natural gas condensate on reproduction of the brooding coral *Pocillopora damicornis* were studied in laboratory experiments (Villanueva et al. 2011). Exposure during gametogenesis did not inhibit subsequent production of larvae, but exposure of gravid corals to >25 % WAF during early and late embryogenesis caused abortion and early release of larvae, with higher percentages of larvae expelled by corals in higher concentrations. Aborted larvae were small, had low metamorphic competency, and were white with a pale brown oral end (indicating low density of zooxanthellae).

Sea Anemones

Responses of the sea anemone *Actinia equina* to oil were investigated by Ormond and Caldwell (1982). Exposure to 2.5 ml l^{-1} crude oil for 7 weeks resulted in ejection of increased numbers of the young which are normally brooded within the gastric cavity. Subsequently the numbers of surviving young being produced fell to zero, and the ovaries were found to be regressed and lacking ova.

Polychaetes

Late gametogenic *Nereis virens* were incubated for up to 2.5 months in environmentally relevant concentrations of copper-spiked sediment by Watson et al. (2012). Sediments were spiked with cupric nitrate solutions to give nominal concentrations of 50, 500 and 1,000 mg kg^{-1} (dry weight) and non-spiked sediment was also included as a control. Oocytes were significantly smaller at higher concentrations. Spawning of males took place a number of days earlier in the higher concentrations. Differences in the number of embryos developing normally after *in vitro* fertilizations of oocytes fertilized with sperm from exposed males and non-exposed males showed that sperm were more susceptible to toxicity, but oocytes were also affected at the highest concentration. These results show that there are direct and indirect reproductive consequences of parental exposure to copper with implications for recruitment and colonization of polluted sediments.

5.3 Mating and Fertilization

The majority of marine animals reproduce by spawning, the release of sperm by males and eggs by females into the water column. This requires coordinated timing of gamete release, and fertilization takes place in the water column. Some groups, including decapod crustaceans, have mating, in which males release sperm into females and fertilization takes place internally. There may be elaborate behavior preceding mating, such as release of pheromones in some male crabs to attract females, or waving behavior by male fiddler crabs. Contaminants may affect these processes by interfering with the chemical or visual communication determining timing of gamete release or altering mating behaviors.

The fertilization process is initiated by the acrosome at the anterior end of the sperm contacting the egg. As the sperm approaches the egg, the acrosome reaction occurs – the membrane surrounding the acrosome fuses with the plasma membrane of the egg, releasing the contents of the acrosome. The contents include enzymes that break through the egg coat, allowing fertilization to occur. At this point the egg undergoes a cortical reaction. Cortical granules are secretory vesicles just below the egg's plasma membrane. When the fertilizing sperm contacts the egg plasma membrane, it triggers fusion of the cortical granule membranes with the egg plasma membrane, liberating the contents of the granules into the extracellular space. The granule contents modify a protein coat on the outside of the plasma membrane so that it is released from the membrane and elevates, as the fertilization membrane, which prevents further sperm from penetrating. Contaminants can affect fertilization by impairing sperm swimming or their ability to fertilize eggs. Contaminants can affect egg cells by prematurely triggering a cortical reaction, so that no sperm can fertilize them.

5.3.1 Crustaceans

Seuront (2011) examined whether the WSF of diesel oil (0.01, 0.1 and 1 %) affected male copepods' (*Temora longcornis*) ability to locate, track and mate with females. All concentrations impacted mating behavior and mating success. The ability of males to detect female pheromone trails, follow trails and track a female decreased with increasing oil concentrations, leading to decreased contact and mating (Fig. 5.6). Poulton and Pascoe (1990) devised a behavioral bioassay for pollutant stress based on disrupting the mating behavior (precopula) of amphipods.

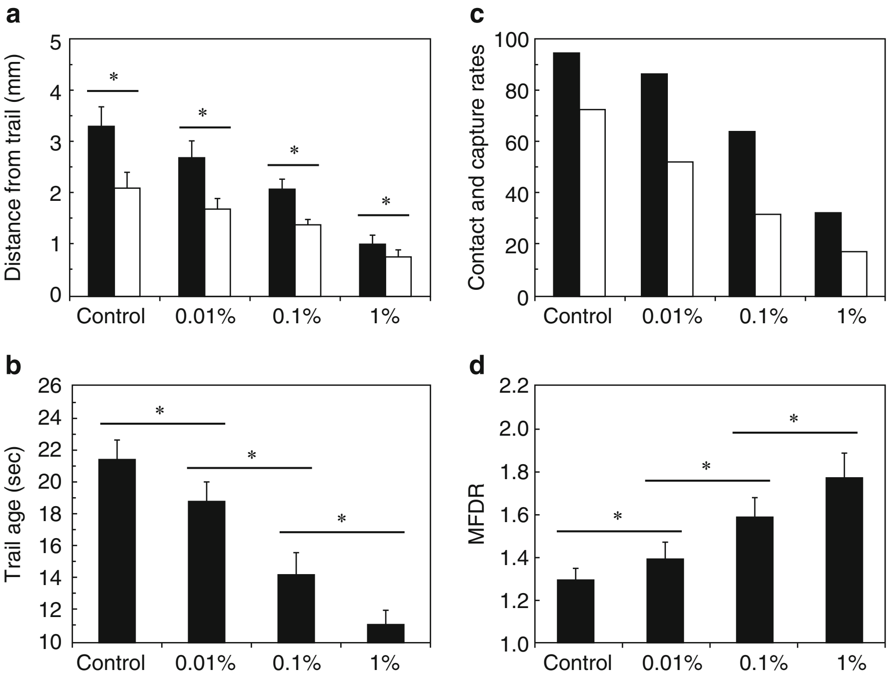


Fig. 5.6 *Temora longicornus* (**a**) Male distance from female track-line at detection of female trail (*black bars*) and during tracking (*white bars*). (**b**) Age of female's trail at detection by male. (**c**) Male–female contact rate (*black bars*) and capture rate (*white bars*). (**d**) Male-to-female displacement ratio (MFDR). Error bars = SD. * = significant differences ($p < 0.05$) (Reprinted from Seuront 2011: art. e26283, Open access)

5.3.2 Mollusks

Treatment of *Mytilus edulis* sperm with Cu or Zn (0.1–3.3 mM) decreased sperm motility; Zn was more inhibitory than Cu and produced greater mitochondrial damage, as revealed by transmission electron microscopy, than Cu (Earnshaw et al. 1986). This is a reversal of the usual relative toxicity of Cu and Zn. The reduction of sperm motility can be explained by respiratory inhibition. However, Zn had a less pronounced effect on sperm motility than on respiration.

5.3.3 Fishes

A particular characteristic of the teleost egg is the chorion, the outer protective membrane that is initially synthesized in the ovary. A canal, the micropyle, forms a pore in the membrane, through which sperm must pass in order to fertilize the eggs. Once a sperm penetrates the egg, a cortical reaction occurs, in which vesicles in the outer layer of the egg release material that blocks the micropyle to prevent polyspermy, and elevates the fertilization membrane, increasing its ability to act as a barrier. There have been relatively few studies of the impact of contaminants on gametes prior to fertilization.

Metals

Exposure of mummichog (*F. heteroclitus*) sperm to 0.01 mg l^{-1} meHg or mercuric chloride for as little as 1 min reduced fertilization success, while similar exposure of eggs had no effect on fertilization or subsequent development (Khan and Weis 1987a, b). Reductions in sperm motility were seen in treated groups. Sperm from fish from Piles Creek (PC) a polluted site in New Jersey, were unaffected by 0.01 or 0.05 mg l^{-1} meHg until they were exposed for 5 min, showing tolerance in fish from this site. Higher concentrations of either $HgCl_2$ or meHg and longer exposures of unfertilized eggs were needed to reduce fertilization and/or produce abnormalities in embryos that were subsequently raised in clean water (Khan and Weis 1987c, d). Thus Hg incorporated prior to fertilization could produce embryonic malformations in eggs that were successfully fertilized, another example of delayed effects. Eggs from PC fish were more tolerant of meHg exposure. The two forms of mercury had different mechanisms to reduce fertilization: meHg triggered a cortical reaction, preventing sperm from entering the micropyle, while $HgCl_2$ caused a swelling of the lip of the micropyle, reducing its diameter and possibly impeding sperm from swimming through (Khan and Weis 1993).

Organics

Pesticides

Exposure of mature male salmon to some currently used pesticides inhibited male olfactory detection of female pheromones that are involved in synchronization of spawning between the sexes (Moore and Waring 1996; Waring and Moore 1997). Exposure for 30 min to 1 $\mu g\ l^{-1}$ diazinon suppressed olfactory responses. Physiological responses of males to female urine, such as increased milt volume and level of sex hormones were reduced after 120 h exposure to 0.3 $\mu g\ l^{-1}$ diazinon or 0.04 $\mu g\ l^{-1}$ atrazine.

Exposure to subchronic levels of the herbicide glyphosate (Roundup) caused a significant decrease in the number of copulations and mating success in male cyprinodontid fish, *Jenynsia multidentata* (Hued et al. 2012). Reproduction of Atlantic salmon was impaired by the pyrethroid insecticide Cypermethrin at 0.1 $\mu g\ l^{-1}$. Affected fish had reduced fertilization rates, as well as lower hormone levels in males (Moore and Waring 2001). This pesticide is used to treat salmonids in aquaculture for sea lice, so farmed fish and nearby wild fish can be exposed to high levels of this chemical.

Oil

Toxicity of dispersed weathered crude oil to early life stages of Atlantic herring (*Clupea harengus*) was tested for short exposures, from 1 to 144 h (McIntosh et al. 2010). Crude oil dispersed with Corexit® 9500 was very toxic to gametes and dramatically impaired fertilization success. For brief exposures, gametes and free-swimming embryos were the most sensitive life stages. Male American plaice (*Hippoglossoides platessoides*) exposed to sediments contaminated with PAHs and PCBs had 30–50 % reduction in hatch of eggs fertilized with their sperm (Nagler and Cyr 1997).

Contaminants of Emerging Concern

Female three-spined sticklebacks were fed with freeze-dried chironomids contaminated with low or high doses of polybrominated diphenyl ethers (PBDEs), polychlorinated naphthalenes (PCNs), or PCBs for 3.5 months (Holm et al. 1993). No significant difference in number of eggs was found, but while spawning success in the controls was 80 %, it was 20 and 25 % in the groups that received high doses of PBDE or PCB, respectively. Levels of PBDE accumulated in the low- and high-dose groups were 861 ± 271 and 1,630 ± 275 mg/kg fat, respectively, whereas the corresponding concentrations of PCN in the PCN groups were 845 ± 43 and 1,929 ± 72 mg/kg fat, respectively. Concentrations of PCB in fish from the PCB groups were 1,972 ± 158 and 3,594 ± 521 mg/kg fat, respectively. Morphological examination of the liver revealed pronounced lipid accumulation in all exposed groups.

5.3.4 Other Taxa

Sea urchin fertilization success is a commonly employed bioassay, so considerable work has been done on *Strongylocentrotus* and other sea urchin species on toxicity of metals and organic contaminants to fertilization (reviewed by Dinnel et al. 1989). A few more recent studies are reviewed here.

Metals

Fertilization in corals can impeded by some metals. Reichelt-Brushett and Harrison (2005) examined effects of Cu, Pb, Zn, Cd, and Ni on fertilization success of gametes of the corals *Goniastrea aspera, Goniastrea retiformis, Acropora tenuis,* and *Acropora longicyathus*. The EC_{50} values (concentration that reduces the fertilization rate by 50 %) for Cu was 15–40 $\mu g\ l^{-1}$, while other metals were much less toxic. Hédouin and Gates (2013) investigated how Cu alters fertilization success of the coral *Montipora capitata* over several nights of spawning and found that gametes are sensitive to Cu, with EC_{50} after 3 h ranging from 16.6 to 31.7 $\mu g\ l^{-1}$. In addition, the sensitivity of the gametes was affected by the night of spawning during which fertilization experiments were performed. This likely reflected changes in the quality of gametes over the spawning period.

Populations of the echinoderms *Asterias rubens* and *Echinus acutus* that occur naturally along a contamination gradient of Cd, Cu, Pb, and Zn in a Norwegian fjord were studied. Sperm motility, a measure of sperm quality, was quantified using a computer-assisted sperm analysis system. The RNA/DNA ratio, a measure of protein synthesis, was also assessed. Although both species accumulated metals at high concentrations, neither sperm motility in *A. rubens* nor the RNA/DNA ratio in either species were affected (Catarino et al. 2008).

Organics

The chlorinated pesticides methoxychlor, dieldrin, and lindane affect fertilization and early development of sea urchin, *Paracentrotus lividus*. Pesando et al. (2004) observed that fertilization decreased when sperm were incubated for various period of time with 100 μM of dieldrin or lindane. Treatment of eggs (1 h with 100 μM) did not prevent fertilization, but increased the rate of polyspermy, delayed or blocked the first mitotic divisions, and altered early embryonic development.

Oil mixed with dispersants is highly toxic to coral early life stages. Coral are extremely sensitive to the combined effects, with fertilization failure in the presence of dispersant and dispersed oil, compared with mostly successful fertilization in the presence of oil alone (Negri and Heyward 2000; Shafir et al. 2007; Epstein et al. 2000). Negri and Heyward (2000) studying *Acropora millepora* found that 20 % v/v PFW (production formation water) inhibited fertilization by 25 %. This was equivalent 0.0721 mg l^{-1} total hydrocarbon (THC). Crude oil WAF did not inhibit fertilization unless dispersant was added. Dispersed oil was slightly more toxic to fertilization than dispersant alone, suggesting toxicity may be additive. The minimum concentration of dispersed oil which inhibited fertilization was 0.0325 mg l^{-1} THC.

To study effects of chronic exposure to produced water (an oil production effluent) on gametogenesis and gamete performance of the purple sea urchin (*Strongylocentrotus purpuratus*) Krause (1994) caged urchins at varying distances from an outfall. Those living closer to the outfall produced larger gonads. Gamete performance was measured using a fertilization bioassay that held eggs constant and varied the amount of sperm added. The proportion of eggs fertilized under each sperm concentration increased with distance from the outfall, indicating that although the exposed adults had larger gonads, they showed a marked decrease in gamete performance.

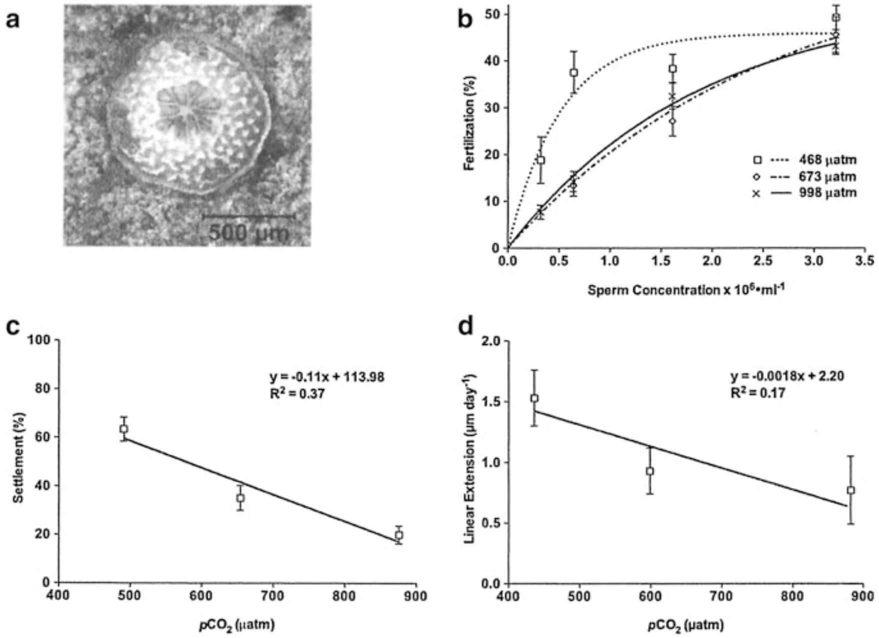


Fig. 5.7 Results of fertilization, settlement and growth experiments (mean ± SE). (**a**) 26-day old *A. palma* juveniles reared under ambient CO_2. (**b**) Nonlinear regressions of fertilization data by CO_2 treatment. (**c**) Percent settlement, (**d**) Linear growth (*u*m day^{-1}) of juveniles over 50 days (Reprinted from Albright et al. 2010: 20401), courtesy National Academy of Sciences)

Climate Change/Acidification

For many marine invertebrates, sperm flagellar motility is likely initiated when intracellular pH is elevated and suppressed when it decreases. The fertilization potential of eggs may also be influenced by changes to internal pH.

Corals

Elevated pCO_2 negatively affected fertilization success of the coral, *Acropora palmata*, but the effect was due to reduced sperm concentration. As sperm concentration declined, the effect of pCO_2 was exacerbated – higher sperm concentrations were required to achieve comparable fertilization rates to controls (Fig. 5.7) (Albright et al. 2010).

Nakamura and Morita (2012) investigated effects of different pCO_2 conditions (300, 400, and 1,000 mg l^{-1}) on sperm motility of *Acropora digitifera,* and found that acidification could suppress the flagellar motility. They calculated that sperm motility will likely decline by ~30 %, which may impact fertility.

Echinoderms

Sperm concentration effects of pCO_2 on fertilization success have also been found for the sea urchin, *Strongylocentrotus franciscanus,* in which fertilization efficiency decreased. Elevated pCO_2 reduced the ability of sea urchin eggs to block polyspermy, which inhibits successful embryo development (Reuter et al. 2011). Acidification-induced changes may be due to effects on the sperm, egg, or both.

Interactive effects of warming and acidification were observed on fertilization and embryonic development of the sea urchin *Sterechinus neumayeri* in elevated temperature (+1.5 and 3 °C) and decreased pH (−0.3 and −0.5 pH units) treatments (Ericson et al. 2012). Fertilization was resilient to acidification at ambient temperature, but at elevated temperatures, there was a negative interaction of temperature and pH on percentage fertilization (11 % reduction at +3 °C). Cleavage stage embryos, showed a significant, but small reduction (6 %) in the percentage of normal embryos at pH 7.5. For blastulae, a 10–11 % decrease in normal development occurred in the +3 °C treatments at all pHs. The results highlight the importance of considering the impacts of both temperature and pH in assessing responses to climate change. Interactive effects of near-future ocean warming and acidification on fertilization of a variety of intertidal and shallow subtidal echinoids (*Heliocidaris erythrogramma*, *H. tuberculata*, *Tripneustes gratilla*, *Centrostephanus rodgersii*), an asteroid (*Patiriella regularis*) and an abalone (*Haliotis coccoradiata*) were examined. Eggs from multiple females were fertilized by sperm from multiple males in combinations of three temperature and three pH/PCO_2 treatments (Byrne et al. 2010) based on near-future conditions for southeast Australia. No significant effects were seen, indicating that fertilization is robust to temperature and pH/PCO_2 fluctuation. This may reflect adaptation to fluctuations in temperature and pH in their shallow water habitats.

5.4 Conclusions

From endocrine effects to gametogenesis, mating, and fertilization, reproduction can be impaired in many different ways by contaminants. It can be seen that there is continuity and overlap of effects from gametes to fertilization to embryonic development which is discussed in the following chapter. Effects initiated by endocrine disruption, for example can be followed through the life cycle. Reproduction is clearly a process with obvious repercussions at the population level. However, many marine organisms normally produce enormous numbers of embryos, so it is not clear what degree of reproductive impairment would be required to lead to population level effects, which are rare. One clear example of population level effects is that of TBT on dog whelks. Nevertheless, additive effects of impairments at numerous stages of reproduction and development imply that overall reproductive success in many taxa could be severely compromised in a number of pollution scenarios.

References

Abidli S, Santos MM, Lahbib Y, Costa Castro LF, Reis-Henriques MA, El Menif NT (2011) Tributyltin (TBT) effects on *Hexaplex trunculus* and *Bolinus brandaris* (Gastropoda: Muricidae): imposex induction and sex hormone levels insights. Ecol Indic 13:13–21

Akcha F, Spagnol C, Rouxel J (2010) Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. Aquat Toxicol 106–107:104–113

Albright R, Mason B, Miller M, Langdon C (2010) Ocean acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata*. Proc Natl Acad Sci U S A 107:20400–20404

Armoza-Zvuloni R, Kramarsky-Winter E, Rosenfeld H, Shore LS, Segal R, Sharon D, Loya Y (2012) Reproductive characteristics and steroid levels in the scleractinian coral *Oculina patagonica* inhabiting contaminated sites along the Israeli Mediterranean coast. Mar Pollut Bull 64:1556–1563

Baussant T, Ortiz-Zarragoitia M, Cajaraville MP, Bechmann RK, Taban I, Ingrid C, Sanni S (2011) Effects of chronic exposure to dispersed oil on selected reproductive processes in adult blue mussels (*Mytilus edulis*) and the consequences for the early life stages of their larvae. Mar Pollut Bull 62:1437–1445

Bergey L, Weis JS (2008) Aspects of population ecology in two populations of fiddler crabs, *Uca pugnax*. Mar Biol 154:435–442

Black DE, Gutjahr-Gobell R, Pruell RJ, Bergen B, Mills L, McElroy AE (1998a) Reproduction and polychlorinated biphenyls in *Fundulus heteroclitus* (Linnaeus) from New Bedford Harbor, Massachusetts, USA. Environ Toxicol Chem 17:1405–1414

Black DE, Gutjahr-Gobell R, Pruell RJ, Bergen B, Mills L, McElroy AE (1998b) Effects of a mixture of non-*ortho*- and mono-*ortho*-polychlorinated biphenyls on reproduction in *Fundulus heteroclitus* (Linnaeus). Environ Toxicol Chem 17:1396–1404

Brouwer M, Brown-Peterson NJ, Larkin P, Patel V, Denslow N, Manning S, Hoexum Brouwer T (2007) Molecular and whole animal responses of grass shrimp, *Palaemonetes pugio*, exposed to chronic hypoxia. J Exp Mar Biol Ecol 341:16–31

Brown CL, Parchaso F, Thompson J, Luoma SN (2003) Assessing toxicant effects in a complex estuary: a case study of effects of silver on reproduction in the bivalve, *Potamocorbula amurensis*, in San Francisco Bay. Hum Ecol Risk Assess 9:95–119

Bryan GW, Gibbs PE, Hummerstone LG, Burt GR (1986) The decline of the gastropod *Nucella lapillus* around south-west England: evidence from the effect of tributyltin antifouling paints. J Mar Biol Assoc UK 66:611–640

Bryan GW, Burt GR, Gibbs PE, Pascoe PL (1993) *Nassarius reticulatus* (Nassariidae: Gastropoda) as an indicator of tributyltin pollution before and after TBT restrictions. J Mar Biol Assoc UK 73:913–929

Bugel S, White LA, Cooper KR (2009) Impaired reproductive health of killifish (*Fundulus heteroclitus*) inhabiting Newark Bay, NJ, a chronically contaminated estuary. Aquat Toxicol 96:182–193

Bugel S, White LA, Cooper KR (2010) Impaired reproductive health of killifish (*Fundulus heteroclitus*) inhabiting Newark Bay, NJ, a chronically contaminated estuary. Aquat Toxicol 96:188

Bugel S, White LA, Cooper KR (2011) Decreased vitellogenin inducibility and 17β-estradiol levels correlated with reduced egg production in killifish (*Fundulus heteroclitus*) from Newark Bay, NJ. Aquat Toxicol 105:1–12

Byrne ME, Soars NA, Ho MA, Wong E, McElroy D, Selvakumaraswamy P, Dworjanyn SA, Davis AR (2010) Fertilization in a suite of coastal marine invertebrates from SE Australia is robust to near-future ocean warming and acidification. Mar Biol 157:2061–2069

Casillas E, Misitano D, Johnson LL, Rhodes LD, Collier TK, Stein JE, McCain BB, Varanasi U (1991) Inducibility of spawning and reproductive success of female English sole (*Parophrys vetulus*) from urban and nonurban areas of Puget Sound, Washington. Mar Environ Res 31:99–122

Castro IB, Arroyo MF, Costa PG, Fillmann G (2012) Butyltin compounds and imposex levels in Ecuador. Arch Environ Contam Toxicol 62:68–77

Catarino AI, Cabral HN, Peeters K, Pernet P, Punjabi U, Dubois P (2008) Metal concentrations, sperm motility, and RNA/DNA ratio in two echinoderm species from a highly contaminated fjord (the Sørfjord Norway). Environ Toxicol Chem 27:1553–1560

Celestial DM, McKenney C (1994) The influence of an insect growth regulator on the larval development of the mud crab *Rhithropanopeus harrisii*. Environ Pollut 85:169–173

Chandra K, Bosker T, Hogan N, Lister A, MacLatchy D, Currie S (2012) Sustained high temperature increases the vitellogenin response to 17α-ethynylestradiol in mummichog (*Fundulus heteroclitus*). Aquat Toxicol 118–119:130–140

Cheek A (2011) Diel hypoxia alters fitness in growth-limited estuarine fish (*Fundulus grandis*). J Exp Mar Biol Ecol 409:13–20

Cheek A, Landry CA, Steele SL, Manning S (2009) Diel hypoxia in marsh creeks impairs the reproductive capacity of estuarine fish populations. Mar Ecol Prog Ser 392:211–221

Christiansen ME, Costlow JD, Monroe RJ (1977a) Effects of the JH mimic ZR-512 (Altozar) on larval development of the mud-crab *Rhithropanopeus harrisii* at various cyclic temperatures. Mar Biol 39:281–288

Christiansen ME, Costlow JD, Monroe RJ (1977b) Effect of the JH mimic ZR-515 (Altosid) on larval development of the mud-crab *Rhithropanopeus harrissii* in various salinities and cyclic temperatures. Mar Biol 39:269–279

Cooper KR, Wintermyer M (2009) A Critical Review: 2,3,7,8 –Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) effects on gonad development in bivalve mollusks. J Environ Sci Health C 27:226–245

Cripe GM, McKenney CL, Hoglund MD, Harris PS (2003) Effects of fenoxycarb exposure on complete larval development of the xanthid crab, *Rhithropanopeus harrisii*. Environ Pollut 125:295–299

Cross JN, Hose JE (1988) Evidence for impaired reproduction in white croaker (*Genyonemus lineatus*) from contaminated areas off Southern California. Mar Environ Res 24:185–188

DeLong RL, Gilmartin WG, Simpson JG (1973) Premature births in California sea lions: association with high organochlorine pollutant residue levels. Science 181:1168–1169

Depew DC, Basu N, Burgess NM, Campbell LM, Devlin EW, Drevnick PE, Hammerschmidt CR, Murphy CA, Sandheinrich MB, Wiener JG (2012) Toxicity of dietary methylmercury to fish: derivation of ecologically meaningful threshold concentrations. Environ Toxicol Chem 31:1536–1547

Dinnel PA, Link JM, Stober QJ, Letourneau MW, Roberts WE (1989) Comparative sensitivity of sea urchin sperm bioassays to metals and pesticides. Arch Environ Contam Toxicol 18:748–755

Dong W, Wang L, Thornton C, Scheffler BE, Willett KL (2008) Benzo(a)pyrene decreased brain and ovarian aromatase mRNA expression in *Fundulus heteroclitus*. Aquat Toxicol 88:289–300

Dubé MG, MacLatchy DL (2000) Endocrine responses of *Fundulus heteroclitus* to effluent from a bleached-kraft pulp mill before and after installation of reverse osmosis treatment of a waste stream. Environ Toxicol Chem 19:2788–2796

Earnshaw MJ, Wilson S, Akberali HB, Butler RD, Marriott KR (1986) The action of heavy metals on the gametes of the marine mussel, *Mytilus edulis* (L.)—III. The effect of applied copper and zinc on sperm motility in relation to ultrastructural damage and intracellular metal localization. Mar Environ Res 20:261–278

Epstein N, Bak RPM et al (2000) Toxicity of third generation dispersants and dispersed Egyptian crude oil on Red Sea coral larvae. Mar Pollut Bull 40:497–503

Ericson J, Ho M, Miskelly A, King C, Virtue P, Tilbrook B, Byrne M (2012) Combined effects of two ocean change stressors, warming and acidification, on fertilization and early development of the Antarctic echinoid *Sterechinus neumayeri*. Polar Biol 35:1027–1034

Eriksson SP, Weeks JM (1994) Effects of copper and hypoxia on two populations of the benthic amphipod *Corophium volutator* (Pallas). Aquat Toxicol 29:73–81

Fernandes D, Schnell S, Porte C (2011) Can pharmaceuticals interfere with the synthesis of active androgens in male fish? An *in vitro* study. Mar Pollut Bull 62:2250–2253

Fitzer SC, Caldwell GS, Close AJ, Clare AS, Upstill-Goddard RC, Bentley MG (2012) Ocean acidification induces multi-generational decline in copepod naupliar production with possible conflict for reproductive resource allocation. J Exp Mar Biol Ecol 418–419:30–36

Geeraerts C, Focant J-F, Eppe G, De Pauw E, Belpaire C (2011) Reproduction of European eel jeopardised by high levels of dioxins and dioxin-like PCBs? Sci Total Environ 409:4039–4047

Ghekiere A, Verslycke T, Janssen CR (2006) Effects of methoprene, nonylphenol and estrone on the vitellogenesis of the mysid *Neomysis integer*. Gen Comp Endocrinol 147:190–195

Ghosh S, Thomas P (1995) Antagonistic effects of xenobiotics on steroid-induced final maturation of Atlantic croaker oocytes *in vitro*. Mar Environ Res 39:159–163

Gibbs PE, Bryan GW (1986) Reproductive failure in populations of the dog-whelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. J Mar Biol Assoc UK 66:767–777

Gould E, Grieg RA, Rusanowsky D, Marks BC (1985) Metal-exposed sea scallops, *Placoplecten magellanicus* (Gmelin): a comparison of the effects and uptake of cadmium and copper. In: Vernberg FJ, Thurberg FP, Calabrese A, Vernberg WB (eds) Marine pollution and physiology: recent advances. University of South Carolina Press, Columbia, pp 157–198

Guo F, Wang L, Wang W-X (2012) Acute and chronic toxicity of polychlorinated biphenyl 126 to *Tigriopus japonicus*: effects on survival, growth, reproduction, and intrinsic rate of population growth. Environ Toxicol Chem 31:639–645

Guzmán H, Holst I (1993) Effects of chronic oil-sediment pollution on reproduction of the Caribbean reef coral *Siderastrea sidereal*. Mar Pollut Bull 26:276–282

Hansen P-D, von Westernhagen H, Rosenthal H (1985) Chlorinated hydrocarbons and hatching success in Baltic herring spring spawners. Mar Environ Res 15:59–76

Hansson T, Lindesjöö E, Förlin L, Balk L, Bignert A, Larsson A (2006) Long-term monitoring of the health status of female perch (*Perca fluviatilis*) in the Baltic Sea shows decreased gonad weight and increased hepatic EROD activity. Aquat Toxicol 79:341–355

Hédouin L, Gates RD (2013) Assessing fertilization success of the coral *Montipora capitata* under copper exposure: does the night of spawning matter? Mar Pollut Bull 66:221–224

Heintz RA, Rice SD, Wertheimer AC, Bradshaw RF, Thrower FP, Joyce JE, Short JW (2000) Delayed effects on growth and marine survival of pink salmon *Oncorhynchus gorbuscha* after exposure to crude oil during embryonic development. Mar Ecol Prog Ser 208:205–216

Hogan NS, Wartman CA, Finley MA, van der Lee JG, van den Heuvel MR (2008) Simultaneous determination of androgenic and estrogenic endpoints in the threespine stickleback (*Gasterosteus aculeatus*) using quantitative RT-PCR. Aquat Toxicol 90:269–276

Hogan NS, Currie S, LeBlanc S, Hewitt LM, McLatchy DL (2010) Modulation of steroidogenesis and estrogen signaling in the estuarine killifish (*Fundulus heteroclitus*) exposed to ethinylestradiol. Aquat Toxicol 98:148–156

Holm G, Norrgren L, Andersson T, Thuren A (1993) Effects of exposure to food contaminated with PBDE, PCN or PCB on reproduction, liver morphology and cytochrome P450 activity in the three-spined stickleback, *Gasterosteus aculeatus*. Aquat Toxicol 27:33–50

Hook SE, Fisher NS (2001) Reproductive toxicity of metals in calanoid copepods. Mar Biol 138:1131–1140

Hose JE, Cross JN, Smith SG, Diehl D (1989) Reproductive impairment in a fish inhabiting a contaminated coastal environment off Southern California. Environ Pollut 57:139–148

Hued AC, Oberhofer S, Bistoni M (2012) Exposure to a commercial glyphosate formulation (Roundup$^{®}$) alters normal gill and liver histology and affects male sexual activity of *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes). Arch Environ Contam Toxicol 62:107–117

Ito M, Mochida K, Ito K, Onduka T, Fujii K (2013) Induction of apoptosis in testis of the marine teleost mummichog *Fundulus heteroclitus* after *in vivo* exposure to the antifouling biocide 4,5-dichloro-2-*n*-octyl-3(2H)-isothiazolone (Sea-Nine 211). Chemosphere 90:1053–1060

Jensen LK, Carroll J (2010) Experimental studies of reproduction and feeding for two Arctic-dwelling *Calanus* species exposed to crude oil. Aquat Biol 10:261–271

Jenssen BM (2006) Endocrine-disrupting chemicals and climate change: a worst-case combination for Arctic marine mammals and seabirds? Environ Health Perspect 114(S-1):76–80

Johnson LL, Casillas E, Collier TK, McCain BB, Varanasi U (1988) Contaminant effects on ovarian development in English sole (*Parophrys vetulus*) from Puget Sound, Washington. Can J Fish Aquat Sci 45:2133–2146

Johnson LL, Misitano D, Sol SY, Nelson GM, French B, Ylitalo M, Hom T (1998) Contaminant effects on ovarian development and spawning success in rock sole from Puget Sound, Washington. Trans Am Fish Soc 127:375–392

Johnson LL, Lomax DP, Myers MS, Olson OP, Sol SY, O'Neill SM, West JE, Collier TK (2008) Xenoestrogen exposure and effects in English sole (*Parophrys vetulus*) from Puget Sound, WA. Aquat Toxicol 88:29–38

Katsiadaki I, Scott AP, Hurst M, Matthiessen P, Mayer I (2002) Detection of environmental androgens: A novel method based on enzyme-linked immunosorbent assay of spiggin, the stickleback (*Gasterosteus aculeatus*) glue protein. Environ Toxicol Chem 21:1946–1954

Khan AT, Weis JS (1987a) Effects of methylmercury on sperm and egg viability of two populations of killifish, *Fundulus heteroclitus*. Arch Environ Contam Toxicol 16:499–505

Khan AT, Weis JS (1987b) Effects of mercuric chloride on sperm and egg viability in two populations of the mummichog *Fundulus heteroclitus*. Environ Pollut 48:263–273

Khan AT, Weis JS (1987c) Effects of mercuric chloride on eggs and juvenile viability in two populations of killifish. Mar Pollut Bull 18:504–505

Khan AT, Weis JS (1987d) Effects of methylmercury on egg and juvenile viability in two populations of *Fundulus heteroclitus*. Environ Res 44:272–278

Khan AT, Weis JS (1993) Differential effects of organic and inorganic mercury on the micropyle of eggs of *Fundulus heteroclitus*. Environ Biol Fishes 37:323–327

Khan IA, Thomas P (1992) Stimulatory effects of serotonin on maturational gonadotropin release in the Atlantic croaker, *Micropogonias undulatus*. Gen Comp Endocrinol 88:388–396

Khan IA, Thomas P (1994) Seasonal and daily variation in plasma gonadotropin II response to a LHRH analog and serotonin in Atlantic croaker (*Micropogonias undulatus*): evidence for mediation by 5-HT2 receptors. J Exp Zool 269:531–537

Khan IA, Thomas P (1997) Aroclor 1254-induced alterations in hypothalamic monoamine metabolism in the Atlantic croaker (*Micropogonias undulatas*): correlation with pituitary gonadotropin release. Neurotoxicology 18:553–560

Khan IA, Thomas P (2001) Disruption of neuroendocrine control of luteinizing hormone secretion by Aroclor 1254 involves inhibition of hypothalamic tryptophan hydroxylase activity. Biol Reprod 64:955–964

Khan IA, Thomas P (2006) PCB congener-specific disruption of reproductive neuroendocrine function in Atlantic croaker. Mar Environ Res 62(suppl):S25–S28

Khan IA, Mathews S, Okuzawa K, Kagawa H, Thomas P (2001) Alterations in the GnRH-LH system in relation to gonadal stage and Aroclor 1254 exposure in Atlantic croaker. Comp Biochem Physiol B Biochem Mol Biol 129:251–259

Knoebl I, Hemmer MJ, Denslow ND (2004) Induction of zona radiata and vitellogenin genes in estradiol and nonylphenol exposed male sheepshead minnows (*Cyprinodon variegatus*). Mar Environ Res 58:547–551

Krause PR (1994) Effects of an oil production effluent on gametogenesis and gamete performance in the purple sea urchin (*Strongylocentrotus purpuratus* Stimpson). Environ Toxicol Chem 13:1153–1161

Kuhnhold WW, Everich D, Stegeman JJ, Lake J, Wolke RE (1978) Effects of low levels of hydrocarbons on embryonic, larval, and adult winter flounder (*Pseudopleuronectes americanus*). In: Proceedings of the conference on assessment of ecological impacts of oil spills, Keystone CO. American Institute of Biological Sciences, Washington, DC, pp 677–711

LeBlanc G (2007) Crustacean endocrine toxicology: a review. Ecotoxicology 16:61–81

Leblanc J, Couillard CM, Brethês J-C (1997) Modifications of the reproductive period in mummichog (*Fundulus heteroclitus*) living downstream from a bleached kraft pulp mill in the Miramichi Estuary, New Brunswick, Canada. Can J Fish Aquat Sci 54:2564–2573

Lewis C, Watson G (2012) Expanding the ecotoxicological toolbox: the inclusion of polychaete reproductive endpoints. Mar Environ Res 75:10–22

Li J, Sun L, Zuo Z, Chen M, Geng H, Wang C (2012) Exposure to paclobutrazol disrupts spermatogenesis in male *Sebastiscus marmoratus*. Aquat Toxicol 122–123:120–124

Loomis AK, Thomas P (1999) Binding characteristics of estrogen receptor (ER) in Atlantic croaker (*Micropogonias undulatus*) testis: different affinity for estrogens and xenobiotics from that of hepatic ER. Biol Reprod 61:51–60

Loomis AK, Thomas P (2000) Effects of estrogens and xenoestrogens on androgen production by Atlantic croaker testes in vitro: evidence for a nongenomic action mediated by an estrogen membrane receptor. Biol Reprod 62:995–1004

Loya Y, Rinkevich B (1980) Effects of oil pollution on coral reef communities. Mar Ecol Prog Ser 3:167–180

Lye CM, Frid CLJ, Gill ME (1998) Seasonal reproductive health of flounder *Platichthys flesus* exposed to sewage effluent. Mar Ecol Prog Ser 170:249–260

MacLatchy DL, Courtenay SC, Rice CD, Van der Kraak GJ (2003) Development of a short-term reproductive endocrine bioassay using steroid hormone and vitellogenin end points in the estuarine mummichog (*Fundulus heteroclitus*). Environ Toxicol Chem 22:996–1008

Matthiessen P, Allen Y, Bamber S, Craft J, Hurst M, Hutchinson T, Feist S, Katsiadaki I, Kirby M, Robinson C, Scott S, Thain J, Thomas K (2002) The impact of oestrogenic and androgenic contamination on marine organisms in the United Kingdom—summary of the EDMAR programme. Mar Environ Res 54:645–649

Maunder RJ, Matthiessen P, Sumpter JP, Pottinger TG (2007) Impaired reproduction in three-spined sticklebacks exposed to ethinyl estradiol as juveniles. Biol Reprod 77:999–1006

McGuiness CL, Encarnacao PC, Crivello JF (2012) Dibutyltin (DBT) an endocrine disrupter in zebrafish. J Exp Mar Biol Ecol 430–431:43–47

McIntosh S, King T, Wu D, Hodson PV (2010) Toxicity of dispersed weathered crude oil to early life stages of Atlantic herring (*Clupea harengus)*. Environ Toxicol Chem 29:1160–1167

McKenney CL (2005) The influence of insect juvenile hormone agonists on metamorphosis and reproduction in estuarine crustaceans. Integr Comp Biol 45:95–105

McKenney CL, Celestial DM (1993) Variations in larval growth and metabolism of an estuarine shrimp *Palaemonetes pugio* during toxicosis by an insect growth regulator. Comp Biochem Physiol 105C:239–245

Meier S, Morton HC, Andersson E, Geffen AJ, Taranger GL, Larsen M, Petersen M, Djurhuus R, Klungsøyr J, Svardal A (2011) Low-dose exposure to alkylphenols adversely affects the sexual development of Atlantic cod (*Gadus morhua*): acceleration of the onset of puberty and delayed seasonal gonad development in mature female cod. Aquat Toxicol 105:136–150

Misitano DA, Schiewe MH (1990) Effect of chemically contaminated marine sediment on naupliar production of the marine harpacticoid copepod *Tigriopus californicus*. Bull Environ Contam Toxicol 44:636–642

Moore CG, Stevenson JM (1991) The occurrence of intersexuality in harpacticoid copepods and its relationship with pollution. Mar Pollut Bull 22:72–74

Moore CG, Stevenson JM (1994) Intersexuality in benthic harpacticoid copepods in the Firth of Forth, Scotland. J Nat Hist 28:1213–1230

Moore A, Waring CP (1996) Sublethal effects of the pesticide diazinon on olfactory function in mature male Atlantic salmon (*Salmo salar* L) parr. J Fish Biol 48:758–775

Moore A, Waring CP (2001) The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.). Aquat Toxicol 52:1–12

Nagler JJ, Cyr DG (1997) Exposure of male American plaice (*Hippoglossoides platessoides*) to contaminated marine sediments decreases the hatching success of their progeny. Environ Toxicol Chem 16:1733–1738

Nakamura M, Morita M (2012) Sperm motility of the scleractinian coral *Acropora digitifera* under preindustrial, current, and predicted ocean acidification regimes. Aquat Biol 15:299–302

Negri AP, Heyward AJ (2000) Inhibition of fertilization and larval metamorphosis of the coral *Acropora millepora* (Ehrenberg, 1834) by petroleum products. Mar Pollut Bull 41:420–427

Nelson DA, Miller JE, Rusanowsky D, Grieg RA, Sennefelder GR, Mercado-Allen R, Kuropat C, Gould E, Thurberg FP, Calabrese A (1991) Comparative reproductive success of winter flounder in Long Island Sound: a three-year study (biology, biochemistry, and chemistry). Estuaries 14:318–331

Oberdörster E, Brouwer M, Hoexum-Brouwer T, Manning S, McLachlan JA (2000) Long-term pyrene exposure of grass shrimp, *Palaemonetes pugio*, affects molting and reproduction of exposed males and offspring of exposed females. Environ Health Perspect 108:641–646

Ohji M, Arai T, Miyazaki N (2003) Chronic effects of tributyltin on the caprellid amphipod *Caprella danilevskii*. Mar Pollut Bull 46:1263–1272

Ormond RF, Caldwell S (1982) The effect of oil pollution on the reproduction and feeding behaviour of the sea anemone *Actinia equine*. Mar Pollut Bull 13:118–122

Pang C, Selck H, Misra SK, Berhanu D, Dybowska A, Valsami-Jones E, Forbes VE (2012) Effects of sediment-associated copper to the deposit-feeding snail, *Potamopyrgus antipodarum*: a comparison of Cu added in aqueous form or as nano- and micro-CuO particles. Aquat Toxicol 106–107:114–122

Pesando D, Robert S, Huitorel P, Gutknecht E, Pereira L, Girard J-P, Ciapa B (2004) Effects of methoxychlor, dieldrin and lindane on sea urchin fertilization and early development. Aquat Toxicol 66:225–239

Petersen GI, Gerup J, Nilsson L, Larsen JR, Schneider R (1997) Body burdens of lipophilic xenobiotics and reproductive success in Baltic cod (*Gadus morhua* L.). International Council for the Exploration of the Sea, Copenhagen, ICES CM 1997/U:10, p 22

Peterson CH, Rice SD, Short JW, Esler D, Bodkin JL, Ballachey BE, Irons DB (2003) Long-term ecosystem response to the *Exxon Valdez* oil spill. Science 302:2082–2086

Poulton M, Pascoe D (1990) Disruption of precopula in *Gammarus pulex* (L.) – development of a behavioural bioassay for evaluating pollutant and parasite-induced stress. Chemosphere 20:403–415

Qiu JW, Chan KM, Leung KM (2011) Seasonal variations of imposex indices and butyltin concentrations in the rock shell *Thais clavigera* collected from Hong Kong waters. Mar Pollut Bull 63:482–488

Reichelt-Brushett AJ, Harrison PL (2005) The effect of selected trace metals on the fertilization success of several scleractinian coral species. Coral Reefs 24:524–534

Reijnders PJ (1986) Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature 324:456–457

Reuter KE, Lotterhos KE, Crim RN, Thompson CA, Harley CDG (2011) Elevated pCO_2 increases sperm limitation and risk of polyspermy in the red sea urchin *Strongylocentrotus franciscanus*. Glob Change Biol 17:163–171

Roos A, Bäcklin BM, Helander BO, Rigét FF, Eriksson UC (2012) Improved reproductive success in otters (*Lutra lutra*), grey seals (*Halichoerus grypus*) and sea eagles (*Haliaeetus albicilla*) from Sweden in relation to concentrations of organochlorine contaminants. Environ Poll 170:268–275

Sanchez W, Sremski W, Piccini B, Palluel O, Maillot-Maréchal E, Betoulle S, Jaffal A, Aït-Aïssa S, Brion F, Thybaud E, Hinfray N, Porcher J-M (2011) Adverse effects in wild fish living downstream from pharmaceutical manufacture discharges. Environ Int 37:1342–1348

Sant'Anna BS, dos Santos DM, de Marchi MR, Zara FJ, Turra A (2012) Effects of tributyltin exposure in hermit crabs: *Clibanarius vittatus* as a model. Environ Toxicol Chem 31:632–638

Sebire M, Katsiadaki I, Taylor NG, Maack G, Tyler CR (2011) Short-term exposure to a treated sewage effluent alters reproductive behaviour in the three-spined stickleback (*Gasterosteus aculeatus*). Aquat Toxicol 105:78–88

Segner H, Caroll K, Fenske M, Janssen CR, Maack G, Pascoe D, Schafers C, Vandenberg GF, Watts M, Wenzel A (2003) Identification of endocrine-disrupting effects in aquatic vertebrates and invertebrates: report from the European IDEA project. Ecotoxicol Environ Saf 54:302–314

Seuront L (2011) Hydrocarbon contamination decreases mating success in a marine planktonic copepod. PLoS One 6(10):art. e26283

Shafir S, Van Rijn J, Rinkevich B (2007) Short and long term toxicity of crude oil and oil dispersants to two representative coral species. Environ Sci Technol 41:5571–5574

Shimasaki Y, Kitano T, Oshima Y, Inoue S, Imada N, Honjo T (2003) Tributyltin causes masculinization in fish. Environ Toxicol Chem 22:141–144

Siah A, Pellerin J, Amiard J-C, Pelletier E, Viglino L (2003) Delayed gametogenesis and progesterone levels in soft-shell clams (*Mya arenaria*) in relation to in situ contamination to organotins and heavy metals in the St. Lawrence River (Canada). Comp Biochem Physiol C 135:145–156

Siegel PR, Wenner AM (1984) Abnormal reproduction in the sand crab *Emerita analoga* in the vicinity of nuclear generating station in Southern California. Mar Biol 8:341–345

Sol SY, Johnson LB, Horness H, Collier TK (2000) Relationship between oil exposure and reproductive parameters in fish collected following the EXXON VALDEZ oil spill. Mar Pollut Bull 40:1139–1147

Sopinka NM, Fitzpatrick JL, Taves JE, Ikonomou MG, Marsh-Rollo SE, Balshine S (2012) Does proximity to aquatic pollution affect reproductive traits in a wild-caught intertidal fish? J Fish Biol 80:2374–2383

Spies RB, Rice DW, Montagna PA, Ireland RR (1985) Reproductive success, xenobiotic contaminants and hepatic mixed-function oxidase (MFO) activity in *Platichthys stellatus* populations from San Francisco Bay. Mar Environ Res 17:117–121

Subramanian A, Tanabe S, Tatsukawa R, Saito S, Miyazaki N (1987) Reduction in the testosterone levels by PCBs and DDE in Dall's porpoises of the northwestern North Pacific. Mar Pollut Bull 18:643–646

Sun L, Zuo Z, Luo H, Chen M, Zhong Y, Chen Y, Wang C (2011) Chronic exposure to phenanthrene influences the spermatogenesis of male *Sebastiscus marmoratus*: U-shaped effects and the reason for them. Environ Sci Technol 45:10212–10218

Sundt RC, Björkblom C (2011) Effects of produced water on reproductive parameters in prespawning Atlantic cod (*Gadus morhua*). J Toxicol Environ Health A 74:543–554

Thomas P (1989) Effects of Aroclor 1254 and cadmium on reproductive endocrine function and ovarian growth in Atlantic croaker. Mar Environ Res 28:499–503

Thomas P, Rahman MS, Kummer JA, Lawson S (2006) Reproductive endocrine dysfunction in Atlantic croaker exposed to hypoxia. Mar Environ Res 62:S249–S252

Thomas P, Rahman MS, Khan IA, Kummer JA (2007) Widespread endocrine disruption and reproductive impairment in an estuarine fish population exposed to seasonal hypoxia. Proc R Soc B 274:2693–2702

Tlili S, Métais I, Ayache N, Boussetta H, Mouneyrac C (2011) Is the reproduction of *Donax trunculus* affected by their sites of origin contrasted by their level of contamination? Chemosphere 84:1362–1370

Van der Kraak G, Pankhurst NW (1997) Temperature effects on the reproductive performance of fish. In: Wood CM, McDonald DG (eds) Global warming: implications for freshwater and marine fish. Cambridge University Press, Cambridge, pp 159–176

Vaschenko MA, Syasina IG, Zhadan PM, Medvedeva LA (1997) Reproductive function state of the scallop *Mizuhopecten yessoensis* Jay from polluted areas of Peter the Great Bay, Sea of Japan. Hydrobiologia 352

Villanueva RD, Yap HT, Montaño MNE (2011) Reproductive effects of the water-accommodated fraction of a natural gas condensate in the Indo-Pacific reef-building coral *Pocillopora damicornis*. Ecotoxicol Environ Saf 74:2268–2274

von Westernhagen H, Rosenthal H, Dethlefsen V, Ernst W, Harms U, Hansen P-D (1981) Bioaccumulating substances and reproductive success in Baltic flounder *Platichthys flesus*. Aquat Toxicol 1:85–99

Waring CP, Moore A (1997) Sublethal effects of a carbamate pesticide on pheromonal mediated endocrine function in mature Atlantic salmon (*Salmo salar*) parr. Fish Physiol Biochem 17:203–211

Watson GJ, Pini J, Leach A, Fones G (2013) Long-term incubation of adult *Nereis virens* (Annelida: Polychaeta) in copper-spiked sediment: the effects on adult mortality, gametogenesis, spawning and embryo development. Aquat Toxicol 128–129:1–12

Wenner AM (1988) Crustaceans and other invertebrates as indicators of beach pollution. In: Soule DF, Keppel GS (eds) Marine organisms as indicators. Springer, New York, pp 199–229

Weydmann A, Søreide JE, Kwasniewski S, Widdicombe S (2012) Influence of CO_2-induced acidification on the reproduction of a key Arctic copepod *Calanus glacialis*. J Exp Mar Biol Ecol 428:39–42

Wiig O, Derocher AE, Cronin MM, Skaare JU (1998) Female pseudohermaphrodite polar bears at Svalbard. J Wildl Dis 34:792–796

Wiklund AE, Sundelin B (2001) Impaired reproduction in the amphipods *Monoporeia affinis* and *Pontoporeia femorata* as a result of moderate hypoxia and increased temperature. Mar Ecol Prog Ser 222:131–141

Wintermyer M, Cooper DR (2007) The development of an aquatic bivalve model: evaluating the toxic effects on gametogenesis following 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) exposure in the eastern oyster (*Crassostrea virginica*). Aquat Toxicol 81:10–26

Wu RS, Or YY (2005) Bioenergetics, growth and reproduction of amphipods are affected by moderately low oxygen regimes. Mar Ecol Prog Ser 297:215–223

Zhang J, Zuo Z, Xiong J, Sun P, Chen Y, Wang C (2013) Tributyltin exposure causes lipotoxicity responses in the ovaries of rockfish, *Sebastiscus marmoratus*. Chemosphere 90:1294–1299

Zucchetta M, Cipolato G, Pranovi F, Antonetti P, Torricelli P, Franzoi P, Malavasi S (2012) The relationships between temperature changes and reproductive investment in a Mediterranean goby: insights for the assessment of climate change effects. Estuar Coast Shelf Sci 101:15–23

Chapter 6
Embryonic Development

Abstract Embryonic stages of marine animals have been used extensively in explorations of effects of contaminants and toxicity testing. This chapter provides a review and summary of the nature of observed adverse effects and toxic levels on embryonic development for important classes of chemical pollutants. Early life stages are generally more susceptible to environmental contaminants than later stages, so many studies focus on embryos or larvae. Embryos can be exposed to developmental toxicants during oogenesis in exposed females, during the brief period between shedding of gametes and fertilization, and after fertilization. Studies have shown that chemicals incorporated into the egg during oogenesis can produce malformations in the embryos that subsequently develop from these eggs. In most experimental studies, however, embryos are exposed to chemicals after fertilization. Exposures can be throughout embryonic development or during shorter time periods. Although many toxicity tests still use hatching success as the endpoint of interest, common responses include delayed development and formation of abnormalities. Chemicals can affect morphogenetic movements such as gastrulation, tissue interactions such as induction, growth, and degeneration or cell death, which is an inherent part of embryonic development. Some responses do not become apparent until larval or juvenile stages.

Keywords Delay • Egg • Embryo • Hatch • Malformation • Terata

6.1 Crustaceans

Many crustacean embryos develop in egg sacs held by the pleopods of the female, after which time they hatch into a swimming larval stage. Exposures can be done on gravid females or on eggs that have been removed and cultured separately. While there are many studies on Daphnia embryos, the amount of literature on embryonic responses of marine crustaceans, especially investigations of responses other than

J.S. Weis, *Physiological, Developmental and Behavioral Effects of Marine Pollution*,
DOI 10.1007/978-94-007-6949-6_6,

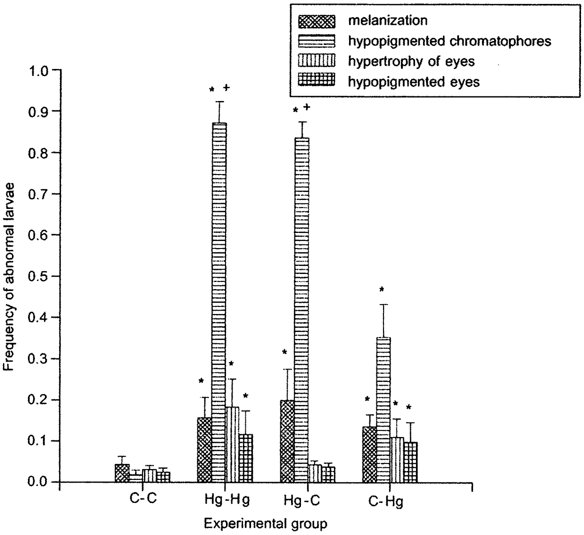


Fig. 6.1 Mean frequencies of larvae with abnormalities after mercury exposure during early, late, or entire egg incubation period (Hg- C, C- Hg, and Hg-Hg respectively; C-C = control in clean water). * = significant difference with respect to control group. + = significant difference between the C- Hg group and any other experimental group (Reprinted from Sanchez et al. 2005: 77, courtesy of Elsevier Publishing Co.)

hatching success, is surprisingly small. With their numerous appendages, setae, etc. crustaceans provide extensive opportunities for investigating developmental malformations and asymmetry (Allenbach 2011), yet relatively few recent studies examine these phenomena.

6.1.1 Metals

Exposure to Cu and Cd (at 3.1 and 0.25 $\mu g\ l^{-1}$ respectively) inhibited development of blue crab (*Callinectes sapidus*) embryos, with effects more pronounced at lower salinity (Lee et al. 1996). In contrast, 0.2 and 2 mg l^{-1} Cd increased the hatching rate of king crab (*Lithodes santolla*) embryos but increased the percent of abnormal larvae (Amin et al. 1998). Pb (0.16 and 1.6 mg l^{-1}) produced abnormalities and decreased the proportion of larvae hatching. When stage 3 blue crab embryos were exposed to 4 $\mu g\ l^{-1}$ Hg, all stage 7 embryos had no heart beat. Abnormal eye spots were also produced (Lee and Oshima 1998). The crab *Chasmagnathus granulatus* appears to be relatively insensitive to Hg, as. 0.1 mg l^{-1} produced only minor effects – developmental delay, reduced pigmentation of the body and the eyes, and hypertrophy of the eyes (Fig. 6.1) (Sanchez et al. 2005). The effects on pigmentation were attributed to interference with pigment-controlling hormones of the eyestalks. A loss of pigmentation has been noted in embryos of this species exposed to higher concentrations of Zn and Pb (Lavolpe et al. 2004).

Lopez-Greco et al. (2002) investigated toxicity of copper sulfate (1 mg l^{-1}) during embryonic development of the grass shrimp *Palaemonetes pugio*. One group of embryos was exposed throughout development, a second during the first half of development, and a third group during the second half. The group continuously exposed to Cu and that exposed during the second half of development had higher mortality and fewer hatched larvae than controls in clean water. Thus, the later embryonic period was more sensitive than the early one. Deformed eyespots were seen in all Cu-exposed groups, while underdeveloped setae were noted in hatched larvae after exposure during the entire or the second half of development. Lee et al. (2000) exposed grass shrimp (*P. pugio*) embryos to chromium(III) chloride, sodium chromate, and mercuric chloride and found a reduced hatching rate. Stage 4 embryos were more affected after exposure than stage 7 embryos. This is the opposite result from that of the Lopez-Greco et al. study above, in which later embryonic stages were more sensitive to Cu than earlier ones. This difference deserves future investigation.

Though not a crustacean, horseshoe crab, *Limulus polyphemus* embryos are very tolerant to TBT. Greater toxicity was seen following exposure of larvae. Acute exposures increased the time required by larvae to molt into the first-tailed stage. Horseshoe crabs are highly tolerant of TBT compared to early developmental stages of other marine arthropods (Botton et al. 1998).

6.1.2 Organics

Pesticides

The pesticides fenvalerate, chlorpyrifos, cypermethrin, diflubenzuron at 1.8–5.9 $\mu g\ l^{-1}$ inhibit hatching in blue crabs (Fig. 6.2) (*C. sapidus*) (Lee and Oshima 1998).

Ovigerous grass shrimp, *Palaemonetes pugio*, were exposed to the chitin-inhibiting pesticide diflubenzuron for 4 days, which did not cause immediate effects on embryo development or on hatching. However, delayed effects occurred, included stunting and swelling in the larvae that hatched from exposed embryos ($>0.3\ \mu g\ l^{-1}$), along with reduced viability (Wilson et al. 1995). Toxicity was reduced when sediment was present and in old solutions of the pesticide. Behavioral differences were also seen in larvae.

Methoprene, a juvenile hormone analog, at 2–10 μM reduced hatching of blue crab embryos; surviving larvae were lethargic (Horst and Walker 1999). Wirth et al. (2001) exposed reproductively active grass shrimp to 200 ng l^{-1} endosulfan or 1 mg l^{-1} methoprene and they were allowed to produce embryos. The resulting

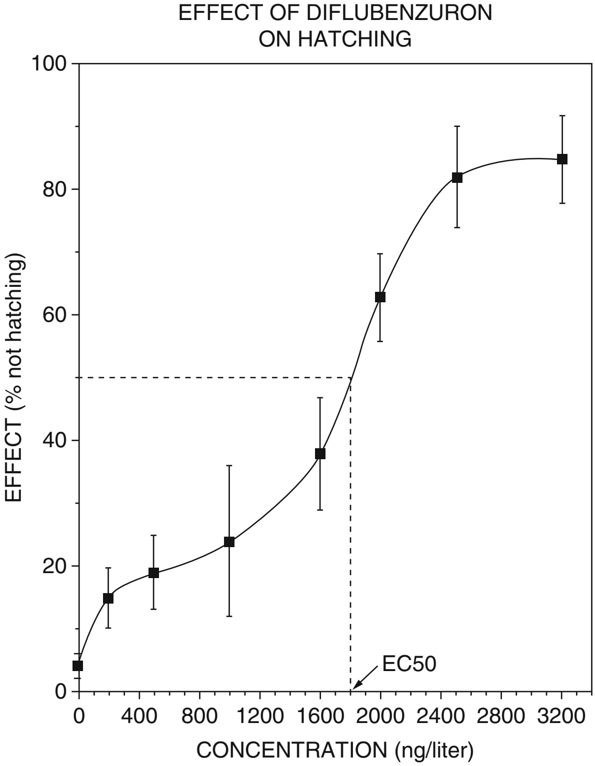


Fig. 6.2 Effect of different concentrations of diflubenzuron on hatching of blue crabs. Stage 3 embryos were exposed (Reprinted from Lee and Oshima 1998: 481, courtesy Elsevier Publishing Co.)

embryos were assessed for potential sublethal toxicity. There were no observed differences in the percent successfully hatching or larval mortality 3-days post hatch among treatments, but endosulfan-treated embryos had a significantly increased hatching time (9.76 days compared to 8.72 days in controls). Methoprene treated embryos also took longer to hatch (9.55 days), but this delay was not significantly different from controls.

Embryos of the mysid *Neomysis integer* were removed from the marsupium in which they normally develop, and exposed to nominal concentrations of 0.01, 1, and 100 μg methoprene l^{-1}. Average percentage survival, hatching success, total development time and duration of each developmental stage were analyzed. Embryos exposed to 1 and 100 μg methoprene l^{-1} had a significantly lower hatching success and lower survival rates (Ghekiere et al. 2007).

A number of studies have compared sensitivity of embryos, larvae, and adults of grass shrimp and found embryos less sensitive than other stages. Key et al. (2003) examined the fungicide chlorothalonil and found embryos were the least sensitive with a 96 h LC50 of 396.0 μg l^{-1}. Larvae were the most sensitive with a 96 h LC50 of 49.5 μg l^{-1}. Surviving larvae in lower concentrations required more molts to reach postlarvae than the control. Thus, larvae are the most sensitive stage. However, this study did not examine embryos for sublethal effects. Larvae were also the most sensitive life stage for the pyrethroid insecticide Etofenprox, with 96-h LC50 of 0.89 μg l^{-1}, compared with 1.26 μg l^{-1} for adults and 100 μg l^{-1} for embryos. Etofenprox exposure (100 μg l^{-1}) increased time to hatch (DeLorenzo and DeLeon 2010). McKenney et al. (2004) found that fenoxycarb at 888 μg l^{-1} significantly inhibited embryonic development to hatching and extended the embryonic developmental period. Exposure to 502 μg l^{-1} had no significant effect on embryos, but at 4 μg l^{-1} significantly fewer larvae metamorphosed. Larvae were thus found to be much more sensitive to this juvenile hormone agonist than embryos. Unfortunately, these comparative studies did not investigate subtle responses such as malformations, asymmetry, or delayed effects of embryonic exposure on larval behavior, such as are described in Wilson et al. (1995), above and in Chap. 9.

Oil

Lee and Nicol (1980) studied toxicity of fuel oil to embryos and juveniles of two amphipods. *Parhyale hawaiensis* eggs could develop in 10 % (2 mg l^{-1})–40 % (8 mg l–^{1}) water soluble fraction (WSF), but hatching success and juvenile survival were reduced at concentrations $\geq$10 % WSF. Juveniles that hatched from those eggs in higher WSFs had more mortality than those that hatched from eggs in lower WSFs. Survival of juveniles from late-exposed eggs was also greater than those from early-exposed eggs. *Amphithoe valida* eggs were more sensitive than *P. hawaiensis*; <10 % of the eggs hatched in 4 mg l^{-1} WSF compared with 66 % of *P. hawaiensis* eggs at the same concentration. Authors concluded that toxicity was related to concentration and duration of exposure, and to developmental stage and species, possibly due to the permeability of the egg case.

Fisher and Foss (1993) tested embryos of grass shrimp *P. pugio*, with two commercial oil dispersants (Corexit® 7664 and Corexit 9527), and the WSF of Number 2 fuel oil prepared with and without the dispersants. *P. pugio* embryos were similar to previously measured life stages in their sensitivity to WSF prepared without dispersants. They were ten times more sensitive to WSF of dispersed oil, which may have been due to the increases in total hydrocarbons, which were measured. Temperature and salinity affected toxicity of WSF prepared with dispersants, the most obvious effect being earlier mortalities at higher temperatures. Differences observed in the onset of mortalities with WSF prepared with and without dispersants implicated egg-case permeability as a factor in toxicity.

Toxicity tests using *P. pugio* exposed to WSF of Number 2 fuel oil were developed (Rayburn et al. 1996). The original test, a 12-day embryo exposure period in glass tubes, was compared with modified 4-day and 12-day tests in plastic tissue culture plates. Comparison of LC_{50} values, coefficients of variation and time to hatch indicated no significant change in sensitivity due to the plastic containers. Sensitivity was reduced, however, by shortening the test to a 4-day exposure initiated 9 days after oviposition.

Two years after the *Deepwater Horizon* blow-out in the Gulf of Mexico, abnormalities were found in white shrimp in 2012, which was considered evidence of transgenerational effects in the second generation. Shrimp had defective eye stalks, pleopods, and pereiopods. These anatomical defects were found in the markedly reduced white shrimp population, though this has not yet been published in the refereed literature nor has it been definitely linked to the oil (Darryl Felder, Professor, Louisiana State University 2013, pers. commun.).

Contaminants of Emerging Concern

To investigate how polybrominated diphenyl ethers (PBDEs) affect grass shrimp (*P. pugio*) embryos in different developmental stages, hatching rates were measured by Lee et al. (2012) after PBDE exposure of early- and late-stage embryos. BDE-47, -99, and -209 at 5, 50 and 100 $\mu g\ l^{-1}$ concentrations were used on stage 4 and stage 8 embryos. PBDEs at these concentrations had no clear influence on the hatching rate. Also, to investigate on the reproductive and genetic toxicities of UV-exposed PBDEs, the hatching rate and DNA damage of stage 7 embryos were also measured. In most cases, PBDEs had no significant impact on the hatching rate or DNA damage, but in the case of UV-exposed BDE-47, the hatching rate was decreased, suggesting potential reproductive toxicity.

P. pugio embryos exposed to 2-methyl-1,2-naphthoquinone (MNQ) (Vitamin K) had a reduced hatching rate (Lee et al. 2000). Development of stage 4 embryos was more affected by MNQ exposure than stage 7 embryos. The hatching rates of stage 4 and 7 embryos exposed to MNQ (172 $\mu g\ l^{-1}$) were 0 and 90 %, respectively. DNA damage was measured by the comet assay. Thus, exposure of early embryos to MNQ prevented full development, while development of exposed later stages was not affected. It may be that the DNA repair systems are more efficient in later embryo stages than in early stages.

6.1.3 Acidification

Weydman et al. (2012) examined effects of different pH (8.2, 7.6 and 6.9) on the reproduction of *Calanus glacialis*, an Arctic shelf-water copepod, and found that CO_2-induced seawater acidification did not affect egg production, but a reduction in pH to 6.9 significantly delayed hatching and reduced overall hatching success.

In active swimmers, exercise-produced increases in metabolic rate require efficient ion-regulatory machinery for CO_2 excretion and acid–base regulation, especially when anaerobic metabolism is used. These ion-transport systems, which are located in gill epithelia, enable compensation of pH disturbances during low pH. So far, performance at higher seawater *p*CO2 (>0.3–0.6 kPa) has been observed in adults/juveniles of active, high metabolic species. However, while these taxa are adapted to cope with elevated pCO_2, their embryos and gametes, which lack specialized ion-regulatory epithelia, may be vulnerable – even in tolerant taxa (Melzner et al. 2009).

6.1.4 Hypoxia

Oxygen can be limiting in embryo masses of marine invertebrates, but brooding female crabs can ventilate and provide oxygen to the embryo masses on their abdomens. Brooding females of *C. setosus* performed abdominal flapping, which increased oxygen availability to the brood mass; the frequency of abdominal flapping increased with embryonic development, as oxygen demand of embryos increased (Baeza and Fernandez 2002). Oxygen consumption of brooding females also increased throughout embryonic development. Female lobsters, *Nephrops norvegicus*, carrying eggs in late developmental stages exhibited brood irrigation in normoxic conditions. In hypoxic conditions (30 % saturation, or sat) the behavior was initiated in females with early eggs. Both early and late embryos survived acute exposure to 5–95 % sat. Early embryos also survived chronic progressive exposure down to 5 % sat, while late embryos displayed premature hatching at <16 % sat and decreased survival at <7 % sat (Eriksson et al. 2006).

Regardless of female ventilatory behavior, there is less oxygen available in the center than at the periphery of embryo masses. Fernández et al. (2003) investigated differences in patterns of oxygen supply to the periphery and the center of embryo masses of the crabs *Cancer setosus* and *Homalaspis plana*. Oxygen availability at the center vs periphery of the embryo masses was very different during early development, but the differences decreased over time. Inner embryos spent a greater proportion of the time exposed to low pO_2 levels throughout development. pO_2 affected oxygen consumption of the inner and outer embryos in the same fashion, but the oxygen demand of inner embryos was lower. Development of inner embryos was delayed, probably due to oxygen limitation, which affected their oxygen consumption.

6.1.5 Polluted Environment

Sundelin and Eriksson (1997) studied the Baltic amphipod *Monoporeia affinis* from fertilization to hatching. Between 2 and 6 % malformed, 0–5 % undifferentiated and 0–6 % dead eggs and embryos were observed at sites in the northern Baltic and the Bothnian Sea without contaminant discharges. Gravid females were sampled near pulp mills and an aluminum smelter. Frequency of malformed embryos was higher at these industrial sites, with the highest frequencies (15.3 %) near the smelter. The number of enlarged embryos with edema was higher near the pulp mills particularly one with a bleaching stage, where 25–40 % embryos per female were affected. Background frequencies of enlarged embryos were only 0.7–3.5 %. Undifferentiated and dead eggs did not correlate to the exposure situation, suggesting they are of limited value in monitoring. The frequency of malformed embryos was more sensitive than fecundity or reproductive success. Thus, this variable was particularly sensitive to toxicants.

6.2 Fishes

Exposures can affect morphogenetic movements such as gastrulation, tissue interactions such as induction, growth, and degeneration or cell death, all of which can produce abnormalities in the developing embryos. Fish embryos tend to become abnormal in certain ways, regardless of the chemical they are exposed to. The most sensitive systems are the developing skeletal system, circulatory system, and craniofacial system. Another common response is a general retardation of development. A slower development rate can allow teratogenic chemicals to work for a longer time during sensitive periods (critical stages) and thus produce more severe anomalies. A number of investigators have proposed using malformations in wild fish embryos and larvae for assessing pollution in local waters (von Westernhagen and Dethlefsen 1997; Kingsford and Gray 1996). Nevertheless, many studies still focus on hatching as an endpoint for bioassays. More detailed and complex analyses of developmental toxicology in fishes are developed for the freshwater zebrafish (*Danio rerio*) for which the entire genome has been analyzed.

6.2.1 Metals

Many of the studies on metals and marine fish embryos are decades old. A review of more recent studies – primarily on freshwater fishes, however – was published by Jezierska et al. (2009).

Stormwater runoff in a coastal urban area (San Diego, CA, USA) produced toxicity to early life stages of silversides (*M. beryllina*) (Skinner et al. 1999). Exposure of embryos to lower concentrations (5–25 %) increased the incidence of abnormal swim bladder inflation and other teratogenic responses, while higher concentrations caused mortality or failure to hatch. Correlation of EC50s with concentrations of individual pollutants (including Cd, Cr, Cu, Pb, Ni, and Zn) was low, but the correlation with total metals was high and corresponded with exceedences of Water Quality Criteria for Cd, Cu, Pb, and Zn.

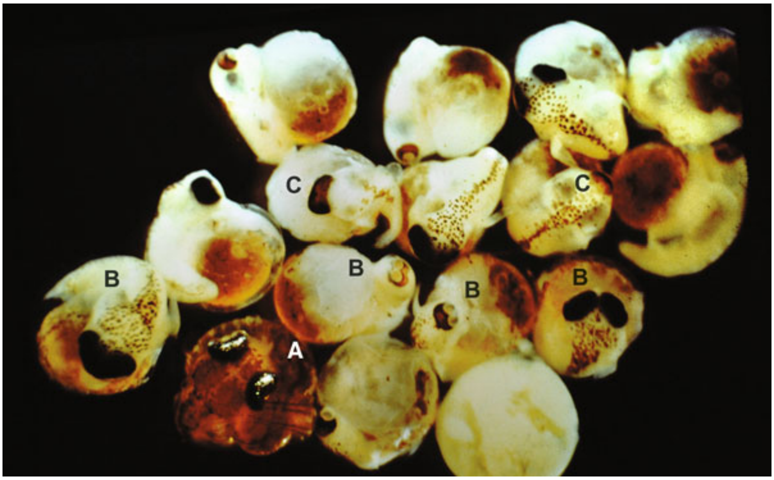


Fig. 6.3 Craniofacial abnormalities of varying severity leading to cyclopia in *F. heteroclitus* embryos in 0.05 mg l^{-1} meHg. *A* Normal control, *B* fusion of optic vesicles (synophthalmia), *C* cyclopia (Photo courtesy of P. Weis)

Mercury

Weis and Weis (1977a, b) noted cyclopia and intermediate conditions in which eye rudiments converge in *Fundulus heteroclitus* embryos treated with 50 $\mu g\ l^{-1}$ meHg or inorganic Hg (Fig. 6.3).

The mechanism underlying the fusion of the optic vesicles is reduced development of the forebrain, which then permits the eye rudiments to approach each other in the anterior midline of the embryo. The critical period for development of this anomaly was gastrulation, which is the time of induction of the forebrain and well before the actual formation of the optic cups, so this is not basically a defect in eye development, but rather one of craniofacial development. Defects of the cardiovascular system, including thin atrial and ventricular walls, a failure of the heart tube to differentiate or to bend, hemostasis, and pericardial swelling have been observed in fish embryos including *F. heteroclitus*. These effects can be produced by inorganic or meHg. Axial malformations are also common, ranging from slight bending of the skeletal axis to the extreme condition of no axis formation at all. Flexures and stunting were observed in *F. heteroclitus*. Indices of severity can grade embryos in terms of the degree of severity of anomalies, a more accurate evaluation than the percentage of affected embryos (Weis and Weis 1977a, b). Using such scales and separating out eggs produced by different females, it was seen that eggs produced by different females were very different in susceptibility to meHg; some females produced eggs that were very susceptible, others produced very resistant ones (Weis et al. 1982). Traits of the females producing tolerant eggs included different meristic characters (fin ray counts), suggesting that it is genetic. Females from a polluted site (Piles Creek in New Jersey) produced highly tolerant eggs, an indication of tolerance in this population (Weis et al. 1981). Fin ray counts in the PC population were similar to those females of the reference population that produced more tolerant embryos. Mutagenic effects were also observed in meHg-treated embryos; and those with more severe teratogenic responses had more severe mutagenic responses (Perry et al. 1988).

Cadmium

Cd is much less embryotoxic and teratogenic than Hg. Herring embryos (*Clupea harengus*) in 100 μg Cd l^{-1} had accelerated hatching (Rosenthal and Sperling 1974), which was attributed to reduced embryonic movements preventing the normal distribution of hatching enzyme, causing it to concentrate in the head region where the capsule ruptured. von Westernhagen et al. (1975) reported that pectoral fin movements of garpike embryos (*Belone belone*) were reduced after exposure to 5 mg l^{-1} Cd, and that lower salinity increased toxicity. Effects of a 2 h pulse-exposure of Cd on early life stages of Australian crimson spotted rainbow fish (*Melanotaenia fluviatilis*) were investigated on 3, 46, and 92 h old embryos (Williams and Holdway 2000). The 3-h-old embryos had the highest percentage of deformities with 27 % for embryos pulse-exposed to 3.3 mg l^{-1} Cd. Exposed 9–10-day-old larvae were more tolerant. Pulse-exposure to Cd caused reduced hatch, spinal deformities, and toxicity in larvae.

Tributyltin

Tributyltin (TBT) (a commonly used ingredient in antifouling paints for boats until banned in many countries because of its developmental and reproductive effects) produced eye and skeletal defects and delayed hatching in *F. heteroclitus* exposed to concentrations as low as 3 μg l^{-1} (Weis et al. 1987). Exposure to <10 μg l^{-1} induced craniofacial cartilage defects in rockfish (*Sebastiscus marmoratus*) embryos by perturbing the proliferation and differentiation of cartilage cells and disturbing calcium metabolism (Zhang et al. 2012). It also produced dorsal curvature, severely twisted tails and pericardial edema.

Fertilized cod eggs exposed to 5 μg TBT l^{-1} had increased larval mortality, but those exposed to 0.004–0.8 μg l^{-1} did not show altered respiration or teratogenic effects (Granmo et al. 2002).

Copper

Herring (*Clupea harengus*) incubated in 30 μg l^{-1} Cu had high mortality as embryos, and those that hatched were deformed. If exposure was delayed until 4 days post-fertilization, no deformities occurred, indicating that the early stages were most sensitive (Blaxter 1977). European seabass, *Dicentrarchus labrax* embryos had reduced hatching after exposure to 5 μg l^{-1} Cu (Cosson and Martin 1981). Exposure to 11.5 μg l^{-1} reduced hatching, prolonged incubation time, and produced abnormalities, such as bent axis and cyclopia, in embryos of cod, *Gadus morhua* (Swedmark and Granmo 1981).

Zinc

Zinc tends to be less toxic and teratogenic than the other metals, usually requiring higher concentrations to produce effects. Exposure to 50 μg l^{-1} Zn caused craniofacial and skeletal malformations in herring (*Clupea harengus*) embryos (Somasundaram et al. 1984), while 5 mg l^{-1} increased the frequency of skeletal abnormalities in cod (*Gadus morhua*) embryos (Swedmark and Granmo 1981). A 2 h pulse-exposure to 33.3 mg l^{-1} Zn of 3-h old Australian crimson spotted rainbow fish (*Melanotaenia fluviatilis*) embryos produced 27 % spinal deformities and reduced hatch. Zn affected development, growth, and survival of red sea bream (*Pagrus major*) embryos and larvae (Huang et al. 2010). Concentrations ≥0.5 mg l^{-1} reduced hatching rate, caused high mortality and morphological abnormalities; time-to-hatch was delayed in ≥1.0 mg l^{-1}. Larval length at the end of the study was reduced in the 1.0 and 2.0 mg l^{-1} groups.

Silver

Klein-McPhee et al. (1984) found that winter flounder, *Pseudopleuronectes americanus* larvae hatched from Ag-exposed eggs were shortened and curved; their yolk sacs were smaller and showed abnormal shape and inclusions.

6.2.2 Organics

Pesticides

Adult *Cyprinodon variegatus* surviving exposure to the organochlorine pesticide Kepone were spawned, and embryonic development, hatching, and survival and growth of fry were monitored in 36 days exposure to <33 μg l^{-1}. A significant number of embryos from adult fish exposed to 1.9 μg l^{-1} developed abnormally and died even when incubated in clean water. The length of juveniles was reduced by embryonic exposure to 0.08 μg l^{-1} and some fish developed scoliosis (Hanson et al. 1977a).

A given pesticide can produce different types of malformations in different species, showing that particular species are especially prone to certain types of malformations. DDT, malathion, and parathion induced cardiovascular defects in embryos of the killifish, *Fundulus heteroclitus* (Weis and Weis 1974). In contrast, these insecticides produced primarily optical malformations (microphthalmia) in embryos of the silversides, *Menidia menidia* (Weis and Weis 1976a) and malathion produced primarily skeletal malformations in developing sheepshead minnows, *Cyprinodon variegatus* (Weis and Weis 1976b). However, exposure of this species to 0.31 μg l^{-1} endrin caused early hatching, stunting, and some mortality, but no major abnormalities (Hanson et al. 1977b). The organophosphate terbufos at 50 μg l^{-1} produced fused vertebrae and lesions in silversides (*Menidia beryllina*) embryos (Middaugh et al. 1990). Hemmer et al. (1990) found effects of temperature and salinity on these responses, but the percentage of hatched larvae with normal vertebrae was significantly reduced from controls at terbufos concentrations of 25, 50 and 100 μg l^{-1} for the three temperatures (20, 25, and 30 °C) tested. Vertebral anomalies occurred across all temperature and salinity combinations, and were observed at concentrations as low as 12.5 μg l^{-1} terbufos. Chlorpyrifos was studied by Humphrey and Klumpp (2003) on various life history stages of the eastern rainbowfish *Melanotaenia splendida splendida*. Chlorpyrifos was toxic to all stages and became more toxic to embryos and larvae as the temperature increased. The LC_{50} values increased from 0.02 mg l^{-1}, for eggs and sperm prior to fertilization, to 2.02 mg l^{-1}, for eggs exposed after fertilization and hardening of the chorion. Sublethal tests revealed that larval length and otolith perimeter were the most sensitive indicators of stress, with significant effects at 0.006 mg l^{-1}. Thus, these sublethal effects were seen at orders of magnitude less pesticide than the $LC_{50.}$

Arufe et al. (2010) examined the toxicity of the organophosphorus pesticide parathion on embryos and yolk-sac larvae of gilthead seabream (*Sparus aurata*), and investigated effects on cholinesterase and carboxylesterase activity of larvae. The 72-h LC50 for yolk-sac larvae (0.523 mg l^{-1}) was about twofold lower than the 48-h LC50 for embryos (1.005 mg l^{-1}). Parathion significantly inhibited the activity of ChE and CaE in yolk sac larvae. Larvae exposed to parathion for 72 h showed a 70 % inhibition of whole body acetylcholinesterase at approximately the LC50. No examination of embryos for malformations or other sublethal effects was performed, however.

Bioassays of degraded pentachlorophenol with *M. beryllina* embryos indicated that the biodegraded samples were toxic or teratogenic, showing that intermediate metabolites of the degradation process or undegraded impurities remained toxic or teratogenic (Middaugh et al. 1993). Goodman et al. (1992) investigated early-life-stage toxicity of fenvalerate, a synthetic pyrethroid, to topsmelt (*Atherinops affinis*), a Pacific coast saltwater fish. In the 30-d ELS test with laboratory-spawned embryos, average measured fenvalerate concentrations were 0.14, 0.34, 0.82, 1.5, and 3.2 μg l^{-1}. Survival to hatching was 94–100 %, with no statistically significant differences among treatments. No fry survived exposure to fenvalerate concentrations $\geq$0.82 μg l^{-1}. There was no mention of sublethal abnormalities or delayed effects on exposed embryos.

Exposure to environmental levels of the insecticides cypermethrin and diazinon (0.05 and 0.10 μg l^{-1} cypermethrin and 0.05 μg l^{-1} diazinon) inhibited embryo development in Atlantic salmon (*Salmo salar*). Even brief exposure of eggs and sperm during fertilization reduced the number of fry that emerged and the time of emergence (Lower and Moore 2003). Following insecticide exposure there was a high incidence of fry deformities and a reduction in the survival of the fry.

PCBs and Dioxins

Extensive work on embryological effects of PCBs and dioxins has been done on freshwater fish such as zebrafish and medakas. Less work has focused on estuarine and marine species. Chambers et al. (2012) investigated sensitivities of shortnose sturgeon, *Acipenser brevirostrum*, and Atlantic sturgeon, *A. oxyrinchus*, to early-life-stage exposure to PCB 126 and 2,3,7,8-TCDD. Morphological alterations of larvae of both species included shortening of the body, reduction in head size, yolk reserves, and eye size. Eye development in both species was delayed with increasing dose for both chemicals. Survival of larvae decreased in both species, with sharp declines occurring at PCB126 and TCDD doses of $\geq$1 and $\geq$0.1 ppb, respectively.

The developing fish heart is particularly sensitive to effects of low concentrations of TCDD, as seen by Prince and Cooper (1995) in *F. heteroclitus*, and reviewed by Kopf and Walker (2009). Early signs of TCDD cardiovascular toxicity are decreased blood flow, yolk sac edema, pericardial edema, hemorrhage, and eventual mortality. While heart rate remains normal during vascular degeneration, heart size of TCDD-exposed sac fry is dramatically reduced. Close examination of the endothelium of capillaries revealed increased vacuolation, separation of the interendothelial spaces, and interstitial fluid accumulation. Other cardiovascular effects include decreased vascular growth, and deformities, such as altered looping, compaction of the ventricle, and elongation of atria. Many of these effects are similar to effects produced by metals.

Yamauchi et al. (2006) characterized early life stage toxicity of TCDD in red seabream (*Pagrus major*) related to the expression of aryl hydrocarbon receptors (AHRs) and cytochrome P 1A (CYP1A). Embryos at 10 hours post fertilization were exposed to 0–100 $\mu g\ l^{-1}$ TCDD for 80 min, which elicited mortality, yolk sac edema, retarded growth, spinal deformity, reduced heart rate, shortened snout, underdeveloped fin, heart, and lower jaw. Interestingly, hemorrhage and pericardial edema, typical defects noticed in other fish, were not found until test termination. The EC 50 s for yolk sac edema, underdeveloped fin, and spinal deformity were 170, 240, and 340 pg g^{-1}, respectively. The LC 50 was 360 pg g^{-1}, indicating that this species is one of the most sensitive fishes to TCDD. Comparing temporal trends of TCDD-induced AHRs and CYP1A expression, and developmental toxicities, the highest expression of AHR2 and CYP1A mRNAs were detected prior to the appearance of most severe malformations. These results suggest that TCDD defects are dependent on the alteration of rsAHR2 and/or rsAHR2-CYP1A signaling pathway that is controlled through their expression levels. This species is also very sensitive to effects of 2,3,7,8 TCDD on the developing nervous system. Iida et al. (2013) found that neuronal outgrowth of the facial nerve was shortened and axon guidance in the glossopharyngeal (IX) and vagus nerves (X) was altered in a dose-dependent manner with a lowest observable effect level of 0.1 $\mu g\ l^{-1}$.

Foekema et al. (2008) investigated effects of the dioxin-like PCB 126 (3,3′,4,4′,5-pentachlorobiphenyl) on early development of the sole (*Solea solea*). Early life stages were exposed until 4, 8, 10 and 15 days post fertilization (dpf) and later development of larvae was in clean water. The LC50s at the start of the free-feeding stage (12 dpf) was 39–83 ng l^{-1} depending on exposure duration, but after fish had metamorphosed, LC50s were 1.7–3.7 ng l^{-1} for groups exposed for 4, 8 and 10 dpf, respectively. Thus, exposure for only 4 days during the egg stage caused adverse effects during a critical phase 2 weeks later. This study indicates that ELS fish tests that end shortly after the fish become free-feeding underestimate the toxic potential of compounds such as PCBs. Foekema's study itself, since it focuses only on mortality, also underestimates toxicity since it does not examine sublethal effects.

Palstra et al. (2006) noted that during maturation of female European eels (*Anguilla anguilla*), about 60 g fat per kg eel is incorporated in the oocytes, along with dioxin-like PCBs. The total dioxin-like toxic potency of the individual gonads was determined as 2,3,7,8-TCDD equivalents (TEQs). Observed differences in development and survival showed a negative correlation with TEQ levels in the gonads. The inverse relationship between the TEQ level and survival of the fertilized eggs strongly suggests that the current levels of dioxin-like compounds are impairing reproduction of the European eel.

Oil/PAHs

Oil spill effects on fish eggs have been intensively studied, with studies initially focusing on the water-soluble fraction (WSF) containing mostly 1- and 2-ringed aromatic hydrocarbons (Rice et al. 2001). *F. heteroclitus* embryos exposed to the WSF of number 2 fuel oil had reduced growth, decreased vertebral counts, skeletal malformations, and decreased yolk utilization during development, effects which were intensified at higher temperature (Linden et al. 1980). Pollock embryos (*Theragra chalcogramma*) exposed to WSF of Cook Inlet crude oil were shorter, and had malformations of eye, brain, jaw and intestine in addition to skeletal malformations. Many did not hatch, but those that did had abnormalities (Carls and Rice 1989). Kocan et al. (1996a) found genetic damage was the most sensitive response in Pacific herring (*Clupea pallasi*) embryos exposed to oil–water dispersions of Prudhoe Bay crude oil, followed by deformities, reduced mitotic activity, and premature hatch. Exposures at blastodisc and gastrula stages had the greatest effects.

Among the common embryonic malformations induced by oil are heart defects. Cardiac malformations produced by PAH were found to be preceded by deficiencies in cardiac function (Incardona et al. 2004). Hicken et al. (2011) found that crude oil produces a lethal syndrome of heart failure in fish embryos. Transient embryonic exposure to very low concentrations causes delayed toxicity that is not counteracted by protective effects of cytochrome P450 induction. Nearly a year

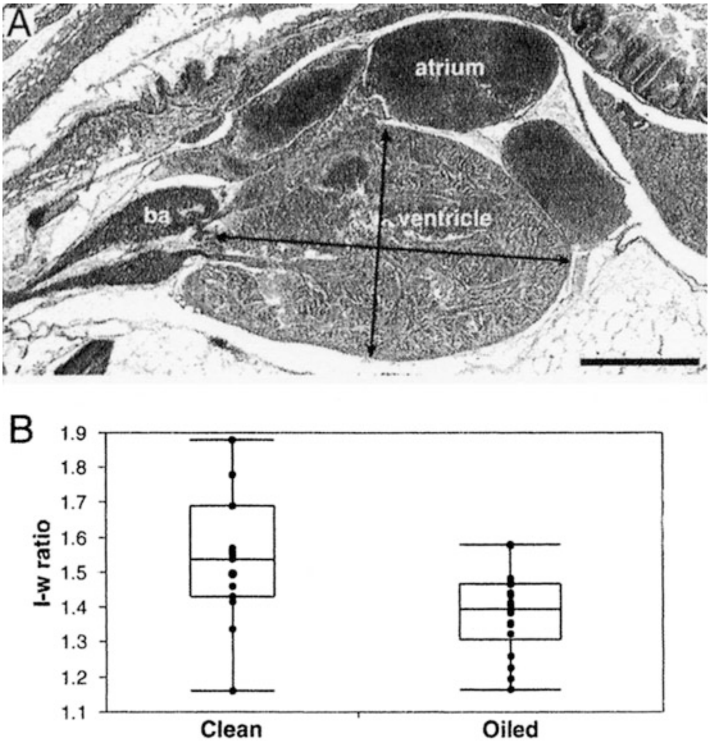


Fig. 6.4 (**a**) Transverse section of adult heart showing atrium, ventricle and bulbus arteriosus. *Arrows* indicate length and width. (**b**) Box plots showing length-width ratios for fish exposed embryonically to clean or oiled gravel effluent (Reprinted from Hicken et al. 2011: 7088, courtesy National Academy of Sciences)

after embryonic exposure, adults showed subtle changes in heart shape and reduced swimming performance, indicating reduced cardiac output. These delayed impacts on cardiovascular performance provide a potential mechanism linking reduced individual survival to population-level responses (Fig. 6.4).

Oil on the sea surface penetrates into floating eggs. Irie et al. (2011) compared responses of floating eggs and sinking eggs from a variety of marine fishes. In the course of development, floating embryos showed abnormal gross morphology, whereas sinking embryos were almost normal. However, the peripheral nervous system of sinking eggs had abnormalities, showing that these embryos were also susceptible. Capelin (*Mallotus villosus*) spawn on beaches and thus eggs can be affected by oil. Newly fertilized eggs were collected from a spawning beach and exposed until hatch (32 days) to the WSF of crude oil or to pyrene. Concentrations of 40 μg l^{-1} crude oil or 55 μg l^{-1} pyrene significantly increased mortality and decreased hatching success, indicating that an oil spill could cause significant impacts. Interestingly, no significant teratological effects were seen (Frantzen et al.

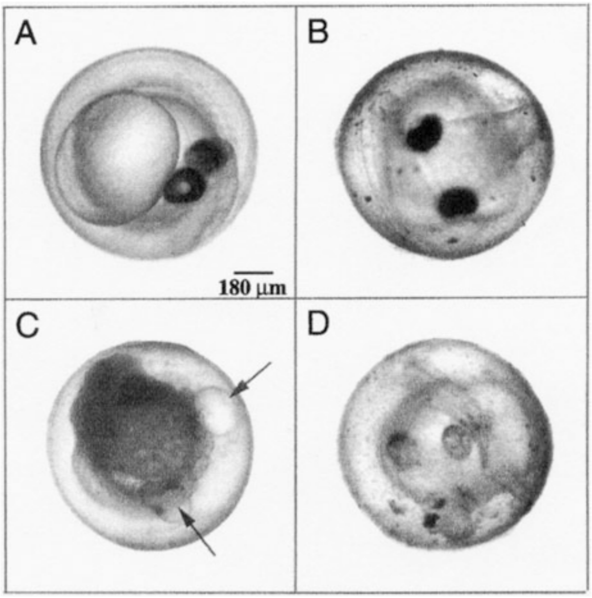


Fig. 6.5 Effects of creosote (1.2 mg l^{-1}) on embryo morphology (**a**) Normal optic vesicle pigmentation in 5-day old control Pacific herring embryo. (**b**) Normal optic vesicle pigmentation in 5-day old embryo exposed to untreated wood (**c**) 2-day old embryo exposed to creosote (*arrows* indicate vacuolization of yolk). (**d**) Delayed optic vesicle pigmentation in 5-day old embryo exposed to creosote (Reprinted from Vines et al. 2000: 231, courtesy Elsevier Publishing Co.)

2012). However, He et al. (2012) found pyrene caused neurodevelopmental defects in the rockfish (*Sebastiscus marmoratus*) by disrupting the cranial innervation pattern, reducing motor nerves and impairing axon growth. Acetylcholine (Ach), a neurotransmitter which can suppress retinal cell neurite outgrowth, was increased by pyrene exposure. Authors suggested PAHs might suppress neurite growth through increasing ACh concentration or depressing NO concentration. Exposure also caused defects in craniofacial cartilage development in this species (Shi et al. 2012).

When a Pacific herring deposited her eggs on creosote-treated wood in a marina in California, all the eggs failed to develop past a few days of incubation (Vines et al. 2000). This fortuitous discovery (for the scientists, not the fish) prompted a further study on embryonic effects of diffusible creosote-derived compounds. Incubations of eggs that were not adhering directly to the wood caused 40–50 % mortality. Survivors had severely reduced heartbeat, tremors, and greatly reduced hatching rates. Those that did hatch had abnormalities including pericardial edema and axial bends (Figs. 6.5, 6.6, and 6.7). The creosote- treated wood in the marina was weathered, and estimated at 40 years old, demonstrating the continued leaching and toxicity of PAHs from the wood over decades.

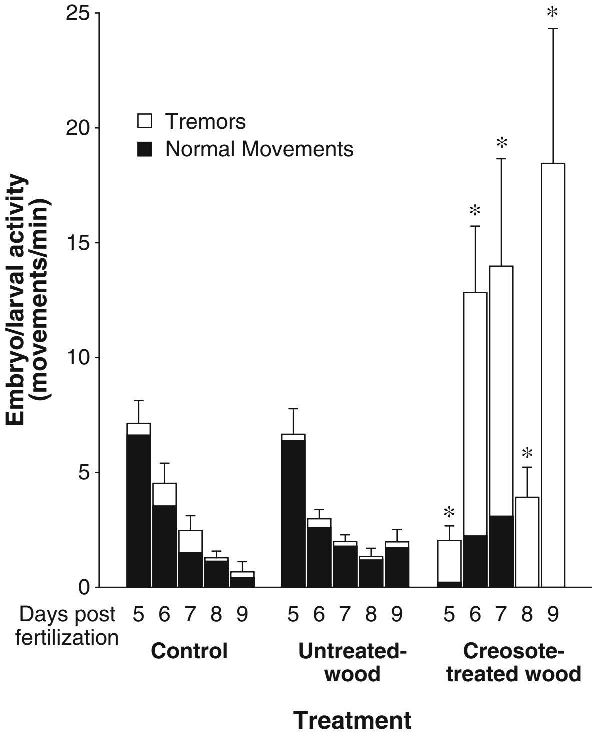


Fig. 6.6 Pre-hatching embryonic-larval activity in untreated and wood control embryos, and embryos exposed to creosote (1.2 mg l^{-1}) from day 5 to 9 of incubation. Normal activity was vigorous movements within the chorion, while abnormal movements were tremors (Reprinted from Vines et al. 2000: 232, courtesy Elsevier Publishing Co.)

Exxon Valdez

The *Exxon Valdez* ran aground on March 24 1989, releasing 11–32 million gallons (42,000–120,000 m^3) of oil into Prince William Sound (PWS), Alaska during the breeding season of many fish. After the spill, fish embryos were chronically exposed to partially weathered oil including 3-, 4-, and 5-ringed hydrocarbons generally missing from standard bioassays (Murphy et al. 1999). These PAHs affected pink salmon and herring eggs at <1 ug l^{-1}, concentrations far lower than had been previously known to be toxic. Abnormal development was seen in herring and salmon after exposure to the oil (Hose et al. 1996; Carls et al. 1999; Marty et al. 1997). After incubating herring egg masses collected from oiled and unoiled beaches, Hose et al. 1996 found more morphological deformities and cytogenetic abnormalities in embryos from oiled than from unoiled locations. By 1990 and 1991 no oil related differences were seen. Exposure to an initial aqueous concentration of 0.7 μg l^{-1} PAHs caused malformations, genetic damage, gonadal cell apoptosis, mortality,

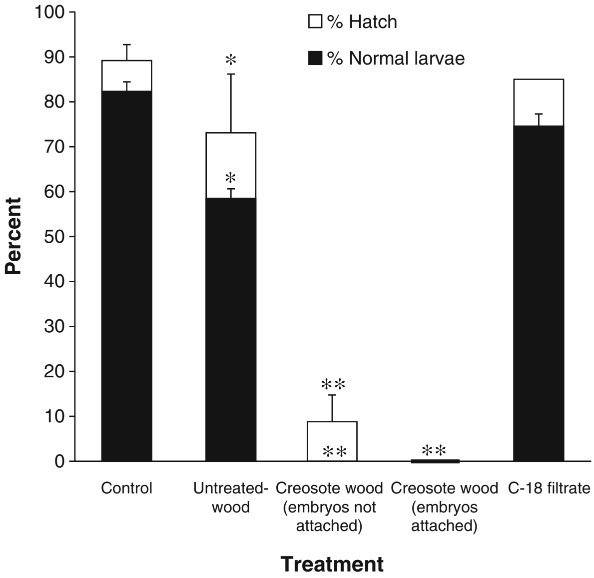


Fig. 6.7 Hatching success and larval morphology of untreated and wood control embryos and embryos exposed to creosote (1.2 mg l^{-1}) and the C-18 extract of creosote. *Bars* = total % hatch, solid area of bars represent % normal hatchlings. * = significant difference in hatching or morphology compared with controls ($P < 0.05$) ** = significant difference from control embryos and embryos exposed to untreated wood (Reprinted from Vines et al. 2000: 233, courtesy Elsevier Publishing Co.)

reduced size, and reduced swimming. Concentrations as low as 0.4 $\mu g\ l^{-1}$ caused yolk sac edema and immaturity consistent with premature hatching. Increased gonadal apoptosis may be related to later reproductive impairment seen in field studies of pink salmon up to 4 years after the spill (Marty et al. 1997). Responses to less weathered oil with fewer high molecular weight PAH, generally paralleled those of more weathered oil, but the lowest effective concentrations were higher (9.1 $\mu g\ l^{-1}$), demonstrating the higher toxicity of heavier PAHs. Biological effects were identical to those seen in PWS in 1989 and support the conclusion that the oil caused significant damage to herring. Previous demonstration that most malformed or precocious larvae die supports the decreased larval production measured after the spill. Pacific herring (*Clupea pallasi*) embryos that were deployed in PWS 3 years after the spill had more abnormalities and lower weight at previously oiled sites than at unoiled sites (Kocan et al. 1996a). Reproductive impairment was also seen in herring 3 years after the spill (Kocan et al. 1996b). Adults from oiled sites had a lower hatch and produced more abnormal larvae than fish from an unoiled site.

Heintz et al. (1999) incubated pink salmon *Oncorhynchus gorbuscha* embryos under three conditions: direct contact with oil-coated gravel, effluent from oil-coated gravel, and direct contact with gravel coated with very weathered oil (VWO). Mortalities and PAH accumulation in direct-contact and effluent exposure

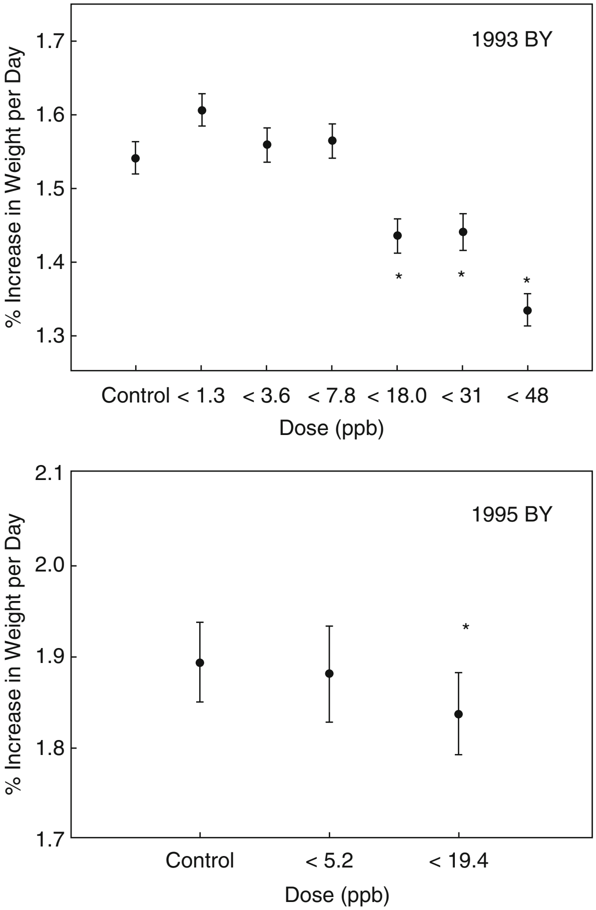


Fig. 6.8 Growth rate of *O. gorbuscha* (M ± SE) of 1993 brood fish 4–6 months after PAH exposures ended and 1995 brood fish during first 10 m after exposure. * = significantly different from control (Reprinted from Heintz et al. 2000: 211, courtesy of Inter-Research)

experiments were not significantly different, indicating that PAH accumulation was mediated by aqueous transport. Mortality of embryos exposed initially to a total PAH of 1.0 $\mu g\ l^{-1}$ was significantly higher than controls when the PAH were derived from VWO, but not from less weathered oil, indicating that toxicity was associated with the heavier PAH.

Delayed effects were noted (Heintz et al. 2000). Pink salmon exposed to 5.4 $\mu g\ l^{-1}$ PAHs as embryos had decreased marine survival compared to unexposed salmon. A delayed effect on growth was found in juveniles that survived embryonic exposure to doses as low as 18 $\mu g\ l^{-1}$ PAH (Fig. 6.8). Reduced growth could account for the reduced marine survival of the released fish.

Bue et al. (1998) investigated transgenerational effects of the *Exxon Valdez* oil spill and found that chronic damage occurred to some populations of pink salmon.

Elevated embryo mortalities were observed from 1989 to 1993 in areas previously contaminated by oil, but not in 1994 and 1995. They collected gametes from adults returning to contaminated and to uncontaminated streams, transported the gametes to a hatchery where crosses were made, and incubated the resulting embryos under identical environmental conditions. Significantly increased mortality was seen in embryos originating from the oil-contaminated lineages in 1993 but not in 1994.

There has been controversy over the embryotoxicity of weathered oil reflecting disagreements over how long after the spill effects could still be seen. While numerous studies demonstrated PAHs dissolved from weathered crude oil affect fish embryos at 0.5–23 $\mu g\ l^{-1}$ this has been challenged by studies that claim much lower toxicity of weathered aqueous PAHs, and say that direct contact with dispersed oil droplets is required for toxicity. For example, Brannon et al. (2009) reported no toxicity of weathered EVO to pink salmon embryos until exposure exceeded 1,500 $\mu g\ l^{-1}$ representing a PAH tissue burden of 7,100 ppb. They stated that after a spill, hydrocarbons drop well below levels that can cause harm over a few weeks, regardless of oiling level. In contrast, other studies demonstrated high toxicity of weathered oil; embryos accumulated dissolved PAHs at low concentrations and were damaged. Shifts in PAH composition (weathering) indicate that PAHs become more toxic because high molecular weight PAHs are more persistent and toxic than low molecular weight PAHs (Carls and Meador 2009). Years after the publication of Heinz et al. (1999), Exxon-funded scientists wrote a letter to the editor of the journal (Page et al. 2012a) complaining that the dose–response plot presented in a figure did not demonstrate PAH causality for the VWO treatment because the dose, as defined as the total extractable oil concentration in the VWO gravel on the columns at the start of the exposure, did not reflect the bioavailable dose, measured as PAHs in water or tissue and suggested that embryo injury may have resulted from exposure to other stressors, e.g., products of microbial degradation of petroleum. In response, Heintz et al. (2012a) replied that when they originally published the work, the idea that high molecular weight PAHs were teratogenic at such low concentrations was novel. Since then, the sensitivity to low concentrations of PAHs has been confirmed for fish embryos exposed to oiled sediments, to dissolved mixtures of PAHs, and to specific high molecular weight PAHs dissolved in water. They reiterated that the most toxic components of oil are the most persistent and become more concentrated as oil weathers because less toxic components are lost. There were additional exchanges (Page et al. 2012b; Heintz et al. 2012b), and Page et al. (2012c) wrote an article criticizing the Heintz study, pointing out that it did not establish consistent dose–response or show that dissolved PAH alone from the weathered oil caused the effects on fish embryos at low concentrations. They advised that these studies should not be relied on for decision-making.

Deepwater Horizon

Killifish (*Fundulus grandis*) from Louisiana marshes showed a variety of sublethal effects following the *Deepwater Horizon* blowout in 2010. Genome expression responses in liver were predictive of the types of responses associated with developmental abnormalities and death observed in previous studies (Whitehead et al. 2011). Furthermore, responses were predictive of reproductive impairment; thus the probability of population impacts is significant. Gills of oiled fish appeared damaged and had altered protein expression long after the visible oil disappeared from the marsh surface. Controlled laboratory exposures of developing embryos to field-collected waters induced similar cellular responses.

Photoenhanced Toxicity

Photoenhanced toxicity of weathered Alaska North Slope crude oil was investigated in eggs and larvae of Pacific herring (*Clupea pallasi*) with and without the dispersant Corexit®. Oil alone was acutely toxic below 50 $\mu g\ l^{-1}$ total PAH and toxicity decreased with time after exposure, but brief exposure to sunlight increased toxicity 1.5- to 48-fold (Barron et al. 2003). Photoenhanced toxicity increased with increasing tissue PAH concentration. The toxicity of dispersed oil was similar to oil alone, but oil + dispersant was more toxic in light treatments. The dispersant appeared to accelerate PAH dissolution, causing more rapid toxicity. Similarly, Incardona et al. (2012) found that the toxicity of bunker oil (thick fuel oil distilled from crude oil) to fish embryos is greatly increased by light. The study analyzed Pacific herring embryos following the *Cosco Busan* spill in San Francisco Bay. Components of the oil accumulated in naturally spawned herring embryos, then interacted with sunlight at low tide to kill the embryos. Three months after the spill, caged embryos at deeper sites in oiled areas had sublethal cardiac toxicity, as expected from exposure to PAHs, but intertidal embryos exposed to light had very high rates of necrosis and mortality unrelated to cardiac toxicity. Embryos sampled 2 years later from oiled sites still had modest heart defects but not increased death rates.

Oil Dispersants

Effects of dispersed oil are of interest since dispersants can modify the toxicity of oil and may also be toxic themselves. Toxicity of dispersed weathered crude oil to early life stages of Atlantic herring (*Clupea harengus*) was tested with 1–144 h exposures (McIntosh et al. 2010). Crude oil dispersed with Corexit® 9500 caused blue sac disease in embryos. Newly fertilized embryos were more sensitive than later stages, but sensitivity increased again after hatch, with signs of narcosis. Dispersed oil dramatically impaired fertilization success. For brief exposures, gametes and free-swimming embryos were the most sensitive stages. Anderson et al. (2009) exposed topsmelt eggs (*Atherinops affinis*) to Prudhoe Bay oil with and without Corexit®. While the water-accommodated fraction produced only minor effects, oil + dispersant produced major cardiovascular and other anomalies. Greer et al. (2012) investigated whether brief exposures of Atlantic herring (*C. harengus*) embryos to dispersed oil prepared by standard mixing procedures was as toxic as oil dispersed in a more realistic model system. Toxicity increased with exposure time, and 2.4 h exposures at realistic concentrations of oil induced blue-sac disease and reduced the percentage of normal embryos at hatch. Overall, the laboratory- and wave tank-derived solutions of dispersed oil had similar toxicity, suggesting that laboratory and wave tank data are a reliable basis for ecological risk assessments of spilled oil.

Dispersants alone are also toxic to fish embryos. Boudreau et al. (2009) studied the toxicity of Orimulsion-400® to Atlantic herring (*C. harengus*) and mummichog (*F. heteroclitus*) embryos. Water-accommodated fractions (WAFs) of no. 6 fuel oil were tested to compare dispersant toxicity with that of a heavy fuel oil. Concentrations of Orimulsion-400® >0.001 % produced 100 % abnormal larvae in herring. Abnormalities, including pericardial edema and spinal deformities, (the same effects caused by fuel oils and PAHs), were produced in both species. Fish exposed to Orimulsion-400® had increased mortality, reduced heart rates, premature hatch, and reduced length. The dispersant was about 300-fold more toxic than the WAFs of fuel oil. Fuller et al. (2004) evaluated the relative toxicity of dispersant, weathered crude oil, and weathered crude oil + dispersant to embryos of *Cyprinodon variegatus* and *Menidia beryllina*, and found that oil + dispersant was equal to or less toxic than the oil alone. Unweathered oil (dominated by soluble hydrocarbon fractions) was more toxic than weathered oil (dominated by colloidal oil fractions). Total hydrocarbons in oil + dispersant media prepared with weathered or unweathered crude oil were dominated by colloidal oil and showed no significant difference in toxicity. These results are contrary to other results of weathered oil on pink salmon and herring.

6.2.3 CECs

Perfluorooctane sulfonate (PFOS) is a persistent organic contaminant that has been widely detected in seawater. Huang et al. (2011) investigated its effects on cardiac development of *Oryzias melastigma* embryos. Embryos 2 days post-fertilization were exposed to 1, 4 and 16 mg l^{-1} PFOS and those in 4 and 16 mg l^{-1} developed an enlarged sinus venosus –bulbus arteriosus distance and altered heart rate. This is similar to effects of chlorinated chemicals, but effects occurred at much higher concentrations. Boudreau et al. (2004) investigated estrogenic (ethylene estradiol, EE2) and antiestrogenic (ZM 189,154, ZM) endocrine disrupting chemicals (10–10,000 ng l^{-1}) for effects on developing *F. heteroclitus* embryos. Skeletal abnormalities were produced at high concentrations of EE2 (1,000 ng l^{-1}). Exposures to ZM produced abnormalities, but also at high concentrations. Authors concluded that developmental abnormalities are not a sensitive indicator of estrogenic or antiestrogenic effects in this species.

6.2.4 Hypoxia

Mejda et al. (2012) studied effects of different levels of DO on embryonic development and hatching success of Greenland halibut (*Reinhardtius hippoglossoides*). Fertilized eggs were exposed to severely hypoxic (10 and 20 %sat [percent saturation]), moderately hypoxic (35 and 50 %sat), and normoxic water. Embryos were tolerant to hypoxia, with hatching occurring at levels down to 20 %sat. However at 10 %sat development was impaired and no hatching occurred. The results suggest that only very severe hypoxia has detrimental effects on the early development of this species. Other species are more sensitive. Black bream embryos (*Acanthopagrus butcheri*) in hypoxic conditions (30 % sat) had reduced survival and no hatching. In moderately hypoxic conditions (45–55 %sat), both precocious and delayed hatching occurred, hatch rates were reduced, and the number of hatched larvae with deformities and reduced length increased. No larvae survived to Day 2 post-hatch when held in hypoxic conditions <55 %sat (Hassell et al. 2008).

Energy exchange between yolk and embryo in dogfish (*Scyliorhinus canicula*) eggs in 100, 50 and 20 % sat, and anoxia for 2 h/day was studied for 10 weeks, starting when eggs were 13–15 weeks old (Diez and Davenport 1990). Exposure to 20 % sat was lethal after 3 weeks; embryos exposed to anoxia for only 2 h/day died after 10 weeks. Animals exposed to normoxia and 50%sat survived the experimental period. Embryos in reduced DO had reduced growth. *S. canicula* embryos utilize different ventilatory methods; small and intermediate embryos use body movements to stir the jelly or sea water in the capsule, larger embryos use pharyngeal pumping to pump water through the case. The effects of reduced DO on ventilation were studied (Thomason et al. 1996) but there was no significant change in ventilatory frequency induced by 2 h hypoxia.

While hypoxia in deep water is likely to be a problem for benthic eggs, there has also been some study of its effects on floating eggs. In order to elucidate the causes of malformations in buoyant eggs, Sawada et al. (2012) estimated the time change of DO and dissolved CO_2 concentrations in the interstitial water within egg aggregations of red sea bream, *Pagrus major*. They estimated that DO decreases below 10 % sat within 21 s and CO_2 increases to more than 135 mg l^{-1} within 60 min in interstitial water, suggesting that hypoxia and hypercapnia might become teratogenic within a short time in aquaculture when eggs aggregate at the surface in containers, or possibly in the wild when clumps of eggs are exposed to harmful algal blooms.

6.2.5 Climate/Ocean Acidification

Changes in pH can disturb calcification, acid–base regulation, blood circulation, respiration, and the nervous system of marine organisms, leading to long-term effects. Early life stages of fishes are vulnerable since they lack the specialized pH regulatory mechanisms that adults have. *Menidia beryllina* embryos were placed in $p\mathrm{CO}_2$ concentrations comparable to current levels (400 mg l^{-1}), those expected by mid-century (600 mg l^{-1}) and levels projected for the end of the century (1,000 mg l^{-1}) (Baumann et al. 2011). Survival was cut in half at 600 and dropped by 74 % at 1,000 mg l^{-1}. In contrast, eggs of the Atlantic herring (*C. harengus*) were fertilized and incubated in acidified seawater ($p\mathrm{CO}_2$ 1,260, 1,859, 2,626, 2,903, 4,635 μatm). Elevated $p\mathrm{CO}_2$ did not affect embryogenesis or hatch rate, and there was no relationship between $p\mathrm{CO}_2$ and total length, dry weight, yolk sac area and otolith area of newly hatched larvae, indicating they are much more tolerant than *M. beryllina* (Franke and Clemmesen 2011). However, RNA concentration was reduced at higher $p\mathrm{CO}_2$ levels, which could lead to a decreased protein synthesis and growth of larvae. Slow growing individuals have a lower survival potential due to lower feeding success and increased predation.

6.2.6 Polluted Sites

Klumpp et al. (2002) sampled fish and eggs from several areas in Xiamen coastal waters with varying levels of pollution. Embryonic malformation rates, which indicate general water quality, varied with location and species of fish, and exceeded background levels for unpolluted waters by up to eightfold at some sites. Generally, sites around Xiamen Harbour showed signs of poor water quality and had the highest levels of embryo deformity (20–30 %) and these decreased towards open waters (Tongan Bay, Eastern Channel) where abnormalities approached background levels.

The sea surface is an important habitat for eggs and larvae of many fishes but it also concentrates anthropogenic contaminants. Herring (*C. harengus*) and turbot (*Psetta maxima*) eggs were used to test effects of the sea-surface microlayer (full strength and 1, 10, 50 % dilutions). Depending on the sampling site and time, the microlayer contained Zn, Cd, Cu, Ni, Fe, Pb and Co, in concentrations 100x above those found in subsurface water and considerable amounts of petroleum hydrocarbons and phthalate esters. Exposure produced concentration-dependent effects on hatching time, percent hatch, and number of abnormalities. Herring eggs were more greatly affected by Helgoland microlayer (which contained high metals) while turbot eggs were more sensitive to Travemünde and Elbe microlayer (with high petroleum hydrocarbons) (von Westernhagen et al. 1987). Planktonic embryos of Atlantic mackerel *Scomber scombrus* collected from more polluted coastal areas had higher frequencies of mitotic abnormalities and malformations than those collected from less polluted regions (Longwell et al. 1992). Abnormalities were associated with higher levels of PAHs, metals, and PCBs.

6.3 Mollusks

Toxicity tests have been developed using embryo/larval stages of bivalves such as hard clams, *Mercenaria mercenaria* and oysters, *Crassostrea virginica* so there is a very large data base on these species, of which only a few examples will be presented.

6.3.1 Metals

Mercury at 11 μg l^{-1} caused 50 % of developing oyster embryos to become abnormal, and growth of larvae was retarded at 4 μg l^{-1} (Beiras and His 1994). Interestingly, metamorphosis of the larvae was a much less sensitive response. Mussel (*Mytilus californianus*) embryos were exposed to barium acetate and strontium chloride (Spangenberg and Cherr 1996). The most sensitive stage was the gastrula, while blastula and trochophore larvae were less sensitive. Ba showed activity at levels comparable to what can be found in some environments (200 μg l^{-1}).

Tributyltin reduced reproduction and development of the clam *Scrobicularia plana*; major population reductions in Northern Europe were attributed the TBT-induced reduction of recruitment (Ruiz et al. 1995). Effects are primarily on reproduction rather than embryo development, however. Many bivalve species suffered reproductive and developmental failure, which was attributed to widespread use of TBT antifouling paints (Thain and Waldock 1986). Recovery followed the ban on TBT.

Mai et al. (2012) evaluated embryotoxicity and genotoxicity of copper and cadmium (Cu and Cd) occurring in Arcachon Bay (SW France) in Pacific oyster (*Crassostrea gigas*) larvae and investigated the relationship between those two endpoints. Embryotoxicity was measured by calculating the percentage of abnormal D-shaped larvae and genotoxicity was evaluated with DNA strand breaks using the comet assay. After 24 h exposure, significant increases of the percentage of abnormal D-larvae and DNA strand breaks were observed from 0.1 μg l^{-1} for Cu and 10 μg l^{-1} for Cd in comparison with controls. A strong positive relationship between embryotoxicity and genotoxicity was recorded for Cu and Cd. This study suggests that copper can induce abnormalities and DNA damage in oysters at environmentally relevant concentrations.

In a rare study on cephalopods, Şen and Sunlu (2007) investigated effects of Cd on embryo development of the squid *Loligo vulgaris* and found little effect of concentrations up to 100 μg Cd l^{-1} on hatching success. At 1,000 μg Cd l^{-1}, however, eggs developed abnormally and eventually all died. Thus this species is much more resistant than oysters.

6.3.2 Organics

Pesticides

Unfertilized eggs and six developmental stages of *M. edulis* were exposed for 1 h to the insecticide Sevin (carbaryl) and its hydrolytic product, 1-naphthol. The most sensitive stage was just after fertilization at the time of appearance of the first polar body, when the EC_{50} was 5.3 mg l^{-1}. Toxicity of 1-naphthol for the first polar body stage was comparable. Developmental effects included disjunction of blastomeres, retardation of development, and unaligned and asynchronous cleavage (Armstrong and Milleman 1974). Toxicity tests were carried out by Beiras and Bellas (2008) on *M. galloprovincialis* embryos, and the toxicity of the surfactant sodium dodecyl sulphate (SDS) and the biocides TBT, chlorpyrifos and lindane were evaluated using embryo success as the end point. The EC_{10} (concentration to affect 10 %) and EC_{50} (50 % affected) values were 161 and 377 ng l^{-1} for TBT, 79 and 154 μg l^{-1} for chlorpyrifos, 495 and 2,353 μg l^{-1} for SDS, 1.41 and 1.99 mg l^{-1} for lindane. Toxicity thresholds were compared to environmental concentrations found in coastal waters, and only TBT risk was unacceptably high.

Mai et al. (2012) evaluated embryotoxicity and genotoxicity of two pesticides, metolachlor and irgarol, occurring in Arcachon Bay (SW France) in Pacific oyster (*Crassostrea gigas*) larvae. Irgarol is a supposedly less toxic substitute for TBT in antifouling paints. Embryotoxicity was measured by calculating the percentage of abnormal D-shaped larvae and genotoxicity was evaluated with DNA strand breaks using the comet assay. After 24 h exposure, significant increases of the percentage of abnormal D-larvae and the DNA strand breaks were observed at 0.01 μg l^{-1} for both irgarol and metolachlor in comparison with the controls. A strong positive relationship between embryotoxicity and genotoxicity was recorded for metolachlor. This study indicates that irgarol and metolachlor can induce abnormalities and DNA damage in oysters at environmentally relevant concentrations. They are, however, less toxic than TBT.

In contrast, glyphosate-related herbicides had very low toxicity to *C. gigas* embryos (Mottier et al. 2013). During embryo-larval development, no mortalities were recorded at any of the concentrations of glyphosate or its by-product aminomethylphosphonic acid, whereas no embryos or D-shaped larvae survived after exposure to Roundup® formulations R_{EX} or R_{AT} at 10,000 μg l^{-1}. No effects on embryo-larval development were recorded up to 1,000 μg l^{-1}, of any of the chemicals tested.

Oil

Bellas et al. (2013) investigated possible changes in toxicity due to weathering of petroleum. They evaluated the toxicity of the water-accommodated fraction (WAF) obtained from a standard fuel oil following an environmentally realistic simulated weathering process for a period of 80 days, using sea urchin (*Paracentrotus lividus*) and mussel (*Mytilus galloprovincialis*) embryos. Reminiscent of the fish embryo studies following the *Exxon Valdez*, this research also showed an increase in WAF toxicity with weathering. The EC_{50} after 80 days was eightfold lower than the EC_{50} at day 1, while the AH concentration slightly decreased. For mussel embryogenesis, the median effective concentrations (EC_{50}) and the 10 % effective concentrations (EC_{10}) were 82, 37, 41 and 12 % WAF dilution and 78, 29, 32 and 9 % WAF dilution for 1, 7, 21 and 80 days. For sea urchin growth the calculated EC_{50} and EC_{10} values decreased from 45 to 20 % WAF dilution, and from 15 to 2 % respectively. The differences in toxicity between fresh and weathered fuels could not be explained by total AH content, but to differences in particular PAHs present.

6.3.3 Contaminants of Emerging Concern

Effects of phthalate esters (PAEs) on abalone (*Haliotis diversicolor*) embryos were investigated by exposing embryos to 0.05, 0.2, 2 and 10 $\mu g\ ml^{-1}$ (Zhou et al. 2011). PAEs at fairly high concentrations reduced hatchability, increased malformations, and suppressed metamorphosis of abalone larvae (Fig. 6.9). The ability of PAEs to affect osmoregulation, induce oxidative stress, damage embryo envelope structure, and alter physiological homeostasis were considered responsible for the embryotoxicity.

The toxicity of nanosized titanium dioxide ($nTiO_2$) aggregates was evaluated on abalone (*H. diversicolor supertexta*) embryos, as well as interactions of $nTiO_2$ aggregates with TBT. No developmental effects of $nTiO_2$ were observed at 2 $mg\ l^{-1}$ but concentrations ≥ 10 $mg\ l^{-1}$ caused hatching inhibition and malformations. The presence of 2 $mg\ l^{-1}$ $nTiO_2$ increased the toxicity of TBT 20-fold compared with TBT alone (Zhu et al. 2011). This enhancement of toxicity may be due to TBT adsorption onto $nTiO_2$ aggregates and internalization of aggregates, indicating that nanoparticles may have important indirect impacts on aquatic organisms by altering the toxicity of coexisting pollutants.

6.3.4 Hypoxia

Chaparro et al. (2009) investigated effects of external stress on conditions in the brood chambers for the gastropod *Crepipatella dilatata* and the bivalve *Ostrea chilensis*. Both species incubate their embryos in the pallial cavity for 4 weeks before the young emerge as larvae (oyster) or juveniles (gastropod). Females were stimulated to isolate the pallial cavity from the external environment by exposing them to reduced salinity. Subsequent changes in salinity, dissolved oxygen, pH, and ammonium within the pallial fluid were recorded. Oxygen availability for both species dropped down to hypoxic levels (<1.5 $mg\ O_2\ l^{-1}$) within 12 h and

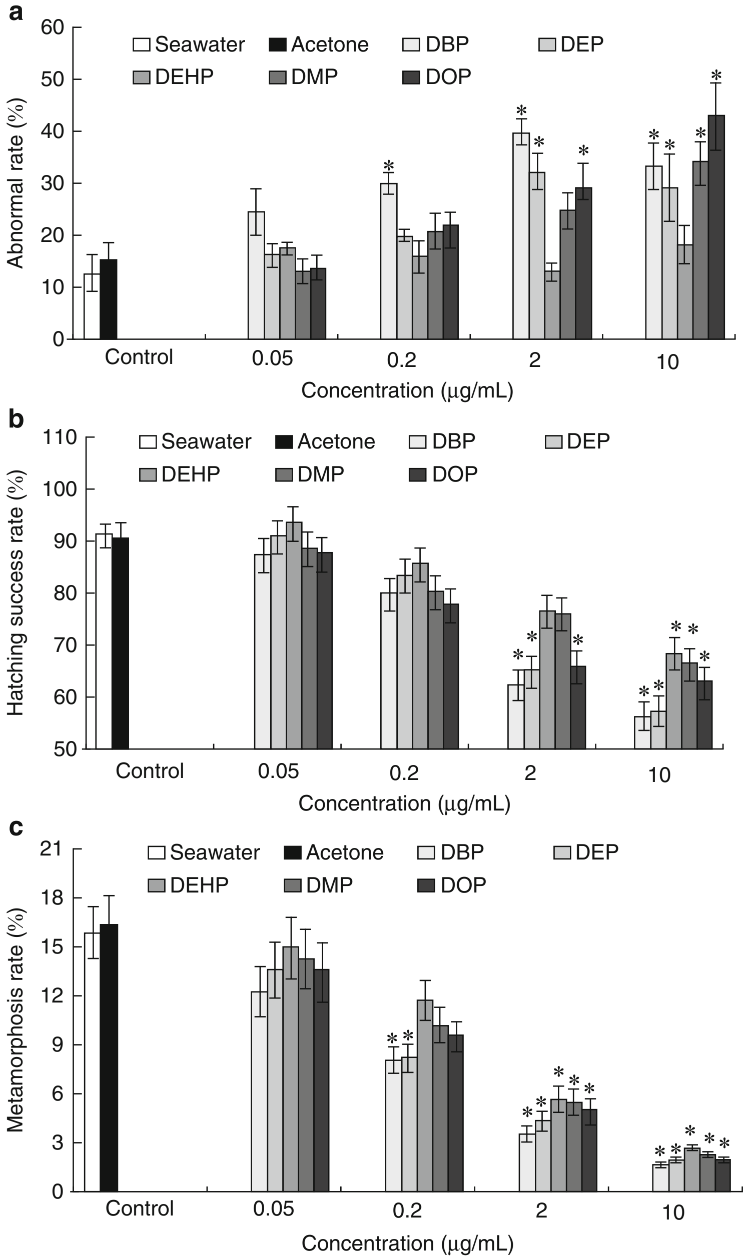


Fig. 6.9 Effects of phthalate esters on abalone embryos (M ± SD) * = significant difference from controls. (**a**–**c**) Abnormal rates, hatching success rate and metamorphosis rate (Reprinted from Zhou et al. 2011: 1118, courtesy Elsevier Publishing Co.)

20 min, respectively and the pH of the intrapallial fluid dropped substantially, suggesting a possible impact on shell formation for brooded veligers. Thus, while the isolation initially protected incubated embryos from low salinity stress, continued isolation resulted in increased hypoxic stress. Hypoxia can also be a risk for deposited egg masses. As with fishes, aggregation of embryos in clutches that lack internal circulation can increase the risk of hypoxia by limiting gas exchange. Hypoxia can slow development, increase mortality, and reduce size at hatching. The risks, however, can be reduced by association with photosynthetic organisms. Fernandes and Podolsky (2011) examined development of *Haminoea vesicula* as influenced by oviposition on eelgrass (*Zostera marina*) and found beneficial effects on development, mediated by light conditions. Under intermediate and high light levels, association with eelgrass accelerated development, while under dim light, the presence of the macrophyte increased development time and reduced hatchling shell size.

Hypoxic risks are increased by warming of the oceans. Impacts of a realistic warming scenario on the physiology of early life stages of cephalopods (squid *Loligo vulgaris* and cuttlefish *Sepia officinalis*) was investigated (Pimentel et al. 2012). During warming conditions the increase in oxygen consumption was much steeper in squid (28-fold increase) than cuttlefish (11-fold increase). Elevated catabolic activity and faster oxygen depletion within egg capsules intensified metabolic suppression in late embryos. Squid late-stage embryos were more affected by metabolic suppression than cuttlefish embryos. Combined effects of DO and temperature affected development of the snail, *Chorus giganteus*. In normoxia increasing water temperature from 12 to 18 °C reduced the time to hatch (Cancino et al. 2003). Hypoxia (50 % sat) slowed development and prevented both shell secretion and hatching from the egg capsule. Experimental transfer at weekly intervals, from normoxia to hypoxia and vice versa did not affect the number of hatched larvae. The inability to produce a shell under hypoxia was considered likely to be due to the combined effect of low external DO added to the intracapsular embryonic oxygen demands, which lowers the O_2 still further. Under such conditions, a decrease in intracapsular pH is likely, and, if so, embryos might divert carbonates away from shell calcification to balance pH changes.

6.3.5 Climate and Ocean Acidification

Early life stages of many marine invertebrates may be at particular risk from effects of global change (Byrne 2011). Laboratory experiments have shown that acidification impairs fertilization, cleavage, larval development, settlement and reproductive stages of marine calcifiers, including echinoderms, bivalves, corals and crustaceans (Figs. 6.10 and 6.11) (Kurihara 2008). The most vulnerable stages differed among species. While larval sea urchins and bivalves seemed to be most vulnerable, the settlement stage was the most severely affected in corals and shrimp.

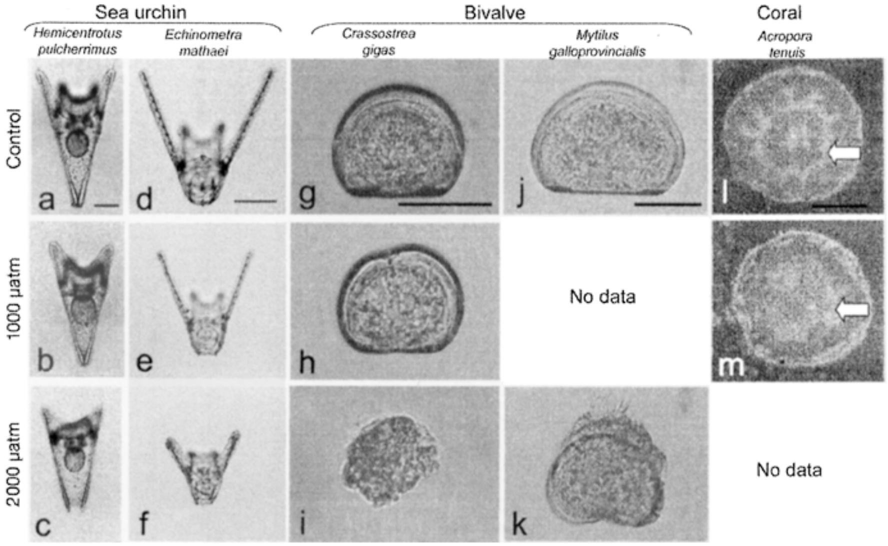


Fig. 6.10 Larval or polyp morphology of sea urchins *Hemicentrotus pulcherrimus* (**a**–**c**) and *Echinometra mathaei* (**d**–**f**), bivalves *Crassostrea gigas* (**g**–**i**) and *Mytilus galloprovincialis* (**j**, **k**) and the coral *Acropora tenuis* (**l**, **m**) incubated in control (**a**, **d**, **g**, **j**, **l**), 1,000 μatm pCO_2 (**b**, **e**, **h**, **m**) and 2,000 μatm pCO_2 (**c**, **f**, **i**, **k**) (Reprinted from Kurihara 2008: 278, courtesy of Inter-Research)

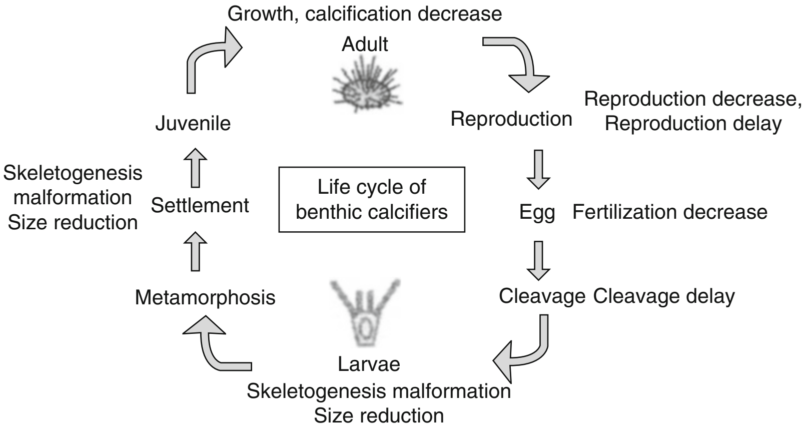


Fig. 6.11 Summary of CO_2 effects at different life cycle stages of benthic calcifiers under CO_2 concentrations expected to occur in the future ocean (380–2,000 μatm pCO_2/pH 8.2–7.3) (Reprinted from Kurihara 2008: 281, courtesy of Inter-Research)

This can be explained since most echinoderms and mollusks begin shell synthesis during larval stages, while corals start calcification at the settlement stage.

Ocean warming also can have negative effects on mollusk embryos. Recently-spawned egg masses of the squid *Loligo pealeii* were collected and reared until hatching at present day and projected near future (+2°C) temperatures. Under the projected near-future warming, the conditions inside the eggs promoted metabolic suppression, followed by premature hatching. Concomitantly, the less developed hatchlings showed more malformations (Rosa et al. 2012). After hatching, the metabolic burst associated with the transition from an embryo to a planktonic larval stage increased linearly with temperature. However, the greater exposure to environmental stress seemed to be compensated by physiological mechanisms that reduce the negative effects. Heat shock proteins (HSP70/HSC70) and antioxidant enzymes activities were stress responses to warming seen in hatchlings but not embryos. Pimentel et al. (2012) compared metabolic physiology of early life stages of squid *Loligo vulgaris* and cuttlefish *Sepia officinalis*. As described in the section on hypoxia, in warmer conditions the increase in oxygen consumption was much steeper in squid (28-fold increase) than cuttlefish (11-fold increase). Increased metabolism depleted oxygen inside egg capsules, which intensified metabolic suppression in late embryogenesis, especially in squid. Unlike benthic cuttlefish newborns, planktonic squid paralarvae use pulsed jet locomotion that requires more energy use. In warmer conditions, larvae will require more food so greater feeding will be crucial.

6.4 Other Taxa

Sea urchin development is a commonly used bioassay for various contaminants, so there is an extensive literature on this group, of which only a small fraction is presented here.

6.4.1 Echinoderms

Metals

Embryogenesis and early larval growth were evaluated after incubation of fertilized eggs of *Paracentrotus lividis* in seawater with metals (Fernandez and Beiras 2001). For individual metals the ranking of toxicity was Hg > Cu > Pb > Cd, with EC_{50} values of 21.9, 66.8, 509 and 9,240 $\mu g\ l^{-1}$, respectively. Lowest observed effect concentrations (LOEC) for early larval growth were approximately three times lower than the EC_{50} values for Hg, Cu and Pb, and more than two orders of magnitude lower for Cd.

King and Riddle (2001) exposed embryos and larvae of the sea urchin *Sterechinus neumayeri* to Cu, Cd, Zn and Pb, and examined effects on development to hatched blastulae after 6–8 days, and to 2-arm plutei after 20–23 days. For all metals, the long-term test to the 2-arm pluteus stage was more sensitive than the short-term test. Cu was the most toxic with EC50s of 11.4 and 1.4 $\mu g\ l^{-1}$

after 6–8 days and 20–23 days exposure respectively. Exposure to Cd >2 mg l^{-1} decreased the proportion of embryos developing normally to hatched blastulae (EC50 $= 6.9$ mg l^{-1}) and concentrations >0.2 mg l^{-1} decreased normal 2-arm plutei. EC50 values for Zn were 2,230 and 326.8 μg l^{-1} for the short- and long-term tests respectively. Pb had no effect up to 3.2 mg l^{-1}. As the concentration of Cu shown to inhibit development is similar to levels found at impacted sites in Antarctic nearshore environments, this metal may have an impact on the development of *S. neumayeri*.

Polluted water from an abandoned lead mine was evaluated using sea urchin (*Anthocidaris crassispina*) bioassays. The water contained elevated Mn, Pb, Cd, Zn, Cr, Ni, Fe, and Cu. The water inhibited, in a dose-dependent manner, the first cleavage and pluteus formation (Kobayashi and Okamura 2004). Some malformations, such as a radialized pluteus, exo-gastrula, and "spaceship Apollo-like" embryos were induced by undiluted test waters. Zinc alone also induced the same anomaly. Zn in the test seawater was one of the metals that caused the anomalies, but not all of the toxicity was caused by Zn. It was speculated that interactive effects of Zn and possibly Mn and Ni, were occurring. Ni also can be teratogenic to sea urchin embryos. Malformations were observed in *Strongylocentrotus intermedius* exposed to 10 mg l^{-1} Ni for 30 h. The most critical stage for triggering effects was thought to be the blastula (Ryu et al. 2012).

TBT at 3.5 μg l^{-1} delayed cleavage, and inhibited echinochrome synthesis in embryonic *Paracentrotus lividus* (Ozretic et al. 1998). Perina et al. (2011) used embryos of *Lytechinus variegatus* to compare effects of TBT and triphenyltin (TPT) (banned antifouling agents), with Irgarol and Diuron (two new commonly used booster biocides). Development was arrested at the gastrula and blastula stages at 1.25 and 2.5 μg l^{-1} TBT, respectively, while development continued to the pluteus stage in the same concentrations of TPT. Embryos reached the prism and morula stages at 5 μg l^{-1} of TPT and TBT, respectively. Effects of Irgarol were more severe than those of Diuron. The pluteus stage was reached at all tested Diuron concentrations (125–8,000 μg l^{-1}) while development was arrested at blastula/gastrula stages at the highest Irgarol concentration (8,000 μg l^{-1}), showing that the newer antifouling agents were less toxic to developing echinoderms. (However, they are very toxic to other organisms). The possibility of delayed effects was not considered. Bellas et al. (2005) investigated effects of the new antifouling compound zinc pyrithione (Zpt) on the embryonic development of sea urchin (*Paracentrotus lividus*). The median effective concentration (EC_{50}) was 7.7 nM. Toxic effects of Zpt on larval growth were detected at 0.5 nM. Predicted environmental concentrations of Zpt in pleasure craft harbors are higher than the predicted no effect concentrations, indicating that Zpt may pose a threat to this species from exposure in the field.

Pesticides and CECs

The chlorinated pesticides methoxychlor, dieldrin, and lindane affect fertilization and early development of sea urchin, *Paracentrotus lividus*. Pesando et al. (2004)

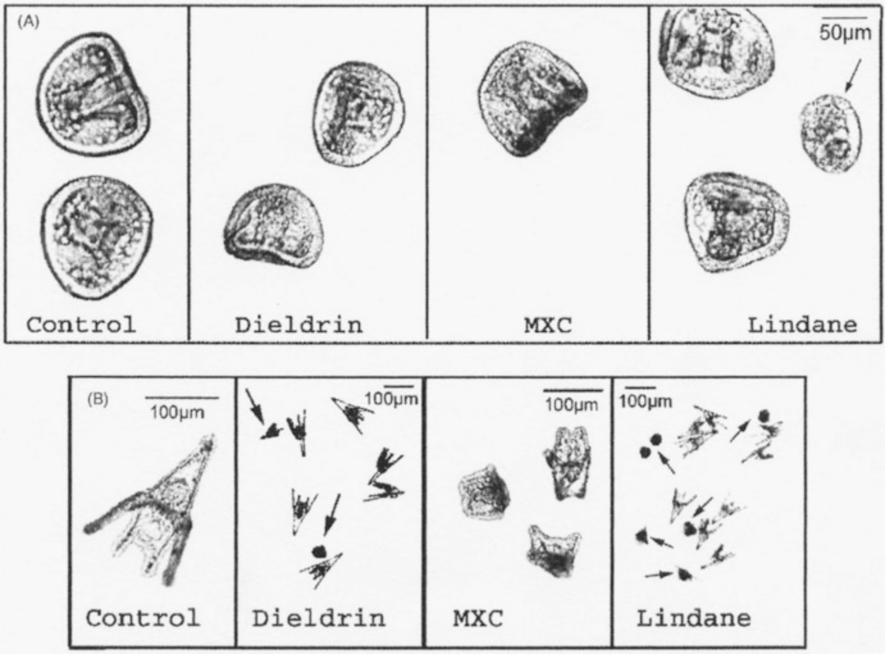


Fig. 6.12 Effects of 100 μM dieldrin, lindane and MXC (methoxychlor) on early sea urchin development. Eggs treated for 1 h with each pesticide, rinsed, fertilized and let to develop. (**a**) Gastrula stage – all normal except for lindane-treated embryos where some were altered (**b**) Pluteus stage – some dieldrin and lindane-treated were abnormal and all MXC treated eggs were abnormal (Reprinted from Pesando et al. 2004: 233, courtesy Elsevier Publishing Co.)

observed that fertilization decreased when sperm were incubated for various periods of time with 100 μM of dieldrin, methoxychlor (MXC), or lindane. Treatment of eggs (1 h with 100 μM) did not prevent fertilization, but increased the rate of polyspermy, delayed or blocked the first mitotic divisions, and altered early embryonic development (Figs. 6.12 and 6.13). All these pesticides altered biochemical pathways that control first mitotic divisions and early development, including intracellular calcium homeostasis, mitosis promoting factor, and formation of the mitotic spindle. Lindane was the most potent of the three pesticides. All these effects were observed at relatively high concentrations.

Buono et al. (2012) examined effects of three current-use pesticides, pentachlorophenol (PCP), azinphos-methyl (AZM), and chlorpyrifos, on *Paracentrotus lividus* early development. PCP at high concentrations modified cytoskeleton assembly, while low concentrations altered the deposition of the larval skeleton. At 500 μg l^{-1} of the organophosphates developmental arrest was produced. The sea urchin *Psammechinus miliaris*, unlike amphibians and fish, was not sensitive to dioxin-like toxicity (Anselmo et al. 2011), but Triclosan >500 nM was acutely toxic. Morphological abnormalities were induced at >50 nM hexabromocyclododecane

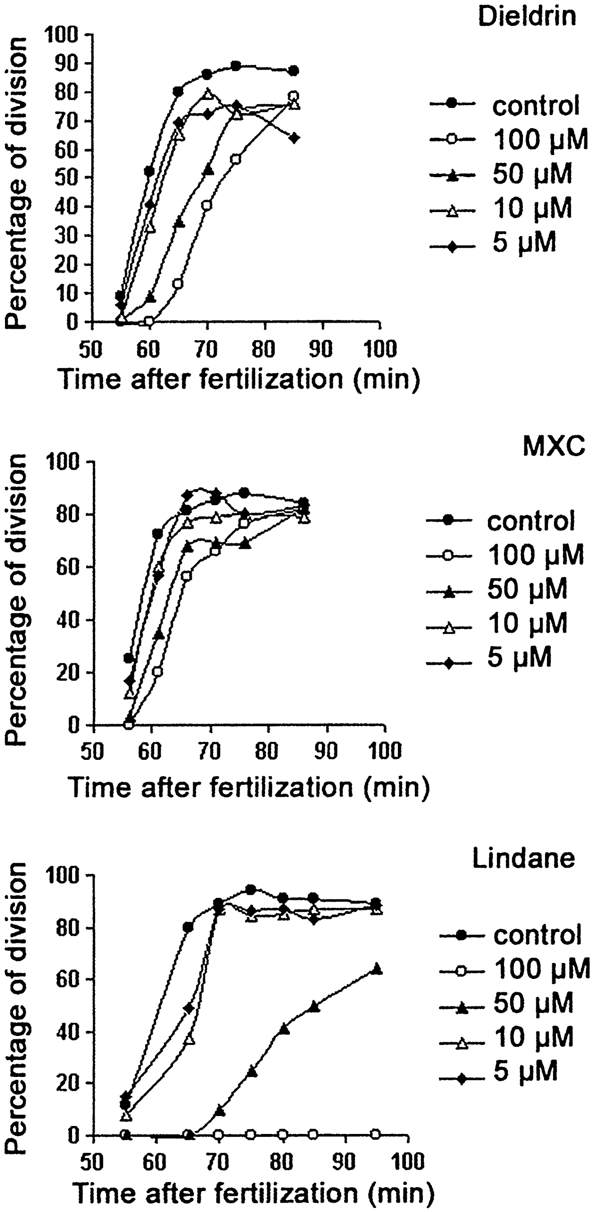


Fig. 6.13 Effects of 100 μM dieldrin (**a**), MXC (methyoxychlor) (**b**) and lindane (**c**) on the first division cycle. Eggs treated for 1 h with each pesticide, rinsed, fertilized and let to develop (Reprinted from Pesando et al. 2004: 230, courtesy Elsevier Publishing Co.)

(HBCD) and 1,000 nM tetrabromobisphenol A (TBBPA). Larval development was delayed in levels >25 nM HBCD and 500 nM TBBPA, and heptadecafluorooctane sulfonic acid (PFOS) exposure accelerated larval development.

Ocean Acidification/Climate Change

Developmental effects of near-future ocean acidification/hypercapnia were investigated by Doo et al. (2012) in the sea urchin *Centrostephanus rodgersii*. Decreased pH (−0.3 to −0.5 pH units) or increased $p\mathrm{CO}_2$ significantly reduced the percentage of normal larvae. Larval growth was reduced, with smaller larvae in the pH 7.6/1,800 ppm treatments. The impact of acidification on development was similar on days 3 and 5, indicating effects early in development. On day 3, increased abnormalities in the pH 7.6/1,600 ppm treatment were seen in aberrant prism stage larvae and arrested/dead embryos. By day 5, echinoplutei in this treatment had smaller arm rods. Observations of smaller larvae may have ecological significance because larval success may be a potential bottleneck for survival. However, Foo et al. (2012) examined genetic variation in tolerance of early development of this species to future ocean conditions. Multiple crosses were used to quantify interactive effects of warming (+2–4 °C) and acidification (−0.3 to 0.5 pH units) across 27 family lines. Acidification, but not temperature, decreased the percentage of cleaving embryos, while temperature, but not acidification decreased the percentage of gastrulation. Cleavage and gastrulation success in response to both stressors was strongly affected by parental identity. Genotypes that did well at lower pH also did well in higher temperatures, indicating the presence of heritable variation in thermal tolerance. The presence of tolerant genotypes indicates the potential to adapt to warming and acidification.

Byrne et al. (2013) examined effects of warming and acidification on development to the non-calcifying larval stage in the sea star *Patiriella regularis*. Fertilization was resilient to both stressors, as were cleavage stage embryos. Warming increased developmental rate across all pHs. For blastulae, there was a complex interaction between stressors, with +4 °C/pH 7.6 lethal to many embryos. A 4 °C warming increased mortality by the gastrula stage by 13–25 % across all pH levels. In conjunction with warming, pH 7.6 increased mortality by 25–27 % across all temperatures. For embryos that reached the 3 day bipinnaria stage, warming reduced the percentage of normal larvae and larval size, with no effect of acidification. These results highlight the importance of considering both warming and acidification. Bipinnaria reared to Day 28 (non-calcifying feeding larvae) were also sensitive. pH 7.6 and 7.8 resulted in smaller larvae and increased mortality. Since acidification reduced growth in larvae that do not calcify, the stunting is influenced by changes in metabolism and teratogenic effects.

6.4.2 Corals

Effects of the water-accommodated fraction (WAF) of a natural gas condensate on the brooding coral *Pocillopora damicornis* were studied (Villaneuva et al. 2011) in short-term (24 h) laboratory experiments. Exposure of gravid corals to >25 % WAF at early and late embryogenesis, resulted in abortion and early release of larvae, respectively, with more larvae expelled in corals treated with higher concentrations. Aborted larvae during early embryogenesis were premature – small size, with low metamorphic competency and low density of zooxanthellae.

Kipson et al. (2012) investigated responses of early life stages of a gorgonian coral, *Paramuricea clavata*, to the highest summer temperature recorded in the Mediterranean study area (25 °C), which simulates the end-of-century predicted warming (+3 °C). The results showed a severe impact on the viability of *P. clavata* embryos and larvae, resulting in reduced survivorship, abnormal embryonic development and impaired metamorphosis. The effect was rapid for embryos (after 7 h), but slower for larvae. This suggests that thermal stress during embryonic development may be the most critical factor for the viability of *P. clavata*.

6.4.3 Sea Turtles

Sea turtles come ashore to lay their eggs in the sand. Maulany et al. (2012) investigated the influences of the thermal nest environment of olive ridley turtles *Lepidochelys olivacea* on emergence success and quality of hatchlings of hatchery nests in Alas Purwo National Park, East Java, Indonesia. Nest temperatures above 34 °C for at least 3 consecutive days during incubation resulted in decreases in emergence success and locomotor performance of hatchlings.

Other problems may arise in sea turtle embryos in a warming world because they have temperature-dependent sex determination (TSD). The temperatures during embryonic development determine the sex of the offspring. Males are generally produced at lower incubation temperatures than females, with this change occurring over a narrow range of temperatures, as little as 1–2 °C. At cooler temperatures of 22.5 and 27 °C mostly males are produced, and at warmer temperatures around 30 °C only females are produced. Janzen (1994) found that annual offspring sex ratio was highly correlated with mean July air temperature and even modest increases in mean temperature may drastically skew the sex ratio. He was concerned that populations with temperature-dependent sex determination may be unable to evolve rapidly enough to counteract the consequences of rapid climate change. Binkley et al. (1998) monitored nests of leatherback turtles in Costa Rica and found that 18 monitored nests in which the temperature was 30^{o} C during the critical period, produced 100 % females. Since many nests reached this temperature, estimated sex ratios for two breeding seasons were >90 % female. However, factors such as increased rainfall and nest site selection may allow for the production of greater numbers of males (Houghton et al. 2007). Another risk for newly hatched turtles is light pollution from buildings near the beaches where they hatch. Sea turtle hatchlings normally navigate toward the sea by orienting away from the elevated, dark silhouette of the landward horizon (Salmon et al. 1992). When there are artificial bright lights on the beach, newly hatched turtles become disoriented and navigate toward the artificial light source, never finding the sea.

6.4.4 Rotifers

The toxicity of dispersed oil from the Gulf of Mexico spill was tested in rotifers. Rico-Martínez et al. (2013) tested the toxicity of oil from the *Deep Water Horizon* spill, dispersant (Corexit®) and mixtures on five strains of rotifers (*Brachionus plicatilis*), small planktonic animals with fast response time and sensitivity to toxicants. The oil-dispersant mixture was much (>50x) more toxic than either the oil or the dispersant alone in causing mortality in adults. Concentrations as low as 2.6 % of the mixture inhibited rotifer egg hatching by 50 %.

6.4.5 Tunicates

Matsushima et al. (2013) exposed embryos of the tunicate (a chordate), *Ciona intestinalis*, to seawater containing bisphenol-a (BPA). Abnormalities increased and the hatching rate decreased in a dose-dependent manner with exposures above 3 μM; swimming of larvae was affected after embryonic exposure to 1 μM BPA. Effects were most severe in embryos exposed early in development – at the 2-cell stage, within 7 h post-fertilization.

6.5 Discussion and Conclusions

It has been known for a long time that early life history stages are particularly vulnerable to environmental insults, and various toxicity tests are based on embryonic exposures. However, many of these tests use hatching success as the metric, while, as has been discussed here, many more subtle effects can be seen, evaluated and quantified, giving much greater insight into effects of contaminants. Another issue, not considered in toxicity tests, is the fact that some impacts of embryonic exposures are not apparent until much later in life, and can only be seen in larvae or adult animals.

A phenomenon seen across many taxa is fluctuating asymmetry (FA, differences between the left and right side of an organism), which is a subtle response that is a very sensitive indicator of stress. Allenbach (2011) reviewed papers over 30 years to evaluate the potential for FA to be an effective biomonitoring tool, and pointed out areas in which FA studies can be improved, including determining baseline FA levels in populations, increasing the number of laboratory studies to corroborate field observations and conducting true replications to validate previous findings. With improved experimental design and data analysis FA may become a powerful tool to assess very subtle effects of environmental degradation.

A recently noted phenomenon that has been studied in mammals, is the transgenerational nature of some contaminant effects. Some exposures during embryological development can induce a permanent epigenetic change in the germ line (sperm) that then transmits epigenetic transgenerational inheritance of abnormalities without any further exposure. The epigenetic transgenerational effects of a pesticide mixture (permethrin and insect repellant DEET), a plastic mixture (bisphenol A and phthalates), dioxin (TCDD) and a hydrocarbon mixture (jet fuel, JP8) were studied by Manikkam et al. (2012). After transient exposure of pregnant rats during the period of embryonic gonadal sex determination, the subsequent F1–F3 generations, with no further exposure, exhibited changes in pubertal onset and gonadal function. Such effects are likely to occur in marine animals as well, and are deserving of study.

References

Allenbach DM (2011) Fluctuating asymmetry and exogenous stress in fishes: a review. Rev Fish Biol Fish 21:355–376

Amin OA, Rodriguez E, Hernando M, Comoglio LI, Lopez LS, Medasani DA (1998) Effects of lead and cadmium on hatching of the Southern king crab *Lithodes santolla* (Decapoda, Anomura). Invert Reprod Dev 33:81–85

Anderson BS, Arenella-Parkerson D, Phillips BM, Tjeerdema RS, Crane D (2009) Preliminary investigation of the effects of dispersed Prudhoe Bay crude oil on developing topsmelt embryos, *Atherinops affinis*. Environ Pollut 157:1058–1061

Anselmo HM, Koerting L, Devito S, van den Berg JH, Dubbeldam M, Kwadijk C, Murk AJ (2011) Early life developmental effects of marine persistent organic pollutants on the sea urchin *Psammechinus miliaris*. Ecotoxicol Environ Saf 74:2182–2192

Armstrong D, Milleman R (1974) Effects of the insecticide sevin and its first hydrolytic product, 1-naphthol, on some early developmental stages of the bay mussel *Mytilus edulis*. Mar Biol 28:11–15

Arufe MI, Arellano JM, Albendín G, Sarasquete C (2010) Toxicity of parathion on embryo and yolk-sac larvae of gilthead seabream (*Sparus aurata* l.): effects on survival, cholinesterase, and carboxylesterase activity. Environ Toxicol 25:601–607

Baeza JA, Fernández M (2002) Active brood care in *Cancer setosus* (Crustacea: Decapoda): the relationship between female behaviour, embryo oxygen consumption and the cost of brooding. Funct Ecol 16:241–251

Barron MG, Carls MG, Short JW, Rice SD (2003) Photoenhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North Slope crude oil to Pacific herring eggs and larvae. Environ Toxicol Chem 22:650–660

Baumann H, Talmage SC, Gobler CJ (2011) Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. Nat Clim Change 2(1):38–41, http://dx.doi.org/10.1038/nclimate1291

Beiras R, Bellas J (2008) Inhibition of embryo development of the *Mytilus galloprovincialis* marine mussel by organic pollutants; assessment of risk for its extensive culture in the Galician Rias. Aquaculture 277:208–212

Beiras R, His E (1994) Effects of dissolved mercury on embryogenesis, survival and metamorphosis of *Crassostrea gigas* oyster larvae. Mar Ecol Prog Ser 113:95–103

Bellas J, Granmo Å, Beiras R (2005) Embryotoxicity of the antifouling biocide zinc pyrithione to sea urchin (*Paracentrotus lividus*) and mussel (*Mytilus edulis*). Mar Pollut Bull 50:1382–1385

Bellas J, Saco-Álvarez L, Nieto Ó, Bayona JM, Albaigés J, Beiras R (2013) Evaluation of artificially-weathered standard fuel oil toxicity by marine invertebrate embryogenesis bioassays. Chemosphere 90:1103–1108

Binkley CA, Spotila JR, Wilson KS, Paladino FV (1998) Sex determination and sex ratios of Pacific leatherback turtles, *Dermochelys coriacea*. Copeia 1998(2):291–300

Blaxter JHS (1977) The effect of copper on the eggs and larvae of plaice and herring. J Mar Biol Assoc UK 57:849–858

Botton M, Hodge M, Gonzalez TI (1998) High tolerance to tributyltin in embryos and larvae of the horseshoe crab, *Limulus polyphemus*. Estuar Coast 21:340–346

Boudreau M, Courtenay SC, MacLatchy DL, Berube CH, Parrott JL, van der Kraak G (2004) Utility of morphological abnormalities during early-life development of the estuarine mummichog, *Fundulus heteroclitus*, as an indicator of estrogenic and anti-estrogenic endocrine disruption. Environ Toxicol Chem 23:415–425

Boudreau PH, Sweezey MJ, Lee K, Hodson PV, Courtenay SC (2009) Toxicity of orimulsion-400® to early life stages of atlantic herring (*Clupea harengus*) and mummichog (*Fundulus heteroclitus*). Environ Toxicol Chem 28:1206–1217

Brannon EL, Collins KM, Brown JS, Neff JM, Parker KR, Stubblefield WA (2009) Toxicity of weathered *Exxon Valdez* crude oil to pink salmon embryos. Environ Toxicol Chem 25:962–972

Bue BG, Sharr S, Seeb JE (1998) Evidence of damage to pink salmon populations inhabiting Prince William Sound, Alaska two generations after the *Exxon Valdez* oil spill. Trans Am Fish Soc 127:35–43

Buono S, Manzo S, Maria G, Sansone G (2012) Toxic effects of pentachlorophenol, azinphos-methyl and chlorpyrifos on the development of *Paracentrotus lividus* embryos. Ecotoxicology 21:688–697

Byrne M (2011) Global change ecotoxicology: identification of early life history bottlenecks in marine invertebrates, variable species responses and variable experimental approaches. Mar Environ Res 76:3–15

Byrne M, Gonzalez-Bernat M, Doo S, Foo S, Soars N, Lamare M (2013) Effects of ocean warming and acidification on embryos and non-calcifying larvae of the invasive sea star *Patiriella regularis*. Mar Ecol Prog Ser 473:235–246

Cancino J, Gallardo J, Torres F (2003) Combined effects of dissolved oxygen concentration and water temperature on embryonic development and larval shell secretion in the marine snail *Chorus giganteus* (Gastropoda: Muricidae). Mar Biol 142:133–139

Carls MG, Meador JP (2009) A perspective on the toxicity of petrogenic PAHs to developing fish embryos related to environmental chemistry. Hum Ecol Risk Assess 15:1084–1098

Carls MG, Rice SD (1989) Abnormal development and growth reductions of Pollock *Theragra chalcogramma* embryos exposed to water-soluble fractions of oil. Fish Bull 88:29–37

Carls MG, Rice SD, Hose JE (1999) Sensitivity of fish embryos to weathered crude oil. Part 1. Low level exposure during incubation causes malformations and genetic damage in larval Pacific herring (*Clupea pallasi*). Environ Toxicol Chem 18:481–493

Chambers C, Davis D, Habeck EA, Roy NK, Wirgin I (2012) Toxic effects of PCB126 and TCDD on shortnose sturgeon and Atlantic sturgeon. Environ Toxicol Chem 31:2324–2337

Chaparro OR, Segura CJ, Montory JA, Navarro JM, Pechenik JA (2009) Brood chamber isolation during salinity stress in two estuarine mollusk species: from a protective nursery to a dangerous prison. Mar Ecol Prog Ser 374:145–155

Cosson RP, Martin J (1981) The effects of copper on the embryonic development, larvae, alevins, and juveniles of *Dicentrarchus labrax*(L.). Rapports Et Procés-verbaux Des Réunions 178:71

DeLorenzo ME, De Leon RG (2010) Toxicity of the insecticide Etofenprox to three life stages of the grass shrimp, *Palaemonetes pugio*. Arch Environ Contam Toxicol 58:985–990

Diez JM, Davenport J (1990) Energy exchange between the yolk and embryo of dogfish (*Scyliorhinus canicula* L.) eggs held under normoxic, hypoxic and transient anoxic conditions. Comp Biochem Physiol B 96:825–830

Doo SS, Dworjanyn SA, Foo SA, Soars NA, Byrne M (2012) Impacts of ocean acidification on development of the meroplanktonic larval stage of the sea urchin *Centrostephanus rodgersii*. ICES J Mar Sci 69:460–464

Eriksson SP, Nabbing M, Sjoman E (2006) Is brood care in *Nephrops norvegicus* during hypoxia adaptive or a waste of energy? Funct Ecol 20:1097–1104

Fernandes D, Podolsky RD (2011) Developmental consequences of association with a photosynthetic substrate for encapsulated embryos of an intertidal gastropod. J Exp Mar Biol Ecol 407:370–376

Fernandez N, Beiras R (2001) Combined toxicity of dissolved mercury with copper, lead and cadmium on embryogenesis and early larval growth of the *Paracentrotus lividus* sea urchin. Ecotoxicology 10:263–271

Fernández M, Ruiz-Tagle N, Cifuentes S, Pörtner H-O, Arntz W (2003) Oxygen-dependent asynchrony of embryonic development in embryo masses of brachyuran crabs. Mar Biol 142:559–565

Fisher WS, Foss SS (1993) A simple test for toxicity of Number 2 fuel oil and oil dispersants to embryos of grass shrimp, *Palaemonetes pugio*. Mar Pollut Bull 26:385–391

Foekema EM, Deerenberg CM, Murk AJ (2008) Prolonged ELS test with the marine flatfish sole (*Solea solea*) shows delayed toxic effects of previous exposure to PCB 126. Aquat Toxicol 90:197–203

Foo SA, Dworjanyn SA, Poore AGB, Byrne M (2012) Adaptive capacity of the habitat modifying sea urchin *Centrostephanus rodgersii* to ocean warming and ocean acidification: performance of early embryos. PLoS One 7(8):art. e42497

Franke A, Clemmesen C (2011) Effect of ocean acidification on early life stages of Atlantic herring (*Clupea harengus* L.). Biogeosciences 8:3697–3707

Frantzen M, Falk-Petersen IB, Nahrgang J, Smith TJ, Olsen GH, Hangstad TA, Camus L (2012) Toxicity of crude oil and pyrene to the embryos of beach spawning capelin (*Mallotus villosus*). Aquat Toxicol 108(42–52):2012

Fuller C, Bonner J, Page C, Ernest A, McDonald T, McDonald S (2004) Comparative toxicity of oil, dispersant, and oil plus dispersant to several marine species. Environ Toxicol Chem 23:2941–2949

Ghekiere A, Fockedey N, Verslycke T, Vincx M, Janssen CR (2007) Marsupial development in the mysid *Neomysis integer* (Crustacea: Mysidacea) to evaluate the effects of endocrine-disrupting chemicals. Ecotoxicol Environ Saf 66:9–15

Goodman LR, Hemmer MJ, Middaugh DP, Moore JC (1992) Effects of fenvalerate on the early life stages of topsmelt (*Atherinops affinis*). Environ Toxicol Chem 11:409–414

Granmo A, Ekelund R, Sneli J-A, Berggren M, Svavarsson J (2002) Effects of antifouling paint components (TBTO, copper and triazine) on the early development of embryos in cod (*Gadus morhua* L.). Mar Pollut Bull 44:1142–1148

Greer CD, Hodson PV, Li Z, King T, Lee K (2012) Toxicity of crude oil chemically dispersed in a wave tank to embryos of Atlantic herring (*Clupea harengus*). Environ Toxicol Chem 31:1324–1333

Hanson D, Goodman LR, Wilson AJ (1977a) Kepone®: chronic effects on embryo, fry, juvenile, and adult sheepshead minnows (*Cyprinodon variegatus*). Chesap Sci 18:227–232

Hanson DJ, Schimel SC, Forester J (1977b) Endrin: effects on the entire life cycle of a saltwater fish, *Cyprinodon variegatus*. J Toxicol Environ Health 3:721–733

Hassell KL, Coutin PC, Nugegoda D (2008) Hypoxia impairs embryo development and survival in black bream (*Acanthopagrus butcheri*). Mar Pollut Bull 57:302–306

He C, Wang C, Li B, Wu M, Geng H, Chen Y, Zuo Z (2012) Exposure of *Sebastiscus marmoratus* embryos to pyrene results in neurodevelopmental defects and disturbs related mechanisms. Aquat Toxicol 116–117:109–115

Heintz R, Short JW, Rice SD (1999) Sensitivity of fish embryos to weathered crude oil: Part II. Increased mortality of pink salmon (*Oncorhynchus gorbuscha*) embryos incubating downstream from weathered *Exxon Valdez* crude oil. Environ Toxicol Chem 18:494–503

Heintz RA, Rice SD, Wertheimer AC, Bradshaw RF, Thrower FP, Joyce JE, Short JW (2000) Delayed effects on growth and marine survival of pink salmon *Oncorhynchus gorbuscha* after exposure to crude oil during embryonic development. Mar Ecol Prog Ser 208:205–216

Heintz RA, Short JW, Rice SD, Carls MG (2012a) The authors' reply. Environ Toxicol Chem 31:472–473

Heintz RA, Short JW, Rice SD, Carls MG (2012b) The authors' second reply. Environ Toxicol Chem 31:475–476

Hemmer M, Middaugh DP, Moore JC (1990) Effects of temperature and salinity on *Menidia beryllina* embryos exposed to terbufos. Dis Aquat Org 8:127–136

Hicken C, Linbo TL, Baldwin DH, Willis ML, Myers MS, Holland L, Larsen M, Stekoll MS, Rice SD, Collier TK, Scholz NL, Incardona JP (2011) Sublethal exposure to crude oil during embryonic development alters cardiac morphology and reduces aerobic capacity in adult fish. Proc Natl Acad Sci USA 108:7086–7090

Horst MN, Walker AN (1999) Effects of the pesticide Methoprene on morphogenesis and shell formation in the blue crab *Callinectes sapidus*. J Crustace Biol 19:699–707

Hose JE, McGurk MD, Marty GT, Hinton DE, Brown ED, Baker TT (1996) Sublethal effects of the *Exxon Valdez* oil spill on herring embryos and larvae: morphological, cytogenetic, and histopathological assessments 1989–1991. Can J Fish Aquat Sci 53:2355–2365

Houghton JD, Myers AE, Lloyd C, King RS, Isaacs C, Hays GC (2007) Protracted rainfall decreases temperature within leatherback turtle (*Dermochelys coriacea*) clutches in Grenada, West Indies: ecological implications for a species displaying temperature dependent sex determination. J Exp Mar Biol Ecol 345:71–77

Huang W, Cao L, Shan X, Xiao Z, Wang Q, Dou S (2010) Toxic effects of zinc on the development, growth, and survival of Red Sea Bream *Pagrus major* embryos and larvae. Arch Environ Contam Toxicol 58:140–150

Huang Q, Fang C, Wu X, Fan J, Dong S (2011) Perfluorooctane sulfonate impairs the cardiac development of a marine medaka (*Oryzias melastigma*). Aquat Toxicol 105:71–77

Humphrey C, Klumpp DW (2003) Toxicity of chlorpyrifos to the early life history stages of eastern rainbowfish *Melanotaenia splendida splendida* (Peters 1866) in tropical Australia. Environ Toxicol 18:418–427

Iida M, Kim E-Y, Murakami Y, Shima Y, Iwata H (2013) Toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the peripheral nervous system of developing red seabream (*Pagrus major*). Aquat Toxicol 128–129:193–202

Incardona JP, Collier TK, Scholz N (2004) Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. Toxicol Appl Pharmacol 196:191–205

Incardona JP et al (2012) Unexpectedly high mortality in Pacific herring embryos exposed to the 2007 *Cosco Busan* oil spill in San Francisco Bay. Proc Natl Acad Sci USA 109:E51–E58

Irie K, Kawaguchi M, Mizuno K, Song J-Y, Nakayama K, Kitamura S-I, Murakami Y (2011) Effect of heavy oil on the development of the nervous system of floating and sinking teleost eggs. Mar Pollut Bull 63:297–302

Janzen F (1994) Climate change and temperature-dependent sex determination in reptiles. Proc Natl Acad Sci USA 91:7487–7490

Jezierska B, Ługowska K, Witeska M (2009) The effects of heavy metals on embryonic development of fish (a review). Fish Physiol Biochem 35:625–640

Key PB, Meyer SL, Chung KW (2003) Lethal and sublethal effects of the fungicide chlorothalonil on three life stages of the grass shrimp, *Palaemonetes pugio*. J Environ Sci Health B 38:539–549

King CK, Riddle MJ (2001) Effects of metal contaminants on the development of the common Antarctic sea urchin *Sterechinus neumayeri* and comparisons of sensitivity with tropical and temperate echinoids. Mar Ecol Prog Ser 215:143–154

Kingsford MJ, Gray CA (1996) Influence of pollutants and oceanography on abundance and deformities of wild fish larvae. In: Schmitt RJ, Osenberg C (eds) Detecting ecological impacts: concepts and applications in coastal habitats. Academic, New York

Kipson S, Linares C, Teixidó N, Bakran-Petricioli T, Garrabou J (2012) Effects of thermal stress on early developmental stages of a gorgonian coral. Mar Ecol Prog Ser 470:69–78

Klein-MacPhee G, Cardin JA, Berry WJ (1984) Effects of silver on eggs and larvae of the winter flounder. Trans Am Fish Soc 113:247–251

Klumpp DW, Humphrey C, Huasheng H, Tao F (2002) Toxic contaminants and their biological effects in coastal waters of Xiamen, China: II. Biomarkers and embryo malformation rates as indicators of pollution stress in fish. Mar Pollut Bull 44:761–769

Kobayashi N, Okamura H (2004) Effects of heavy metals on sea urchin embryo development. 1. Tracing the cause by the effects. Chemosphere 55:1403–1412

Kocan RM, Hose JE, Brown ED, Baker TT (1996a) Pacific herring (*Clupea pallasi*) embryo sensitivity to Prudhoe Bay petroleum hydrocarbons: Laboratory evaluation and in situ exposure at oiled and unoiled sites in Prince William Sound. Can J Fish Aquat Sci 53:2366–2375

Kocan RM, Marty GD, Okihiro MS, Brown ED, Baker TT (1996b) Reproductive success and histopathology of individual Prince William Sound Pacific herring 3 years after the *Exxon Valdez* oil spill. Can J Fish Aquat Sci 53:2388–2393

Kopf PG, Walker M (2009) Overview of developmental heart defects by dioxins, PCBs, and pesticides. J Environ Sci Health C 27:276–285

Kurihara K (2008) Effects of CO2-driven ocean acidification on the early developmental stages of invertebrates. Mar Ecol Prog Ser 373:275–284

Lavolpe M, Lopez Greco L, Kesselman D, Rodriguez E (2004) Differential toxicity of copper zinc, and lead during the embryonic development of *Chasmagnathus granulatus* (Brachyura, Varunidae). Environ Toxicol Chem 23:960–967

Lee WY, Nicol JAC (1980) Toxicity of a fuel oil to the eggs of *Parhyale hawaiensis* and A*mphithoe valida* (Amphipoda). Mar Environ Res 3:297–305

Lee RF, Oshima Y (1998) Effects of selected pesticides, metals and organometallics on development of blue crab (*Callinectes sapidus*) embryos. Mar Environ Res 46:479–482

Lee RF, O'Malley K, Oshima Y (1996) Effects of toxicants on developing oocytes and embryos of the blue crab, *Callinectes sapidus*. Mar Environ Res 42:125–128

Lee RF, Kim GB, Maruya KA, Steinert SA, Oshima Y (2000) DNA strand breaks (comet assay) and embryo development effects in grass shrimp (*Palaemonetes pugio*) embryos after exposure to genotoxicants. Mar Environ Res 50:553–557

Lee RF, Kim GB, Lee HJ (2012) Genotoxicity and development effects of brominated flame retardant PBDEs and UV-exposed PBDEs on grass shrimp (*Palaemonetes pugio*) embryo. Mar Pollut Bull 64:2892–2895

Linden O, Laughlin R, Sharp JR, Neff JM (1980) The combined effect of salinity, temperature and oil on the growth pattern of embryos of the killifish *Fundulus heteroclitus* Walbaum. Mar Environ Res 3:129–144

Longwell AC, Chang S, Hebert A, Hughes JB, Perry D (1992) Pollution and developmental abnormalities of Atlantic fishes. Environ Biol Fishes 35:1–21

Lopez-Greco LS, Rodriguez EM, Bolaños J, Hernandez G, Fingerman M (2002) Toxicity of copper sulphate during early and late embryonic development of a palaemonid shrimp (Crustacea). Invertebr Reprod Dev 41:165–170

Lower N, Moore A (2003) Exposure to insecticides inhibits embryo development and emergence in Atlantic salmon (*Salmo salar* L.). Fish Physiol Biochem 28:431–432

Mai H, Cachot J, Brune J, Geffard O, Belles A, Budzinski H, Morin B (2012) Embryotoxic and genotoxic effects of heavy metals and pesticides on early life stages of Pacific oyster (*Crassostrea gigas*). Mar Pollut Bull 64:2663–2670

Manikkam M, Guerrero-Bosagna C, Tracey R, Haque MM, Skinner MK (2012) Transgenerational actions of environmental compounds on reproductive disease and identification of epigenetic biomarkers of ancestral exposures. PLoS One 7(2):e31901. doi:10.1371/journal.pone.0031901

Marty GD, Hinton DE, Short JW, Heintz RA, Rice SD, Dambach DM, Willits NH, Stegeman JJ (1997) Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome P4501A induction in pink salmon larvae continuously exposed to oil-contaminated gravel during development. Can J Zool 75:989–1007

Matsushima A, Ryan K, Shimohigashi Y, Meinertzhagen IA (2013) An endocrine disruptor, bisphenol A, affects development in the protochordate *Ciona intestinalis*: Hatching rates and swimming behavior alter in a dose-dependent manner. Environ Pollut 173:257–263

Maulany RI, Booth DT, Baxter GS (2012) The effect of incubation temperature on hatchling quality in the olive ridley turtle, *Lepidochelys olivacea*, from Alas Purwo National Park, East Java, Indonesia: implications for hatchery management. Mar Biol 159:2651–2661

McIntosh S, King T, Wu D, Hodson PV (2010) Toxicity of dispersed weathered crude oil to early life stages of Atlantic herring (*Clupea harengus*). Environ Toxicol Chem 29:1160–1167

McKenney CL, Cripe GM, Foss SS, Tuberty SR, Hoglund M (2004) Comparative embryonic and larval developmental responses of estuarine shrimp (*Palaemonetes pugio*) to the juvenile hormone agonist fenoxycarb. Arch Environ Contam Toxicol 47:463–470

Mejda S, Tremblay R, Lambert Y, Audet C (2012) Influence of different levels of dissolved oxygen on the success of Greenland halibut (*Reinhardtius hippoglossoides*) egg hatching and embryonic development. Mar Biol. doi:10.1007/s00227-012-1957

Melzner F, Gutowska MA, Langenbuch M, Dupont S, Lucassen M, Thorndyke MC, Bleich M, Pörtner H-O (2009) Physiological basis for high CO2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences 6:2313–2331

Middaugh DP, Fournie JW, Hemmer MJ (1990) Vertebral abnormalities in juvenile silversides, *Menidia beryllina* exposed to terbufos during embryogenesis. Dis Aquat Org 8:109–116

Middaugh DP, Resnick SM, Lantz SE, Heard CS, Mueller JG (1993) Toxicological assessment of biodegraded pentachlorophenol: Microtox® and fish embryos. Arch Environ Contam Toxicol 24:165–172

Mottier A, Kientz-Bouchart V, Serpentini A, Lebel JM, Jha AN, Costil K (2013) Effects of glyphosate-based herbicides on embryo-larval development and metamorphosis in the Pacific oyster, *Crassostrea gigas*. Aquat Toxicol 128–129:67–78, http://dx.doi.org/10.1016/j.aquatox.2012.12.002

Murphy ML, Heintz RA, Short JW, Larsen ML, Rice SD (1999) Recovery of pink salmon spawning areas after the *Exxon Valdez* oil spill. Trans Am Fish Soc 128:909–918

Ozretic B, Petrovic S, Krajnovic-Ozretic M (1998) Toxicity of TBT-based paint leachates on the embryonic development of the sea urchin, *Paracentrotus lividus*. Chemosphere 37:1109–1118

Page DS, Neff JM, Landrum PF, Chapman PM (2012a) Sensitivity of pink salmon (*Oncorhynchus gorbuscha*) embryos to weathered crude oil. Environ Toxicol Chem 31:469–471

Page DS, Neff JM, Landrum PF, Chapman PM (2012b) Authors response to Heintz et al. Environ Toxicol Chem 31:473–475

Page DS, Chapman PM, Landrum PF, Neff J, Elston R (2012c) A perspective on the toxicity of low concentrations of petroleum-derived polycyclic aromatic hydrocarbons to early life stages of herring and salmon. Hum Ecol Risk Assess 18:229–260

Palstra AP, van Ginneken VJT, Murk AJ, van den Thillart GE (2006) Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama? Naturwissenschaften 93:145–148

Perina D, de Souza Abessa DM, Pinho GL, Fillmann G (2011) Comparative toxicity of antifouling compounds on the development of sea urchin. Ecotoxicology 20:1870–1880

Perry D, Weis JS, Weis P (1988) Cytogenic effects of methylmercury in embryos of the killifish *Fundulus heteroclitus*. Arch Environ Contam Toxicol 17:569–574

Pesando D, Robert S, Huitorel P, Gutknecht E, Pereira L, Girard J-P, Ciapa B (2004) Effects of methoxychlor, dieldrin and lindane on sea urchin fertilization and early development. Aquat Toxicol 66:225–239

Pimentel MS, Trübenbach K, Faleiro F, Boavida-Portugal J, Repolho T, Rosa R (2012) Impact of ocean warming on the early ontogeny of cephalopods: a metabolic approach. Mar Biol 159:2051–2059

Prince R, Cooper KR (1995) Comparisons of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on chemically impacted and nonimpacted subpopulations of *Fundulus heteroclitus*. I. TCDD toxicity. Environ Toxicol Chem 14:579–588

Rayburn JR, Glas PS, Foss SS, Fisher WS (1996) Characterization of grass shrimp (*Palaemonetes pugio*) embryo toxicity tests using the water soluble fraction of number 2 fuel oil. Mar Pollut Bull 32:860–866

Rice SD, Thomas RE, Heintz RA, Wertheimer AC, Murphy ML, Carls MG, Short JW, Moles A (2001) Impacts to pink salmon following the *Exxon Valdez* oil spill: persistence, toxicity, sensitivity, and controversy. Rev Fish Sci 9:165–211

Rico-Martínez R, Snell TW, Shearer TL (2013) Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A® to the *Brachionus plicatilis* species complex (Rotifera). Environ Pollut 173:5–10

Rosa R, Pimentel MS, Boavida-Portugal J, Teixeira T, Trübenbach K et al (2012) Ocean warming enhances malformations, premature hatching, metabolic suppression and oxidative stress in the early life stages of a keystone squid. PLoS One 7(6):e38282. doi:10.1371/journal.pone.0038282

Rosenthal H, Sperling K-R (1974) Effects of cadmium on development and survival of herring eggs. In: Blaxter JH (ed) Early life history of fish. Springer, Berlin, pp 383–396

Ruiz JM, Bryan GW, Wigham GD, Gibbs PE (1995) Effects of tributyltin exposure on the reproduction and embryonic development of the bivalve *Scrobicularia plana*. Mar Environ Res 40:363–379

Ryu TK, Lee G, Rhee Y, Park H-S, Chang M, Lee S, Lee J, Lee T-K (2012) Identification of nickel response genes in abnormal early developments of sea urchin by differential display polymerase chain reaction. Ecotoxicol Environ Saf 84:18–24

Salmon M, Wyneken J, Fritz E, Lucas M (1992) Seafinding by hatchling sea turtles: role of brightness, silhouette and beach slope as orientation cues. Behaviour 122:56–77

Sanchez MV, Cahansky A, Lopez Greco L, Rodriguez EM (2005) Toxicity of mercury during the embryonic development of *Chasmagnathus granulatus* (Brachyura Varunidae). Environ Res 99:72–78

Sawada Y, Higuchi K, Honryo T, Agawa Y, Shiba H, Yokoyama T, Kumai H (2012) Estimated time change of dissolved oxygen and carbon dioxide concentrations in the interstitial water of red sea bream, *Pagrus major*, egg aggregation. Aquaculture 350–353:33–36

Şen H, Sunlu U (2007) Effects of cadmium ($CdCl_2$) on development and hatching of eggs in European squid (*Loligo vulgaris* Lamarck, 1798) (Cephalopoda: Loliginidae). Environ Monit Assess 133:371–378

Shi X, He C, Zuo Z, Li R, Chen D, Chen R, Wang C (2012) Pyrene exposure influences the craniofacial cartilage development of *Sebasticus marmoratus* embryos. Mar Environ Res 77:30–34

Skinner L, de Peyster A, Schiff K (1999) Developmental effects of urban storm water in Medaka (*Oryzias latipes*) and Inland Silverside (*Menidia beryllina*). Arch Environ Contam Toxicol 37:227–235

Somasundaram B, King PE, Shackley SE (1984) Some morphological effects of zinc upon the yolk sac larvae of *Clupea harengus*, L. J Fish Biol 25:333–343

Spangenberg JV, Cherr GN (1996) Developmental effects of barium exposure in a marine bivalve (*Mytilus californianus*). Environ Toxicol Chem 15:1769–1774

Sundelin B, Eriksson AK (1997) Malformations in embryos of the deposit-feeding amphipod *Monoporeia affinis* in the Baltic Sea. Mar Ecol Prog Ser 171:165–180

Swedmark M, Granmo A (1981) Effects of mixtures of heavy metals and a surfactant on development of cod (*Gadus morhua*). Rapp Proces Verb Reun Cons Perm Int Explor Mer 178:95–103

Thain JE, Waldock MJ (1986) The impact of tributyltin (TBT) antifouling paints on molluscan fisheries. Water Sci Technol 18:193–202

Thomason JC, Davenport J, Le Comte E (1996) Ventilatory mechanisms and the effect of hypoxia and temperature on the embryonic lesser spotted dogfish. J Fish Biol 49:965–972

Villaneuva RD, Yap HT, Montaño MN (2011) Reproductive effects of the water-accommodated fraction of a natural gas condensate in the Indo-Pacific reef-building coral *Pocillopora damicornis*. Ecotoxicol Environ Saf 74:2268–2274

Vines CA, Robbins T, Griffin FJ, Cherr GN (2000) The effects of diffusible creosote-derived compounds on development in Pacific herring (*Clupea pallasi*). Aquat Toxicol 51:225–239

von Westernhagen H, Dethlefsen V (1997) The use of malformations in pelagic fish embryos for pollution assessment. Hydrobiologia 352:241–250

von Westernhagen H, Dethlefsen V, Rosenthal H (1975) Combined effects of cadmium and salinity on development and survival of garpike eggs. Helgolander Wiss Meeresunters 27:268–282

von Westernhagen H, Landolt M, Kocan R, Fürstenberg G, Janssen D, Kremling K (1987) Toxicity of sea-surface microlayer: effects on herring and turbot embryos. Mar Environ Res 23: 273–290

Weis P, Weis JS (1974) Cardiac malformations induced by insecticides in embryos of the killifish, *Fundulus heteroclitus*. Teratology 10:263–268

Weis JS, Weis P (1976a) Optical malformations induced by insecticides in embryos of the silversides, *Menidia menidia*. Fish Bull 74:208–211

Weis P, Weis JS (1976b) Abnormal locomotion associated with skeletal malformations in the sheepshead minnow, *Cyprinodon variegatus*, exposed to malathion. Environ Res 12:196–200

Weis JS, Weis P (1977a) The effects of heavy metals on embryonic development of the killifish, *Fundulus heteroclitus*. J Fish Biol 11:49–54

Weis P, Weis JS (1977b) Methylmercury teratogenesis in the killifish, *Fundulus heteroclitus*. Teratology 16:317–327

Weis JS, Weis P, Heber M, Vaidya S (1981) Methylmercury tolerance of killifish (*Fundulus heteroclitus*) embryos from a polluted vs nonpolluted environment. Mar Biol 65:283–287

Weis JS, Weis P, Heber M (1982) Variation in response to methylmercury by killifish (*Fundulus heteroclitus*) embryos. In: Pearson JG, Foster R, Bishop WE (eds) Aquatic toxicology and hazard assessment: fifth conference. ASTM STP 766, pp 109–119. American Society for Testing and Materials, Philadelphia

Weis JS, Weis P, Wang F (1987) Developmental effects of tributyltin on the fiddler crab, *Uca pugilator* and killifish, *Fundulus heteroclitus*. In: Oceans '87 Proceedings of the international organotin symposium, Halifax, vol 4, pp 1456–1460

Weydman A, Søreide JE, Kwasniewski S, Widdicombe S (2012) Influence of CO_2-induced acidification on the reproduction of a key Arctic copepod *Calanus glacialis*. J Exp Mar Biol Ecol 428:39–42

Whitehead A, Dubansky B, Bodinier C, Garcia TI, Miles S, Pilley C, Raghunathan V, Roach JL, Walker N, Walter RB, Rice CD, Galvez F (2011) Science applications in the *Deepwater Horizon* oil spill special feature: genomic and physiological footprint of the *Deepwater Horizon* oil spill on resident marsh fishes. Proc Natl Acad Sci USA. doi:10.1073/pnas.1109545108

Williams ND, Holdway DA (2000) The effects of pulse-exposed cadmium and zinc on embryo hatchability, larval development, and survival of Australian crimson spotted rainbow fish (*Melanotaenia fluviatilis*). Environ Toxicol 15:165–173

Wilson JE, Cunningham P, Evans D, Costlow JD (1995) Using grass shrimp embryos to determine the effects of sediment on the toxicity and persistence of diflubenzuron in laboratory microcosms. In: Hughes JS, Biddinger G, Mones E (eds) Environmental toxicology and risk assessment: third volume, ASTM, STP 1218. ASTM Publication, Philadelphia

Wirth EF, Lund SA, Fulton MH, Scott GI (2001) Determination of acute mortality in adults and sublethal embryo responses of *Palaemonetes pugio* to endosulfan and methoprene exposure. Aquat Toxicol 53:9–18

Yamauchi M, Kim E-Y, Iwata H, Shima Y, Tanabe S (2006) Toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in developing red seabream (*Pagrus major*) embryo: an association of morphological deformities with AHR1, AHR2 and CYP1A expressions. Aquat Toxicol 80:166–179

Zhang J, Zuo Z, Sun P, Wang H, Yu A, Wang C (2012) Tributyltin exposure results in craniofacial cartilage defects in rockfish (*Sebasticus marmoratus*) embryos. Mar Environ Res 77:6–11

Zhou J, Cai Z-H, Xing K-Z (2011) Potential mechanisms of phthalate ester embryotoxicity in the abalone *Haliotis diversicolor supertexta*. Environ Pollut 159:1114–1122

Zhu X, Zhou J, Cai Z (2011) TiO_2 Nanoparticles in the marine environment: impact on the toxicity of tributyltin to abalone (*Haliotis diversicolor supertexta*) embryos. Environ Sci Technol 45:3753–3758

Chapter 7
Larval Development

Abstract After hatching, an organism leaves a relatively closed and protected system for life in a larger environment. Many studies have been performed exposing larval stages directly to contaminants or examining larvae after embryonic exposure. Larvae may be more sensitive than embryonic stages of the same organism, since embryos are protected by an outer membrane that may reduce contaminant uptake (e.g. chorion) that is no longer present in larvae. Larvae also must usually swim and obtain food. Most benthic invertebrates have planktonic larvae, which at a certain stage of development settle to the bottom to metamorphose into a juvenile stage in an appropriate habitat. Larval exposures to contaminants can lead to impaired settlement in the benthic environment and/or to delayed physiological disturbances as juveniles or adults.

Keywords Abnormalities • Delay • Development • Growth • Molting • Morphology • Settlement • Stages

Most marine animals hatch out as small planktonic organisms with little resemblance to the adult form that they will eventually become. Planktonic larvae are common even in taxa that are benthic as adults, such as decapod crustaceans, echinoderms, corals, and most bivalve and gastropod mollusks. The larvae of many species have some yolk, so they need not feed immediately, but most larvae eventually do need to acquire food, (generally smaller phytoplankton) which is a critical point in development. The transition to feeding requires the maturation of a number of organ systems – not only the digestive system, but the nervous system for detecting food and the musculo-skeletal system for moving to the food. There are some species, however, such as deep sea king crabs, in which the larvae are provided with enough yolk that they don't have to feed during their entire larval life (which may be as long as a year). For species that are benthic as adults, planktonic larvae enable greater dispersal. However, being small and floating, they are very vulnerable to predation and only a tiny percentage survive long enough to undergo metamorphosis. Metamorphosis is another critical stage; larvae must

J.S. Weis, *Physiological, Developmental and Behavioral Effects of Marine Pollution*,
DOI 10.1007/978-94-007-6949-6_7,

find an appropriate substrate on which to metamorphose. Contaminants are yet additional stresses on larvae, and the process of metamorphosis is very sensitive. In addition, pollution is probably a greater threat at the time when larvae are settling to metamorphose, as they are likely to encounter higher concentrations of contaminants at the bottom than in the water column. Delayed metamorphosis is a common response to environmental stressors.

7.1 Crustaceans

Since crustaceans go through a series of several larval stages (e.g., nauplius, zoea, megalopa) that undergo a series of molts as they develop, exposures can be performed on particular larval stages that may have differential sensitivity to particular contaminants. In general, later stage larvae are more tolerant than earlier ones. One very common response is a delay in molting from one stage to another and in metamorphosis to the juvenile form.

Molting and its hormonal regulation will be discussed in greater detail in the next chapter.

Deformities are also be produced by some contaminants. Delayed metamorphosis can itself produce "carry-over" effects in juveniles. Simith et al. (2013) found that delayed metamorphosis affected early juvenile survival and growth of mangrove crabs, *Ucides cordatus*. After delayed metamorphosis, survival of juveniles was 11–31 % lower and intermolt periods were 1.5–4.2 days longer than in controls. They also were smaller and had lower growth rates than juveniles derived from non-delayed megalopae. Most effects were observed in all five crab stages studied, indicating that the costs of delayed metamorphosis may persist throughout early juvenile stages.

7.1.1 Metals

Stage II nauplii of the barnacle *Balanus improvisus* were exposed to Cu and Cd by Lang et al. (1981). Cu in concentrations as low as 10 μg l^{-1} caused a dose related delay in molting to stage III nauplii and these had deformed appendages and loss of setae. Cd at 100 μg l^{-1} caused a molting delay but no abnormalities. Similar retarding effects of Cu on the shrimp *Metapenaeus ensis* were reported by Wong et al. 1995 (Fig. 7.1).

Rosenberg and Costlow (1976) exposed blue crab megalopae (the last larval stage of crabs) to 50 and 150 μg l^{-1} Cd and found delayed development from the megalopa to third crab stage. The effect was more pronounced at lower salinities, those preferred by the species. These authors also found that mud crab *Rhithropanopeus harrisii* zoea were more susceptible to Cd than the (older) megalopae. Lopez Greco et al. (2001) found differential responses of different larval stages of the pea crab *Tunicotheres moseri* to Cu (0.5–1,000 μg l^{-1}). The zoea I

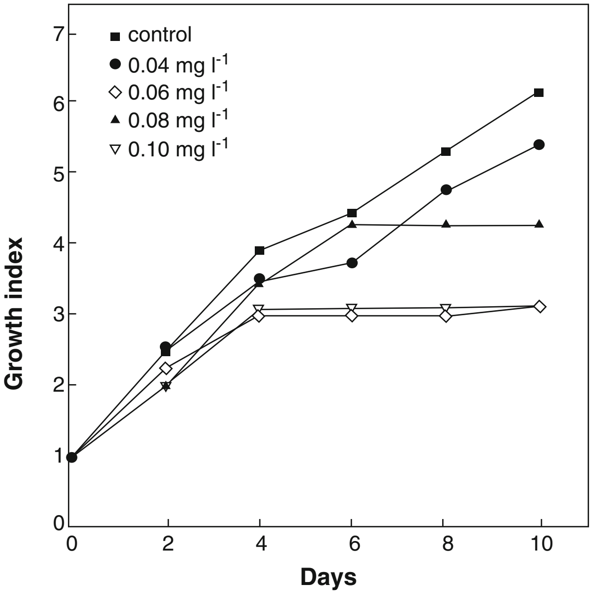


Fig. 7.1 Growth index of *Metapenaeus ensis* larvae during a 10 day exposure to copper. (Reprinted from Wong et al. 1995: 417, courtesy Elsevier Publishing Co.)

stage was the most sensitive, and those that molted to zoea II in 100 μg l^{-1} had abnormal setae causing reduced swimming. However, the abnormality could be reversed if they were maintained in clean water for subsequent molts to megalopa. Cu at 100 μg l^{-1} retarded the duration of zoea I stage but did not affect the duration of zoea II, and accelerated the molt from megalopa to first crab.

Developmental rates of blue crab megalopae were prolonged by exposure to Hg or to low salinity. While optimum time was 8 days, the period was extended to 10 days by salinity of 10 psu, and to 13 days at a salinity of 10 psu plus 20 μg l^{-1} Hg (McKenney and Costlow 1981). After metamorphosis, the first crab stage was more resistant. Exposure of *R. harrisii* and *Callinectes sapidus* larvae to hexavalent chromium slowed zoeal development from hatching to megalopa or to first crab stage (Bookhout et al. 1984). Swimming speed of *R. harrisii* larvae was modified; speed was elevated at low sublethal concentrations (7.2 mg l^{-1}) but depressed at higher concentrations (14 mg l^{-1}). Mortimer and Miller (1994) reported Cr, Ni Zn, and Cu effects on larvae of the crab *Portunus pelagicus* were inhibition of molting, increase in duration of developmental stages, and reduced size. Relative toxicities were $Cu^{2+} > Cd^{2+} \geq Zn^{2+} > Ni^{2+} > Cr(V1)$.

7.1.2 Organics

Pesticides

Effects of the chlorinated hydrocarbon mirex on zoeae of the crabs *Menippe mercenaria* and *Rhithropanopeus harrisii* included retardation and production of an extra (6th) zoeal stage (Bookhout and Costlow 1974). The duration of zoeal stages and development to 2nd crab stage of *R. harrisii* increased significantly with an increase in concentration of mirex from 0.01 to 10.0 μg l^{-1}. The organophosphate malathion at 0.02–0.08 mg l^{-1} also prolonged development time in these species (Bookhout and Monroe 1977). In contrast, Key et al. (1998) found that 30.0 μg l^{-1} malathion reduced the number of larval instars in the grass shrimp, *Palaemonetes pugio*.

Snyder and Mulder (2001) exposed lobster, *Homarus americanus*, larvae for 24 h to heptachlor (333 μg l^{-1}), a known endocrine disruptor, on different days of the 1st larval instar and followed the larvae for effects on timing of ecdysis to 2nd stage, molting hormone titers, and alterations in the levels of cytochrome P450 CYP45 and HSP70 proteins. Control larvae molted on Day 8–9 with 96 % survival. Larvae treated with heptachlor for 24 h on Day 3 molted successfully (92 %) on Day 10, a delay of 2 days. Molting days for other 24 h heptachlor treatments were: Day 1 treatment – molt on Day 11, 90 % success, Day 2 treatment – molt on Day 12, 85 % success, and Day 4 treatment – molt on Day 13, 65 % success. Larvae treated on days 5–6 never molted successfully, while those treated during the late premolt on Day 7 molted on Day 8 with the same survival percentage as controls. The effects of 24 h heptachlor treatment were thus very different, depending on the day of exposure and the sensitive period was days 4–6.

On days 1 or 2, heptachlor treatment caused a significant elevation in ecdysteroid levels the day after treatment, which corresponded to a delay in the premolt ecdysteroid peak prior to ecdysis. Larvae treated on day 3 had no immediate effect on ecdysteroid levels, although the premolt peak was delayed. Those treated on days 4 or 5 had diminished ecdysteroid levels for several days. Day 4 larvae had a 4–5 day delay in the premolt peak, while day 5- or day 6-treated larvae never showed any premolt ecdysteroid peak and all had died by day 16. CYP45 and HSP70 levels were elevated for several days following exposure. Delays in molting were correlated with alterations in ecdysteroid levels, suggesting that this pesticide may function as an endocrine disruptor in crustaceans.

Osterberg et al. (2012) investigated toxicity of a number of pesticides to megalopae of blue crabs. LC_{50} values ranged from 0.22 μg l^{-1} for megalopae in lambda-cyhalothrin to 316,000 μg l^{-1} for juveniles exposed to Roundup®. Treatment of intermolt megalopae with LC_{20} levels of Roundup® (5,500 μg l^{-1}) reduced the time to metamorphosis, but no effects resulted from treatment with the four active ingredient insecticides (lambda-cyhalothrin, imidacloprid, aldicarb, and acephate). Acephate, aldicarb, imidacloprid, and Roundup® increased mortality of juveniles shortly after molting. The sensitivity of molting crabs to these pesticides indicates that frequently molting animals are particularly vulnerable.

It is not surprising that insect growth regulators should affect crustacean larvae. Exposure to the juvenile hormone mimic methoprene caused crabs in suboptimal salinity to be unable to complete metamorphosis (Bookhout and Costlow 1974). The time required for development was not altered, however, by sublethal concentrations. Methoprene exposure of grass shrimp (*P. pugio*) larvae (McKenney and Matthews 1990) reduced completion of larval metamorphosis at 100 μg l^{-1}.

The first two larval stages and the final premetamorphic larval stage were more sensitive than intermediate stages. The total number of larval stages was not affected. Dimilin, a chitin synthesis inhibitor, was highly toxic to larvae of *R. harrisii*, *Sesarma reticulatum*, and *C. sapidus*, causing morphological abnormalities that became apparent at molt (Costlow 1979). Significant increases were seen in swimming speed of Stage I, II and III *R. harrisii* zoeae, with 0.3 μg l^{-1} being the lowest effective concentration. However, it did not delay larval development. Walker et al. (2005) found that low levels of methoprene had adverse effects on lobster larvae. It was toxic to stage II larvae at 1 μg l^{-1}. Stage IV larvae were more resistant, but had significant increases in molt frequency at 5 μg l^{-1}. Environmental concentrations of methoprene inhibited almost all protein synthesis in the hepatopancreas.

Crustacean larvae are often more sensitive than embryos. Larval development of *P. pugio*, was over two orders of magnitude more sensitive to disruption by methoprene and fenoxycarb than was embryonic development (McKenney 2005). Mud crab larvae *R. harrisii*, exhibited reduced metamorphic success at lower concentrations of methoprene and pyriproxyfen than grass shrimp larvae, suggesting that the more rigidly controlled metamorphic process in crabs is more sensitive to endocrine disruptors than the more flexible metamorphic pattern in shrimp. The final crab larva, the megalopa, was more sensitive to methoprene and fenoxycarb than earlier zoeal stages.

PCBs

Roesejadi et al. (1976) tested PCBs (Aroclor 1254) on larvae of *Palaemonetes pugio*. At concentrations above 3.2 μg l^{-1}, there was significant mortality. At 3.2 and 1.0 μg l^{-1}, there was not significant mortality but the duration of development increased and metamorphosis was delayed.

Oil

Zoea larvae of *R. harrisii* were exposed to low concentrations of the water soluble fraction (WSF) of jet fuel for the first 5 days of development. At some low levels there were no negative effects but increased megalopal weight (Laughlin et al. 1981). This was an early reported example of hormesis, a phenomenon that is now widely reported in diverse groups of organisms. The PAHs, phenanthrene and naphthalene, were also tested at 100–200 μg l^{-1}. Phenanthrene-exposed larvae had a decreased development rate, while naphthalene-exposed larvae developed faster than controls (Laughlin and Neff 1979).

Lee et al. (1970) studied effects of freshly prepared WSF of Number 2 fuel oil and WSF exposed to air for 48 h on larval shrimp *Lucifer faxoni*. Based on survival for 14 days, critical levels of toxicities of fresh oil were about 0.2 mg l^{-1}, while in weathered oil they were around 2 mg l^{-1}. A similar trend was found in studies of feeding and activity – fresh extracts were more toxic and effects on feeding were immediate and irreversible, while air-exposed WSF was less toxic, and the effect on feeding was delayed and reversible. Alkyl benzenes, indans, and naphthalenes were rapidly lost from the exposed solution, with negligible concentrations remaining after 24 h. The data suggest that the higher toxicity of fresh WSF was due to volatile aromatic hydrocarbons and there is reduced toxicity following evaporation. This finding is the opposite of that found with fish embryos in Alaska following the oil spill, discussed in the previous chapter. Respiration rates during an 8 h exposure to fresh WSF rose with increasing concentrations up to 30 % of WSF, then fell with further increases of WSF. This may have been hormesis, as later described by Laughlin et al. (1981).

The water accommodated fraction of Number 2 fuel oil (0.1 mg l^{-1}) was tested on *Cancer irroratus* zoea larvae by Johns and Pechenik (1980). Along with decreased survival to megalopae, larvae exhibited reduced food consumption and growth, while metabolic costs of maintenance increased. Larvae of the coonstripe shrimp (*Pandalus hypsinotus*) and king crab (*Paralithodes camtschaticus*) were exposed to solutions of the water-soluble fraction (WSF) of Cook Inlet crude oil in bioassays on intermolt stages I and II and the molt period from stage I to stage II (Mecklenberg et al. 1977). Molting larvae were more sensitive than intermolt larvae, and molting coonstripe shrimp larvae were more sensitive than molting king crab larvae. When molting larvae were exposed to high concentrations of the WSF for as little as 6 h, molting success was reduced by 10–30 % and some deaths occurred. When larvae were exposed to these high concentrations for 24 h or longer, molting declined 90–100 % and the larvae usually died. The lowest concentrations tested did not inhibit molting, but many larvae died after molting.

7.1.3 Contaminants of Emerging Concern

Various hormones and known endocrine disruptors were tested for effects on larval development in the copepod *Acartia tonsa* (Anderson et al. 2001). Tamoxifen (EC50 of 0.049 mg l^{-1}), 17α-ethinylestradiol (EC50 of 0.088 mg l^{-1}) and *p*-octylphenol (EC50 of 0.013 mg l^{-1}) were potent inhibitors of naupliar development. Other estrogens, 17β-estradiol, estrone, and bisphenol A, had little effect. Testosterone and progesterone did not inhibit development, but the antiandrogen flutamide (EC50 of 0.48 mg l^{-1}) had inhibitory effects. Juvenile hormone III (EC50 of 0.102 mg l^{-1}) was a potent inhibitor, as would be expected, but 20-hydroxyecdysone had no effect. Six of the 17 test compounds had LC50: EC50 ratios higher than 10, suggesting that naupliar development, as a parameter, is very sensitive to hormonal disrupters.

Key et al. (2008) examined the effects of a polybrominated diphenyl ether (flame retardant) compound, PBDE-47, on adult and larval stages of grass shrimp (*P. pugio*). The 96-h LC50 was 23.60 μg l^{-1} for larvae and 78.07 μg l^{-1} for adults. Four physiological biomarkers: glutathione (GSH), lipid peroxidation (LPx), cholesterol (CHL) and acetylcholinesterase (AChE) were assessed. GSH, LPx and AChE were not affected at concentrations up to 50 μg l^{-1} for 96 h. CHL levels were elevated at the lowest exposure concentrations tested, but significant differences were found only in adults. Effects thus were observed only at levels well above those reported in the environment, but these investigators did not examine development rate, which has been shown to be very sensitive. Breitholtz and Wollenberger (2003) examined effects of PBDE- 47, -99, and -100s on the larval development of the particle-feeding copepod *Nitocra spinipes*. Larval development rate significantly decreased in copepods exposed for 6 days to nominal concentrations of 0.013 mg l^{-1} BDE-47 and 0.03 mg l^{-1} BDE-99. Partitioning experiments showed that the major fractions were associated with particulate material, showing that development and reproduction in *N. spinipes* are sensitive to PBDEs and that ingestion of particle-adsorbed PBDEs is most likely the major route of exposure.

Chiu et al. 2012 investigated responses of barnacle larvae *Balanus amphitrite* to PBDEs, and demonstrated that chronic exposure to BDE-47 (up to 1,000 ng l^{-1}) throughout the entire larval stage did not affect settlement, development or growth, despite documented bioaccumulation.

7.1.4 Hypoxia

The ability to regulate O_2 uptake during declining DO co-occurred with metamorphosis from a planktonic to a benthic existence in the Norway lobster *Nephrops norvegicus*. The onset of this regulation appears to be related to the development of hypoxia-related ventilation by pleopods of exchange surfaces on the telson and uropods and a shift of hemocyanin from low to high O_2 affinity (Spicer and Eriksson 2003). This is experimental evidence for the use of uropods/telson by larval lobsters as supplemental gas exchange surfaces. The change occurred with calcification of the exoskeleton at metamorphosis, which restricts gas exchange over the general body surface. Pre-exposure of larvae to reduced DO resulted in the "adult" pattern of regulation being established before metamorphosis. Accelerating ontogeny of this regulation was a result of a shift from a low to a high hemocyanin O_2 affinity before metamorphosis and an increase in the magnitude of the hyperventilatory response in the planktonic larval stages. Thus, the development of respiratory regulation can be influenced by ambient O_2 levels.

7.1.5 Ocean Acidification/Climate Change

Pansch et al. (2012) investigated responses of the barnacle *Amphibalanus improvisus* to simulated warming and ocean acidification (OA) during early development. Nauplii and cyprids were exposed to 12, 20 and 27 °C and pCO_2 of 400 (current), 1,250 and 3,250 µatm for 8 and 4 weeks, respectively. Warming affected larvae more than OA. Increased temperatures favored survival and development of nauplii but decreased survival of cyprids, the subsequent stage. Acidification had no effect upon survival of nauplii but enhanced their development at low (12 °C) and high (27 °C) temperatures. At intermediate temperature (20 °C), nauplii were not affected, even by 3,250 µatm $p\mathrm{CO}_2$. No treatments affected settlement success, showing tolerance of *A. improvisus* larvae to OA predicted for the end of the century. Effects of warming and acidification were studied on larvae of the spider crab *Hyas araneus* from two locations, Svalbard (farther north) and Helgoland (Walther et al. 2010). Larvae were exposed at 3, 9 and 15 °C to present day conditions (380 mg l^{-1} CO_2) and to $p\mathrm{CO}_2$ conditions predicted for the near or medium-term future (710 and 3,000 mg l^{-1}). Enhanced $p\mathrm{CO}_2$ levels extended the duration of larval development and reduced larval growth and fitness, decreasing C/N ratio, a proxy of lipid content. Effects were greatest in the zoeal stages of Svalbard larvae, and during the megalopa stage of Helgoland larvae. The high sensitivity of megalopae from Svalbard to warming and of those from Helgoland to enhanced CO_2 suggests that this larval stage is a sensitive bottleneck within the life cycle of *H. araneus*. Arnold et al. (2009) investigated effects of acidified sea water ($p\mathrm{CO}_2$ approx. 1,200 mg l^{-1}) on early larval stages of the European lobster *Homarus gammarus*. Acidified water did not significantly affect carapace length or development, but reduced carapace mass during the final stage of larval development was seen along with reduced mineral (calcium and magnesium) content of the carapace. These alterations were considered the result of acidosis or hypercapnia interfering with normal homeostatic function.

7.1.6 Polluted Environment

Béguer et al. (2008) reported on morphological deformities in a population of *Palaemon* shrimp in the Gironde estuary (France). The most frequent abnormalities were of the cephalothorax and rostrum, and to a lesser extent scaphocerites and uropods; few cases of antenna or telson deformities were observed. Reports of morphological abnormalities of crustaceans were found in the literature, but previously described only isolated individuals, while in the Gironde estuary up to 40 % of individuals were affected. Authors considered the deformities to be due to pollutants, such as metals and PCBs.

Fiddler crabs (*Uca pugnax*) from a highly industrialized site contaminated with metals, PCBs, and PAHs near the Arthur Kill in Northern New Jersey (U.S.) produced many eggs, but had proportionately fewer larvae return to settle than in populations from a reference site (Bergey and Weis 2008). Larval life in highly contaminated waterways appeared to be the bottleneck in reducing population density at this site.

7.2 Mollusks

The basic molluscan larva is a trochophore, which feeds with two bands of cilia around its "equator" to sweep food into its mouth. The trochophore stage is often followed by a veliger stage in which the prototroch, the band of cilia nearest the apical tuft, develops into the velum ("veil"), a pair of cilia-bearing lobes with which the larva swims. Eventually, larvae sink to the bottom and metamorphose into the adult form. While most gastropods and bivalves undergo metamorphosis, cephalopods have direct development and hatch as a small form of the adult. There has been hardly any work on effects of toxicants on cephalopods, perhaps because it is not easy to raise them in the laboratory. In contrast, oysters have been used in standardized embryo-larval bioassays (His et al. 1997). In contrast with bivalves, which spawn, releasing eggs and sperm into the water column, gastropods (which are sometimes hermaphroditic) typically mate and have eggs develop in a sheltered site before larvae are released into the water. Growth retardation and abnormalities are common responses of larval mollusks to a variety of toxicants.

7.2.1 Metals

Calabrese et al. (1977) studied a suite of metals (Cu, Zn, Cd) on *Crassostrea virginica* and *Mercenaria mercenaria* larvae. The order of toxicity for oysters was Hg > Ag > Cu > Ni > Zn, while that for clams was Hg > Cu > Ag > Zn > Ni. All retarded shell growth at their LC_{50} concentrations, but Ni retarded shell growth at the LC_5 concentration of 1.1 mg l^{-1}. Toxicities were significantly altered by changing the salinity and temperature (MacInnes and Calabrese 1979). Fertilized eggs of the abalone *Haliotis rubra* were exposed to a range of concentrations of Cd, Cu, Fe, Pb, Hg, and Zn for 48 h after which survival and morphological abnormalities of veliger larvae were recorded (Gorski and Nugegoda 2006). The effective median concentrations affecting morphological development in decreasing order of toxicity were: Cu (7 μg l^{-1}), Hg (21 μg l^{-1}), Zn (35 μg l^{-1}), Fe (4,102 μg l^{-1}), Cd (4,515 μg l^{-1}), and Pb (5,111 μg l^{-1}). It is surprising that Cu was more toxic than Hg, and that Zn was more toxic than Cd and Pb. Effects of Zn on development of oyster (*C. gigas*) larvae were reported by Brereton et al. (1973). At 0.05 mg l^{-1} growth rates of 48-h veliger larvae were slower than controls but development was normal. Increasing concentrations decreased growth and increased the incidence of abnormality and mortality. At concentrations of 0.15 mg l^{-1} veligers were abnormal and had no umbo development.

Exposure of *Mytilus edulis* to 8 μg l^{-1} Cu during the veliger larval or post-larval stages had no significant effects on survivorship or shell growth (Hoare et al. 1995). However, previous exposure to 8 μg l^{-1} Cu during embryonic stages significantly increased veliger growth rate and decreased spat survivorship in a mussels from the Menai Strait, Wales, UK. In this population, Cu caused a significant increase in embryo abnormalities. The embryo exposure effects outweighed the influence of copper during later stages.

Effects of Hg on survival, growth and metamorphosis of *Crassostrea gigas* larvae were examined (Beiras and His 1994). Growth, the most sensitive process, was significantly retarded at 4 μg l^{-1}. Metamorphosis was significantly reduced when competent pediveligers were exposed to 64 μg l^{-1} for 48 h. Larval clams *Mercenaria mercenaria* were exposed to dissolved Cu (LaBreche et al. 2002). Clams exposed to 5 μg Cu l^{-1} and fed *Isochrysis galbana* had similar survival to controls, but those in 14 and 29 μg Cu l^{-1} had increased mortality. Swimming activity decreased exponentially. Dissolved Cu was taken up by *I. galbana*, and ingested algae were a source of Cu toxicity for clams.

7.2.2 Organics

Wang et al. (2012) examined effects of PAHs (benzo[*a*]pyrene – BaP) and PCBs (Aroclor 1254) on embryogenesis and larval development of the bivalve *Meretrix meretrix*. Even at 1,600 μg l^{-1} of BaP and Aroclor1254 only minor reductions in embryo development rates were produced. The most sensitive endpoint was larval metamorphosis, with an EC_{50} value of 20 μg l^{-1} for BaP and 35 μg l^{-1} for Aroclor1254. These results indicate that BaP and Aroclor1254 are not highly toxic to *M. meretrix* embryos and larvae.

The toxic effects of different types of gasoline formulations on *Crassostrea rhizophorae* embryos and larvae were studied by Paixão et al. (2007). Oyster embryos were exposed to water-soluble fractions (WSF) of different gasoline formulations at a range of concentrations (0, 4.6, 10.0, 22.0, 46.0, and 100 %), for 24 h. The EC50-24h (concentration causing abnormalities in 50 % of the exposed embryos) was evaluated. The results showed gasoline formulations with high concentrations of monoaromatic hydrocarbons to be the least toxic, while formulations having higher content of aromatic hydrocarbons of 9 carbon atoms and naphtha were the most harmful.

Ryan et al. (2001) examined hard clam (*Mercenaria mercenaria*) larvae exposed to PCBs. Aroclor 1254, at concentrations near environmentally relevant levels. A dose–response relationship was observed for larval development; at higher concentrations, fewer larvae developed to the normal straight-hinge, or D-shaped stage, relative to the controls, while the number of abnormally shaped larvae increased.

Hanson et al. (1997) investigated effects of the detergent linear alkylbenzene sulphonate (LAS) (0–39 mg l^{-1}), which is found in nearshore areas receiving wastewater from urban treatment plants. They examined effects on swimming, grazing, and growth of mussel larvae, *Mytilus edulis* in the laboratory, and effects on settling and population development in field mesocosms. In the laboratory the larvae had 50 % mortality at 3.8 mg LAS l^{-1} after 96 h. Swimming was affected at 0.8 mg l^{-1} (i.e. smaller diameter of swimming tracks, reduced speed). Feeding was reduced 50 % at 1.4 mg l^{-1}, and specific growth rate was reduced by half at 0.82 mg l^{-1} in 9 days. In mesocosms, the larval population decreased dramatically in 2 days at concentrations as low as 0.08 mg l^{-1}, due to increased mortality and to settling. Settling success was reduced at the same LAS concentration as that which increased mortality. Treated larvae had delayed metamorphosis and reduced shell growth. Authors felt that the larval ciliary apparatus, crucial for swimming, orientation, settling behaviors, and feeding, was damaged by LAS. Data on grazing and growth agreed with video observations of larvae. These effects occurred at LAS levels found in some estuaries. This is an unusually comprehensive study with many ecologically important endpoints evaluated as well as a proposed mechanism for the effects. More studies like this should be done.

Contaminated sediments can also affect mollusk development. Geffard et al. (2003) studied bioavailability and toxicity of sediment-associated PAHs to embryos and larvae of *C. gigas*, exposed to whole sediment and elutriate. Percentages of abnormal larvae and contaminant accumulation were measured. Sediment-associated PAHs were available, as indicated by accumulation in larvae and by abnormalities induced during larval development. The critical body burden of various PAHs was 0.3 $\mu g\ g^{-1}$, above which abnormalities were observed. The bioavailability of PAHs is determined by their solubility; only the soluble fraction is accumulated by the embryos.

Toxicity of glyphosate herbicides to embryo-larval development of oysters (*C. gigas*) was studied by Mottier et al. (2013). Embryo-larval development was quite insensitive to the pure chemical and commercial formulations, but commercial formulations were considerably more toxic, with EC_{50} values of 1,133 and 1,675 $\mu g\ l^{-1}$ for Roundup Express® (R_{EX}) and Roundup Allées et Terrasses® (R_{AT}).

Since both oysters and sea urchins are used in embryo-larval bioassays, the respective sensitivity of oyster (*Crassostrea gigas*) and sea urchin (*Paracentrotus lividus*) embryos and larvae to various pollutants were compared (His et al. 1999). *C. gigas* embryos and larvae were more sensitive to copper and to the herbicide Dinoterbe; the sensitivity of both species to TBT was practically the same, and *P. lividus* was more sensitive to lead and mercury. Authors felt the oyster bioassay is more suitable for estuarine waters, because of the broader salinity tolerance of estuarine bivalve larvae than sea urchin larvae.

7.2.3 Contaminants of Emerging Concern

The effects of the endocrine disruptors nonylphenol (NP) and bisphenol A (BPA), on embryonic and larval development of the abalone *Haliotis diversicolor supertexta*, were investigated by Liu et al. (2011). The respective 96-h EC_{50} values based on completion of metamorphosis were very high, 11.65 and 1.02 g l^{-1}, suggesting very low sensitivity. However, when abalone were exposed for a longer time, or if the benthic diatoms that are both a food source and a settlement substrate for abalone had been exposed and accumulated the chemicals, then there was much greater sensitivity at metamorphosis.

Crassostrea gigas was exposed to a range of concentrations of 4-nonylphenol (0.1, 1, 10, 100, 1,000 and 10,000 μg/l) by Nice et al. (2000). Development to the D-shape larval stage was monitored. This endocrine disruptor delayed development to D-shape, produced abnormalities, and decreased survival rate. Thus oyster larvae are much more sensitive than abalone larvae.

Chiu et al. (2012) investigated effects of the flame retardant PBDE on the gastropod *Crepidula onyx*, and demonstrated that chronic exposure to BDE-47 (up to 1,000 ng l^{-1}) throughout the entire larval stage did not affect settlement, development, or growth despite bioaccumulation.

7.2.4 Hypoxia

Anoxia tolerance of *M. edulis* larvae, (median mortality time) increased from 14 h in early prodissoconch larvae to 38 h in later veliconch larvae. Both embryos and early larvae developed and grew normally at pO_2 values $\geq$3.16 kPa (Wang and Widdows 1991). Feeding activity of early larval stages was maintained or enhanced under hypoxia, but feeding and growth of later stages was depressed at all hypoxic levels examined. Hypoxia had little influence on settlement, but larvae developed eye-spots at a smaller size, indicating an uncoupling of growth and morphogenesis. These responses were supported by calorimetric and respirometric measurements showing that early larvae could maintain their energy metabolism at reduced O_2, while later stages suppressed heat dissipation in moderate hypoxia.

Effects of hypoxia (1.5 mg O_2 l^{-1}, 20 % sat) and anoxia (<0.07 mg O_2 l^{-1}, <1 % sat) on oyster (*Crassostrea virginica*) larval settlement, juvenile growth, and juvenile survival were studied. Settlement was reduced significantly in hypoxic treatments, and almost no settlement took place in anoxia (Baker and Mann 1992). After 96 h, 38 and 4 % of the larvae in hypoxic and anoxic treatments had settled, while 79 % settled in control normoxic treatments. After settlement, juveniles in hypoxia grew one third as much as those in normoxia, while those in anoxia did not grow at all. In response to hypoxic treatments, post-settlement oysters with shell heights of >469 μm maintained normal rates of ingestion but oysters with shell heights of 436 μm reduced ingestion rates to 54–61 % of control rates (Baker and Mann 1994). These oyster sizes differed in the extent of gill development, which may have been responsible for the differential responses. In response to microxic treatments, (<0.4 mg O_2 l^{-1}, <5 % sat) ingestion rates were 1–14 % of normoxic rates and decreased with body size. Authors concluded that oysters have the ability to feed at nearly all stages of settlement and metamorphosis, that hypoxia affects the feeding of only the youngest post-settlement oysters, while microxic conditions will affect all post-settlement oysters.

The embryo/larval development of bay scallop (*Argopecten irradians*) was inhibited at a DO of 1.38–3.64 mg l^{-1} at 23 °C (Wang and Zhang 1995). Tolerance to anoxia increased with larval sizes and was related to their oxygen debt (accumulation of lactic acid).

Gastropod larvae may be more sensitive than the bivalves discussed above. Effects of low DO on early development and swimming behavior of veliger larvae of the snail, *Nassarius festivus* were studied (Chan et al. 2008). Embryonic development was significantly delayed when DO was reduced to 3.0 mg O_2 l^{-1}. Veligers that hatched at 4.5 mg O_2 l^{-1} had smaller velar lobes, shell length and shell width and lower swimming speeds than those in normoxia. The percentage that developed into juveniles was reduced and metamorphosis was delayed at 4.5 mg O_2 l^{-1} while all larvae at 3.5 mg O_2 l^{-1}died before metamorphosis. Juveniles that developed at 4.5 mg O_2 l^{-1} were smaller than those at 6.0 mg O_2 l^{-1}, indicating that DO levels well above hypoxic levels (2.8 mg O_2 l^{-1}) have significant impacts on hatching and larval development in these gastropods.

7.2.5 *Ocean Acidification/CO_2*

Acidification leads to thinner shells in mollusks, which can make them more susceptible to predation. Kurihara et al. (2007) exposed eggs of the oyster, *C. gigas* for 48 h to seawater at pH 7.4, and examined the larval morphology and shell mineralization. Only 5 % of the low pH group developed into normal D-shaped veligers compared with 68 % of the controls, although no difference was seen up to the trochophore stage. Control D-shaped veligers had greater shell length and height 24–48 h after fertilization, while the few D-shaped veligers of the experimental group had no shell growth during that period (Fig. 7.2). Calcification appeared to be particularly affected by low pH and/or the low $CaCO_3$ saturation state of high-CO_2 seawater.

Mussel embryos (*M. galloprovincialis*) were incubated for 6 days in control and high-CO_2 (2,000 mg l^{-1}, pH 7.4) seawater (Kurihara et al. 2008). While embryogenesis was unaffected, development at the trochophore stage was delayed when the shell began to form. Veligers of the high-CO_2 group showed morphological abnormalities, reduced height and length, consistent with the previous findings on the oyster, although the severity of CO_2 effects was less in the mussels, possibly due to differing spawning seasons. In contrast, Gazeau et al. (2007) found that mussels, in this case *M. edulis*, were more sensitive than oysters – calcification rates of *M. edulis* and *C. gigas* declined linearly with increasing pCO_2, but mussels declined more, and projections were that mussel and oyster calcification may decrease by 25 and 10 %, respectively, by the end of the century. Gazeau et al. (2010) found impacts on mussel calcification with a decrease of $\sim$0.5 pH unit during the first 2 days of development. Hatching rates were 24 % lower while D-veliger shells were 13 % smaller at pH 7.6 than at control pH of 8.1. Although larvae developed a shell at this pH, lower hatching and growth could lead to a significant decrease in settlement success.

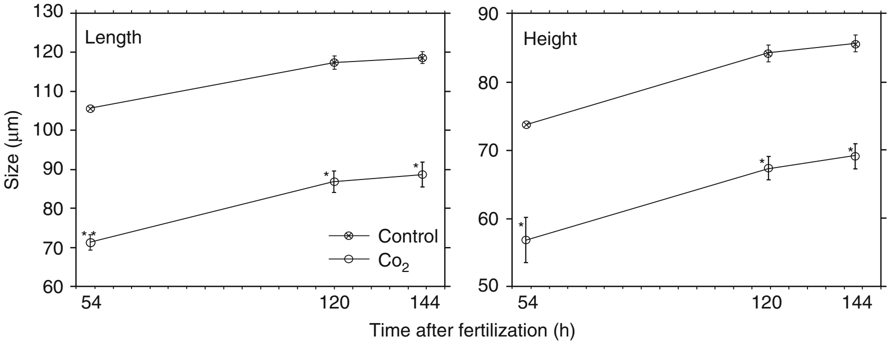


Fig. 7.2 Comparison of shell length and height of D-shaped larvae of *M. galloprovincialis* in control and high CO_2 at 54, 120, and 144 h after fertilization. Error bar = SD, * = significant difference between control and high CO_2 groups (Reprinted from Kurihara et al. 2008: 229, courtesy of Inter-Research)

Hettinger et al. (2012) investigated consequences of decreased pH for early stages of the Olympia oyster (*Ostrea lurida*). Oysters were raised through their larval period and into early juvenile stages at control pH (8.0) as well as 7.9 and 7.8. Larvae at pH 7.8 had a 15 % decrease in shell growth rate, and a 7 % decrease in shell area at settlement. Impacts were greater 1 week after settlement; juveniles that had been larvae in low pH had a 41 % decrease in shell growth rate. Importantly, this was seen in juveniles kept at control pH as well as those that were still in reduced pH, indicating a strong delayed effect from the larval stage. Impacts of early exposure to low pH persisted for at least 1.5 months after juveniles were transferred to control pH. Delayed effects appear to be very important and are overlooked in short-term larval tests that end at metamorphosis.

Endangered northern abalone (*Haliotis kamtschatkana*) larvae were exposed to various levels of CO_2: 400 (ambient), 800, and 1,800 mg l^{-1} (Crim et al. 2011). Larval survival decreased by 40 % in elevated CO_2, but the percent of surviving larvae to metamorphose was unaffected. Shell abnormalities occurred in 40 % of the larvae at 800 mg l^{-1} CO_2 and almost all larvae at 1,800 mg l^{-1} had abnormal or no shells. Zippay and Hoffman (2010) examined the effect of pH on larvae of the red abalone, *Haliotis rufescens*. Low pH (7.87) decreased thermal tolerance of pretorsion and late veliger stages, but not post-torsion and premetamorphic veligers. However, the expression pattern of shell formation genes was not affected in any of the stages.

Since acidification will be accompanied by increasing temperature, Talmadge and Gobler (2011) studied responses of larvae and juveniles of *M. mercenaria*, *C. virginica*, and *Argopecten irradians* to temperatures (24 and 28 °C) and CO_2 levels (~250, 390, and 750 mg l^{-1}). Increased temperature and CO_2 each depressed survival, development, growth, and lipid synthesis of *M. mercenaria* and *A. irradians* larvae and effects were additive (Figs. 7.3 and 7.4). Juvenile

Fig. 7.3 Performance of *M. mercenaria* larvae grown under 250, 390, and 750 ppm CO_2, and 24 and 28 °C. *Letters* represent significant differences (Reprinted from Talmage and Gobler 2011)

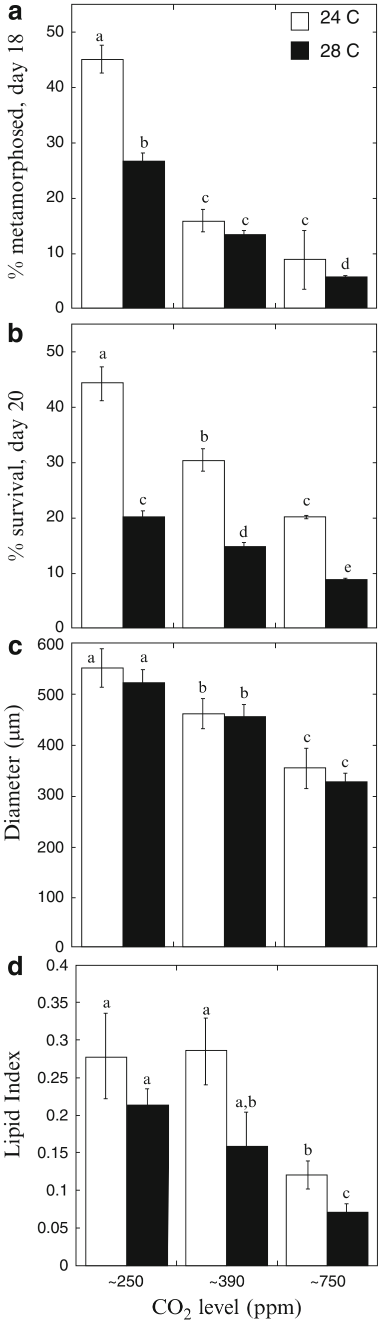
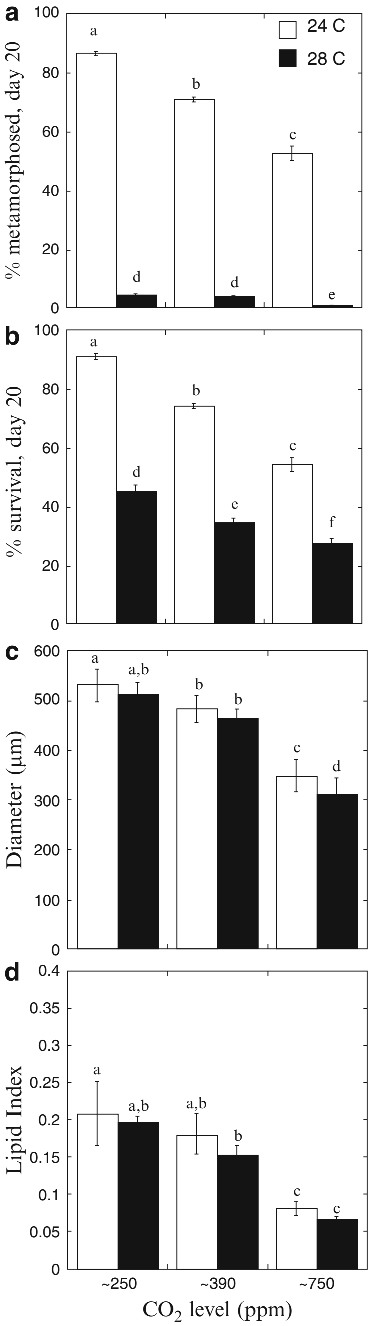


Fig. 7.4 Performance of *Argopecten irradians* larvae grown under 250, 390, and 750 ppm CO_2, and 24 and 28 °C. Letters represent significant differences (Reprinted from Talmage and Gobler 2011)



M. mercenaria and *A. irradians* were negatively affected by higher temperatures while *C. virginica* juveniles were not. *C. virginica* and *A. irradians* juveniles were negatively affected by higher CO_2, while *M. mercenaria* was not. Larvae were more vulnerable to elevated CO_2 than juveniles. Increases in temperature and CO_2 will have combined negative consequences for coastal bivalves.

Impacts have already been seen on oyster larvae. Researchers studied oysters at the Whiskey Creek Hatchery in Oregon after oyster production failures, examining the coastal waters in which the shellfish were raised. For several years larval production collapsed by up to 80 % at shellfish farms. Production failures were linked to CO_2 levels in the water in which the oysters spawned and spent their early lives when they develop into larvae and build their initial shells. Barton et al. (2012) linked the collapse of oyster seed production to increased CO_2 from seasonal upwelling of low pH water, which inhibited larvae from developing shells and growing at a rate that would make commercial production viable.

However, adaptation may be possible. Parker et al. (2012) found that while elevated CO_2 reduced growth, developmental rate and survival of oyster *Sacostrea glomerata* larvae, exposing adults to elevated CO_2 during reproductive conditioning had positive effects. Larvae spawned from adults at elevated CO_2 were larger, developed faster, and had similar survival as larvae from adults at ambient CO_2. Furthermore, they were more resilient to elevated CO_2 than wild larvae, suggesting that they may be able to acclimate or adapt to elevated CO_2.

7.2.6 Polluted Environments

Money et al. (2011) analyzed responses of *C. gigas* larvae exposed to water from the industrialized Tamar estuary (England). A high level of toxicity (up to 100 % abnormal development) was seen at two stations, particularly during periods of the tidal cycle when the influence of more pristine coastal water was lowest. Competitive ligand-exchange Cu titrations showed that natural organic ligands reduced the free cupric ion concentration to levels that were unlikely to have been the sole cause of the observed toxicity. It is probable that combined effects of Cu and other contaminants contributed to the response.

Effects of contaminants along a well-defined North Sea pollution gradient were assessed by McFadzen (1992) using veliger larvae of *C. gigas* and the Manila clam *Tapes philippinarum*. The results demonstrated that larval survival steadily improved further offshore towards the Dogger Bank, with higher mortalities occurring in the surface microlayer and sediment elutriate samples than in the subsurface bulk waters. Clam larvae were more sensitive to contaminants than the younger oyster larvae.

7.3 Fishes

Fish larvae tend not to be quite as different from adult forms as larvae of invertebrates. As they grow, they gradually change shape, instead of having a specific metamorphosis. Exceptions to this are in the flatfish, whose planktonic larvae resemble those of other fishes, but which undergo a major metamorphosis in which one eye (and accompanying nerves) migrate from one side of the head to join the other eye, as the fish settles to the bottom to lie on one side. Eels also undergo a clear metamorphosis. Large numbers of bioassays of contaminants have been done using fish larvae, of which most have been on freshwater species such as zebrafish, fathead minnows, and medaka.

Based on experimental data with sole (*Solea solea*), a bioaccumulation model was adapted to calculate concentrations of persistent organic pollutants in tissues of developing fish (Foekema et al. 2012). Tissue concentrations were predicted to peak at the time when larvae become free-feeding, when lipid reserves are depleted. This may explain delayed effects on larvae that have been observed after egg and embryo exposures. Effects of embryonic exposures on larval behavior are discussed Chap. 9.

7.3.1 Metals

Larvae of garpike (*Belone belone*) exhibited vertebral flexures, reduced activity and swimming ability after incubation in 0.5 mg l^{-1} Cd (Dethlefsen et al. 1975). Toxicity of Cd, Cr (VI) and Cu to *Cyprinodon variegatus* larvae was evaluated in terms of survival and growth over 7 days (Hutchinson et al. 1994). Concentrations affecting survival and growth after 7 days were 0.75 mg Cd l^{-1}, 24.0 mg Cr^{6+} l^{-1} and 0.16 mg Cu l^{-1}. Effects of 2 h pulse-exposure of Cd or Zn on early life stages of Australian crimson spotted rainbow fish (*Melanotaenia fluviatilis*) were investigated by Williams and Holdway (2000). For Cd and Zn, 9–10-day-old larvae were more tolerant than younger ages and Cd was more toxic than Zn. Pulse-exposed metals (3.3 mg l^{-1} of cadmium and 33.3 mg l^{-1} of zinc on 3 h old embryos) caused reduced hatch, spinal deformities, and toxicity in larvae. Continuous exposure LC50 values for 9–10-day-old larvae were 0.01 and 0.27 mg l^{-1} for cadmium and zinc, respectively. Zn at 0.1, 0.5 and 2.0 mg l^{-1} produced deformations of the jaw, head, optic capsules, otic capsules and vertebral column of yolk-sac larvae of herring *Clupea harengus* (Somasundaram et al. 1984). Authors suggested that larvae with moderate deformations, induced by lower concentrations, may survive and continue development, although while this is possible in a laboratory, it seems relatively unlikely to occur in the field where there are predators. However, some adult fish with mild deformities have been collected from field sites.

LC50 values for larvae of red sea bream *Pagrus major* were higher than those of embryos, indicating that embryos were more sensitive to Cd than larvae (Cao et al. 2009). Cd concentrations of ≥ 0.8 mg l^{-1} led to low hatchability, delay in time to hatch, and high mortality, morphological abnormality, and reduced length in the embryos and larvae. Heart beat and yolk absorption of the larvae were significantly inhibited at some high concentrations but they were not as sensitive as other endpoints. Anderson et al. (1991) compared the relative sensitivity of topsmelt (*Atherinops affinis*) sperm, embryos, and larvae to copper chloride. The EC_{50} from 48-h fertilization experiments was 109 μg l^{-1}. The EC_{50} from 12-day embryo development tests was 142 μg l^{-1}, and the mean LC_{50} from 96-h larval mortality tests was 238 μg l^{-1}. Authors concluded that sperm were more sensitive than embryos, and embryos were more sensitive than larvae. However, the larval test was lethality, while the others had sublethal endpoints. It would have been a more valid comparison if sublethal endpoints had been evaluated for larvae as well.

7.3.2 Organics

Pesticides and PCBs

Holdway et al. (1994) studied effects of pulse exposure with two synthetic pyrethroids, fenvalerate and esfenvalerate, on survival of larval Australian crimson-spotted rainbow fish (*Melanotaenia fluviatilis*). Both pesticides were highly toxic with 1-h esfenvalerate pulse-exposure concentrations as low as 0.32 μg l^{-1}, and 1-min fenvalerate pulse-exposure concentrations of 4.5 μg l^{-1}, causing significant mortality. There was a complex relationship between pesticide concentration and time to mortality. At low concentrations of pesticide, most mortality occurred within the first 24 h, while at higher concentrations, mortality continued for 96 h after exposure. The authors suggested that mortality within the first 24 h was due to direct physiological effects on the larvae, while subsequent mortality was primarily due to starvation of larvae unable to recover from the initial insult.

Parental exposure to DDT (2.0 or 10.0 μg per 100 g fish per day in the diet for 1 month) affected behavior of Atlantic croaker, *Micropogonias undulatus*, larvae (Faulk et al. 1999). The proportion of larvae responding to a vibratory stimulus, burst and routine swimming speeds, active duration, and pause duration were affected by parental exposure. Burst speeds in response to the visual stimulus were lower than controls. These changes may decrease survival by increasing predation rates and/or decreasing feeding rates. Additional studies on larval behavior are discussed in Chap. 9.

Monosson et al. (1994) gave adult white perch (*Morone americana*) injections of 3,3′,4,4′-tetrachlorobiphenyl (TCB) at one of three doses (0.2–5.0 mg TCB/kg body weight) approx. 3 months prior to the spawning season and at 3-week intervals. Fewer females receiving the highest dose matured. Those that did mature had a GSI 50 % that of controls. Levels of estradiol-17β, testosterone, and VTG were not altered. By 7 dph survival of larvae from females exposed to 1.0 and 5.0 mg/kg TCB was reduced compared to controls (0, 1 and 54 %, respectively). Thus decreased larval survival was seen at parental doses less than those that decrease ovarian growth, oocyte maturation, or circulating sex steroid hormone and VTG levels in adults.

Olufsen and Arukwe (2011) studied effects of PCB-77 (3,3′,4,4′-tetrachlorobiphenyl) on vascular and bone development of salmon (*Salmo salar*). PCB-77 (1 or 10 ng l^{-1}) produced concentration-dependent increases in the rate of bone tissue formation, dependent on larval age. Evidence of vascular system disruption by the PCB was observed as cardiac edema, anemia and arrhythmia. Foekema et al. (2008) exposed early life stages of sole (*Solea solea*) to dioxin-like PCB 126 until 4, 8, 10 and 15 days post fertilization (dpf), then raised them in clean seawater. The LC50s at the start of the free-feeding stage (12 dpf) was 39–83 ng PCB 126 l^{-1} depending on exposure duration. After fish had completed metamorphosis, the LC50 values were between 1.7 and 3.7 ng PCB 126 l^{-1} depending on exposure duration. Thus exposure of embryos for only 4 days caused adverse effects during a critical developmental phase 2 weeks later. This study indicates that fish tests that are terminated shortly after the fish become free-feeding underestimate the toxicity of compounds such as PCBs.

Larval and metamorphosing summer flounder (*Paralichthys dentatus*) were exposed to the dioxin-like PCB 126, to document effects on metamorphic development (Soffientino et al. 2010). Median lethal doses ranged between 30 and 220 ng g^{-1} wet mass, indicating that this species is very sensitive. Dose-dependent induction of cytochrome P-4501A (CYP1A) at 4 days post-exposure was observed in liver, stomach, intestine, and kidney of metamorphosing larvae. A single sublethal dose (15 ng g^{-1}) delayed metamorphic progress as determined by the degree of eye migration, and resulted in abnormally high levels of cell proliferation and abnormal gastric gland morphology in late metamorphic stages. These results suggest that larval and metamorphic stages of summer flounder are vulnerable to the effects of dioxin-like compounds, including lethality, developmental delay, and malformations.

McCarthy et al. (2003) examined effects of PCBs (Aroclor 1254) on Atlantic croaker larvae. Adult fish were given a dietary administration of 0 (control) or 0.4 (dosed) mg Aroclor 1254 kg^{-1} fish day^{-1} for 2 weeks during the final stages of gonadal recrudescence. Fertilized eggs collected from control and dosed adults immediately after spawning contained 0 and 0.66 μg Aroclor 1254 g^{-1} egg, respectively. Growth rate of dosed larvae was significantly lower than that of control larvae, with dosed larvae showing a 4 day delay in attaining a given size. Routine swimming speed and activity were similar, but there was a difference in response to stimulus. While the percentage of control larvae responding to the stimulus and their burst speeds increased with age, dosed larvae had no such increase. Further studies on larval behavior are described in Chap. 9.

Couillard et al. (2008) investigated the interaction between PCBs and pesticides. The effect of diazinon was evaluated in *Fundulus heteroclitus* larvae produced from eggs differentially treated with PCB126. A few hours after fertilization, eggs

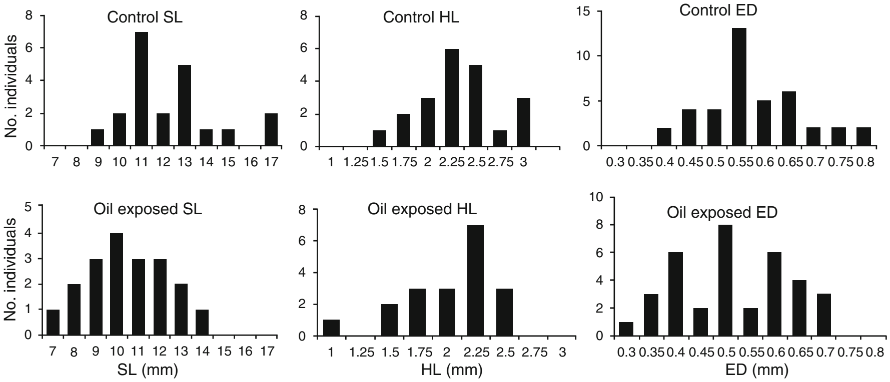


Fig. 7.5 Size distribution of standard length (*SL*) head length (*HL*) and eye diameter (*ED*) of herring larvae in control and oil exposure groups after 8 weeks recovery in clean sea water (Reprinted from Ingvarsdottir et al. 2012: 73, courtesy of Elsevier Publishing Co.)

were treated topically with PCB126 (100 pg ul^{-1}) in dimethyl sulfoxide or not treated. Newly hatched larvae were exposed to diazinon (125–12,900 ng l^{-1}) or seawater alone. Diazinon inhibited cholinesterase activity at 361 ng l^{-1}. Body length was inversely related to diazinon concentration. Embryonic treatment with PCB126 also caused a reduction in body length. The effects of PCB126 and diazinon on body length are cumulative because no significant interactions were observed.

Oil and Dispersants

Atlantic herring (*C. harengus*) larvae were exposed to dispersed Arctic crude oil at 0.129, 0.373, 0.496, 2.486 and 6.019 μg l^{-1} total PAH, and control seawater for 12 days, then transferred to clean water for 8 weeks (Ingvarsdóttir et al. 2012). Higher mortality was found in all oil concentrations after 12 days. There was no difference in mortality during the recovery phase, but after recovery in clean seawater, the oil-exposed larvae exhibited delayed effects including morphological deformities and reduced growth (Fig. 7.5).

Atlantic cod (*Gadus morhua*) larvae were exposed to five concentrations of either artificially weathered dispersed oil containing oil droplets and water-soluble fraction (WSF) or the filtered dispersions containing only the WSF (Olvsvik et al. 2011). The larvae were exposed for 4 days then subjected to transcriptional analysis at 13 days post hatching. The most affected genes were those related to drug metabolism, endocrine system development and function, and lipid metabolism. Oil exposure also increased expression of genes involved in bone resorption, and decreased expression of genes related to bone formation. The altered gene transcription was dominated by the WSF; oil droplets played a smaller role.

Kawaguchi et al. (2011) exposed eggs and larvae of Japanese flounder (*Paralichthys olivaceus*) to heavy oil and investigated neural disorders. In larvae exposed to 8.75 mg l^{-1}, the facial and lateral line nerves partially entered into the incorrect region. Exposed larvae also had abnormal expression of *Sema3A*, an axon guidance molecule, suggesting that the abnormal expression of *Sema3A* caused disruption of the facial nerve scaffolding.

Newly hatched mummichog (*Fundulus heteroclitus*) were exposed to crude oil or water-accommodated fraction (WAF) of dispersed crude oil to evaluate if the dispersant-induced changes in dissolved PAH affected larval survival or body length (Couillard et al. 2005). Weathered Mesa light crude oil (0.05–1 g l^{-1}) with or without Corexit 9500® was used. At 0.2 g l^{-1}, the addition of dispersant caused a two- and fivefold increase in concentrations of total PAH and high-molecular-weight PAH with three or more benzene rings. The highest mortality rates (89 %) were in larvae exposed to dispersed oil. Reduced body length correlated with increased levels of PAH. Thus, dispersion increased total PAH, the proportion of high molecular weight PAH, and overall toxicity.

Contaminants of Emerging Concern

Turbot embryos (*Psetta maxima*) were exposed to BDE-47 and BDE-99 for 6 days. Both compounds caused lethal toxicity as well as non-lethal malformations during embryo development (Mhadhbi et al. 2012). BDE-47 was more toxic than BDE-99 (LC50 values for embryos and larvae, respectively, BDE-47: 27.35 and 14.13 μg l^{-1}; BDE-99: 38.28 and 29.64 μg l^{-1}). PBDEs were teratogenic at concentrations higher than 8.14 and 16.12 μg l^{-1} for BDE-47 and -99 respectively, leading developmental delays and death, as well as malformations and mortality of larvae. Based on environmental concentrations of PBDEs in various aquatic ecosystems, authors concluded that waterborne BDE-47 and BDE-99 pose little risk of acute toxicity to marine fish. However, no sublethal effects on larvae were investigated. More subtle effects of PFOS and PFOA were studied in salmon (*S. salar*) larvae (Arukwe et al. 2013) exposed to100 μg l^{-1} from fertilization for 52 days. PFOS and PFOA body burden increased during the exposure period and affected metabolism and morphometry. PFOA produced increases in heart-, thymus-, liver- and kidney somatic indexes (HSI, TSI, LSI and KSI). PFOA and PFOS decreased whole body dehydroepiandrosterone (DHEA), estrone and testosterone at sampling day 21 and increased cortisol and cholesterol at the end of recovery period (day 56). They observed changes in FA (fatty acid) composition that involved increases in FA methyl esters (FAMEs), mono- and poly-unsaturated FA (MUFA and PUFA) and a decrease in n-3/n-6 PUFA ratio by both PFOA and PFOS. Authors concluded that changes in hormonal and FA profiles may represent cellular and/or physiological adaptation to continuous exposure by increasing membrane fluidity, and/or overt developmental effects.

7.3.3 Hypoxia

Silverside (*Menidia beryllina*) larvae avoid hypoxic water. When larvae sink from an upper normoxic layer into a lower hypoxic layer, they display an avoidance reaction consisting of a burst of fast swimming that ends in an upward direction leading the larva out of the hypoxic region. Each swimming burst lasts for approximately 2–3 s, with a maximum speed of approximately 25 mm s^{-1} (Weltzien et al. 1999). The reaction was seen in larvae from 6 to 64 h post hatching and was correlated with the DO but not with N_2 concentration or salinity. The avoidance response was observed at DO levels from 4.7 to 0.8 mg O_2 l^{-1}.

Tolerance to hypoxic stress and oxygen consumption was studied in the red sea bream, *Pagrus major*, from its early life stage until 42 days post-hatch (Ishibashi et al. 2005). Lethal DO levels and mass-specific metabolic rates increased with larval growth from 2.6 to 5 mm total length (TL), then levels remained high and decreased until about 9.5 mm TL, around the flexion and post flexion stages. In juveniles, lethal DO levels and mass-specific metabolic rates decreased as TL increased. The relationship between lethal DO levels and mass-specific metabolic rates indicated that metabolic rates were highest during metamorphosis, when hypoxia tolerance was lowest. It was presumed that the increasing metabolic rate at metamorphosis decreased the metabolic scope of activity. Similar results were found in bonefish larvae (Pfeiler 2001). Survival times of metamorphosing leptocephali of the bonefish *Albula* sp. placed in hypoxic sea water (0.68 mg O_2 l^{-1}) decreased from 15 to 5 min over the 10 day metamorphic period. Thus they became more sensitive to low DO as they went through metamorphosis. Increased sensitivity to hypoxia again coincided with increased oxygen demand at metamorphosis.

Larvae of the air-breathing teleost *Monopterus* are frequently exposed to periods of hypoxia, which they survive because they have capillary networks in the skin, a small blood-water barrier, pectoral fins that generate a respiratory water current originating from the oxygen-rich surface layer, and a principal flow of blood that runs countercurrent to the water stream. The larva as a whole can be considered functionally comparable to a fish gill lamella (Liem 1981).

7.3.4 Ocean Acidification

Atlantic cod (*Gadus morhua*) larvae were kept at three levels of $p\mathrm{CO}_2$: present day (380 mg l^{-1}), end of next century (about 1,800 mg l^{-1}) and an extreme, coastal upwelling scenario where winds bring CO_2-rich deep water to the surface (about 4,200 mg l^{-1}), in a mesocosm experiment (Frommel et al. 2012). Elevated $p\mathrm{CO}_2$ caused tissue damage in many internal organs, including liver, pancreas, kidney, eye, and gut about 1 month after hatching.

Clownfish *Amphiprion percula* larvae were reared from hatching to settlement at three pH levels (control pH 8.15; intermediate pH 7.8 and extreme: pH 7.6) to test possible effects of ocean acidification on otolith development (Munday et al. 2011a). There was no effect of pH 7.8 on otolith size, shape, chemistry, or symmetry between left and right. However, at pH 7.6 otolith area and maximum length were larger than controls. These results support the hypothesis that pH regulation may cause increased precipitation of $CaCO_3$ in otoliths in elevated CO_2, as suggested by an earlier study, and imply that otolith development is robust to the changes in pH. Newly hatched spiny damselfish *Acanthochromis polyacanthus* were reared for 3 weeks at 4 different levels of pCO_2 from concentrations already experienced in near-reef waters (450 μatm CO_2) to those predicted to occur over the next 50–100 years (600, 725, 850 μatm CO_2). Elevated pCO_2 had no effect on growth, survival or size of skeletal elements (Munday et al. 2011b). Also, otolith size, shape and symmetry between left and right side of the body were not affected by elevated pCO_2, despite the fact that they are composed of aragonite These results suggest that this species is tolerant to increases in environmental CO_2.

7.3.5 Polluted Environments

The sea surface is an important habitat for eggs and larvae of many fishes but it also concentrates contaminants. The microlayer generally has higher levels than the water column of anthropogenic substances which frequently occur at concentrations $10^2 - 10^4$ greater than these in the water column. These include plastics, tar lumps, PAHs, hydrocarbons, chlorinated hydrocarbons, and metals, such as, lead, copper, zinc, and nickel. Studies were conducted to determine the toxicity of the sea surface of Puget Sound to planktonic larval stages (Hardy et al. 1987). Three contaminated urban bays and a rural reference bay were studied. Sand sole (*Psettichthys melanostictus*) embryos and larvae of anchovies and kelp bass were exposed in the field and lab to the sea-surface microlayer. Laboratory exposure to surface microlayer from urban bays increased the incidence of chromosomal aberrations and reduced hatching of sole. *In situ* hatching success of sole eggs was reduced to 50 % in urban bays compared to reference sites. Toxicity was correlated with concentrations of PAHs and metals in the sea-surface microlayer, and was similar in sole, anchovy, and kelp bass. Cross et al. (1987) similarly correlated contaminants in the microlayer in coastal waters off California with toxic effects on kelp bass (*Paralabrax clathratus*) eggs and larvae, which were highest in Los Angeles harbor (with high metals: 17 $\mu g\ l^{-1}$ Ag, 0.26 $\mu g\ l^{-1}$ Cd, 32 $\mu g\ l^{-1}$ Cr, 101 $\mu g\ l^{-1}$ Cu, 100 $\mu g\ l^{-1}$ Pb, and 457 $\mu g\ l^{-1}$ Zn; high chlorinated organics such as 30,708 $ng\ l^{-1}$ Aroclor 1242, and 8,141 $ng\ l^{-1}$ Aroclor 1254; and high PAHs, such as 1,260 $ng\ l^{-1}$ phenanthrene, and 2,178 $ng\ l^{-1}$ benz(a)pyrene). Microlayer samples from farther offshore had lower contaminants and lower toxicity.

There have been studies investigating skeletal abnormalities in adult field-collected fish. These likely originated when the fish were larvae, so are discussed in this chapter. These are relatively subtle abnormalities that allow the fish to survive to adulthood. Fish from metal-polluted waters in the Gulf of Bothnia were examined for morphological anomalies, such as vertebral and spinal deformities and asymmetrical fins and gill rakers (Bengtsson et al. 1985). Fourhorn sculpin (*Myoxocephalus quadricornis*) from polluted sites had high frequencies of skeletal deformities, which decreased as distance from polluted areas increased. Whitefish (*Coregonus lavaretus*) from polluted areas had elevated frequencies of deformed gill rakers and of asymmetrical gill rakers and fins. Kessabi et al. (2013) studied skeletal deformities in natural populations of the Mediterranean killifish *Aphanius fasciatus* from the Tunisian coast. Fish were collected from one reference area and three industrialized polluted areas (S2: industrialized coast of Sfax, S3: coast of Khniss and S4: Hamdoun'Oued), and skeletal deformities were diagnosed with double staining. A total of 1,025 abnormalities were quantified, which were classified into categories of abnormalities on spines, vertebrae, arcs and mandibles. In addition, levels of Cd, Cu and Zn, various PAHs, and estrogenic compounds were measured in water and sediment from the different sites. The frequency of spinal deformities was greatest in fish from S2, the site which had significantly higher levels of metals and PAHs than all the others.

7.4 Other Taxa

Larval studies have been performed with various species of cnidarians, mainly corals. The planula is the free-swimming, ciliated larval form of cnidarians. When ready to metamorphose into a coral, it must find a hard substrate (many may prefer specific substrates) where it anchors and grows into a polyp. Echinoderms (sea urchins) are frequently used in standard embryo-larval bioassays. Sea urchins, sand dollars, and brittle stars have a pluteus larva that uses ciliated bands for swimming and suspension feeding. The larva uses its ciliated arms to sweep food into its mouth as it glides through the water. As the larva develops, it increases the number of arms. The body form changes dramatically with metamorphosis. Many larval structures used during planktonic life are lost, and replaced by appendages adapted to the adult's benthic life. Polychaete worm larvae have also been studied. Polychaetes typically hatch into planktonic trochophore larvae, which eventually metamorphose into the adult form by adding segments.

7.4.1 Metals

Coral larvae are sensitive to metals, especially Cu, which reduced settlement success of *Acropora tenuis* larvae after 48-h compared with controls. The 48-h EC_{50} was 35 $\mu g\ l^{-1}$ (Reichelt-Brushett and Harrison 2000), indicating that moderate Cu concentrations impair settlement of coral larvae.

Brix et al. (2012) tested larvae of the sea echinoderm, *Lytechinus variegatus* in an 18-day study in which larvae were continuously exposed to Ag-laden algae (*Isochrysis galbana*). After 7 days, no significant effects were observed on growth up to the highest concentration tested (10.68 μg g^{-1} dw Ag in algae) but after 18 days, significant effects were observed in all Ag treatments >0.68 μg g^{-1} dw Ag in algae (corresponding waterborne Ag concentration of 0.05–0.07 μg l^{-1}). However, the dose–response relationship was quite flat with a similar growth inhibition (15 %) in all Ag treatments.

7.4.2 *Organics*

Oil and Dispersants

Laboratory experiments were conducted to measure larval settlement and metamorphosis of the polychaete *Streblospio benedicti*, as well as juvenile bioaccumulation and growth rates when exposed to sediment-associated PAHs (Chandler et al. 1997). Larval settlement and metamorphosis was reduced, but not significantly (relative to controls) by IX, 5X and 10X background PAH mixture concentrations of the six most abundant PAHs in urbanized Murrell's Inlet South Carolina (1X = 0.9 μg PAH g dry sediment^{-1}). Bioaccumulation of the most abundant PAH, fluoranthene (FL), was very high in this PAH tolerant species—9.5–13.7X FL sediment concentrations after 28-day exposures. Twenty-eight-day exposures to 0.26 and 2.4 μg FL g^{-1} caused no significant mortality, and produced positive weight gains in *S. benedicti* up to 18 d exposure. However, dramatic weight declines occurred from days 18–28 in both FL treatments. High tolerance of PAH may explain why this species recruits and survives in hydrocarbon-contaminated sediments.

Epstein et al. (2000) investigated short-term effects of five oil dispersants (Inipol IP-90®, Petrotech PTI-25®, Bioreico R-93®, Biosolve® and Emulgal C-100®) on planula larvae of the Red Sea stony coral *Stylophora pistillata* and soft coral *Heteroxenia fuscescens*. Larvae were exposed to WSFs, dispersed oil water accommodated fractions (WAFs) and dispersants dissolved in seawater. While WSF reduced settlement success (Fig. 7.6), dispersants produced a greater decrease in settlement. Dispersed oil showed a dramatic increase in toxicity to both species, suggesting that the low oil: seawater ratio 1:200, that was not lethal to *S. pistillata* larvae in the WSF tests, became highly toxic after dispersion. Dispersants and WAF caused deformation, abnormal swimming and tissue degeneration (Fig. 7.7). Authors suggested dispersants not be used near coral reefs.

Pesticides

Markey et al. (2007) examined effects of two organophosphates (chlorpyrifos, profenofos), an organochlorine (endosulfan), a carbamate (carbaryl), a pyrethroid

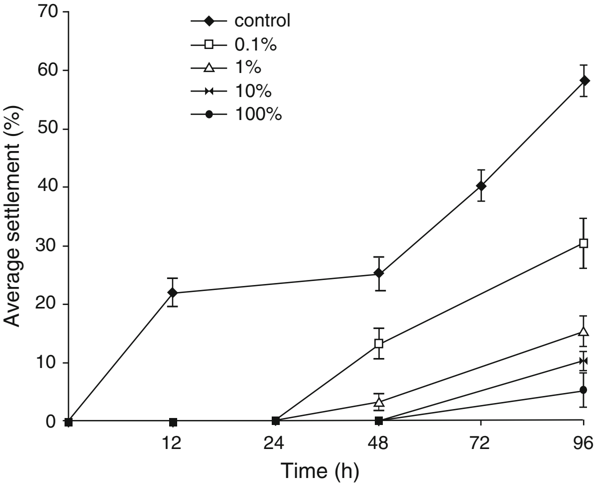


Fig. 7.6 Settlement rates of *S. pistillata* planulae in seawater control and Egyptian crude oil WSF treatments (Reprinted from Epstein et al. 2000: 499, courtesy Elsevier Publishing Co.)

(permethrin), and a fungicide (2-methoxyethylmercuric chloride, MEMC) on embryos and larvae of the coral *Acropora millepora*. Fertilization was not affected by any of the insecticides up to 30 μg l^{-1}, but settlement and metamorphosis were reduced by 50–100 % after 18 h exposure to very low concentrations (0.3–1.0 μg l^{-1}) of each insecticide.

Endosulfan (ES) strongly inhibited larval settlement and early juvenile growth of the polychaete *Streblospio benedicti*. ES concentrations as low as 50 μg kg^{-1} sediment reduced settlement by >50 % relative to control sediments (Chandler and Scott 1991). Higher concentrations closer to actual field levels suppressed colonization completely. Early growth of newly metamorphosed juveniles was depressed 36 and 40 % in 50 and 100 μg kg^{-1}, respectively. This is in contrast with the tolerance of this species for PAHs (above) and tolerance of harpacticoid copepods. When the benthic harpacticoid copepod *Pseudobradya pulchella* was exposed to sediment ES < 200 μg kg^{-1}, survival and egg production were unaffected. Of the *P. pulchella* tested, >95 % survived 200 μg kg^{-1}, and over 98 % of the females produced normal clutches of eggs. Similarly, survival of another common benthic copepod, *Nannopus palustris*, was not affected below 200 μg kg^{-1} ES, but 200 μg kg^{-1} reduced survival.

Contaminants of Emerging Concern

Chiu et al. (2012) investigated effects of flame retardant polybrominated diphenyl ethers (PBDEs) on larvae of the polychaete *Hydroides elegans*, and demonstrated that chronic exposure to BDE-47 (up to 1,000 ng l^{-1}) throughout the entire larval stage did not affect settlement, development or growth despite bioaccumulation.

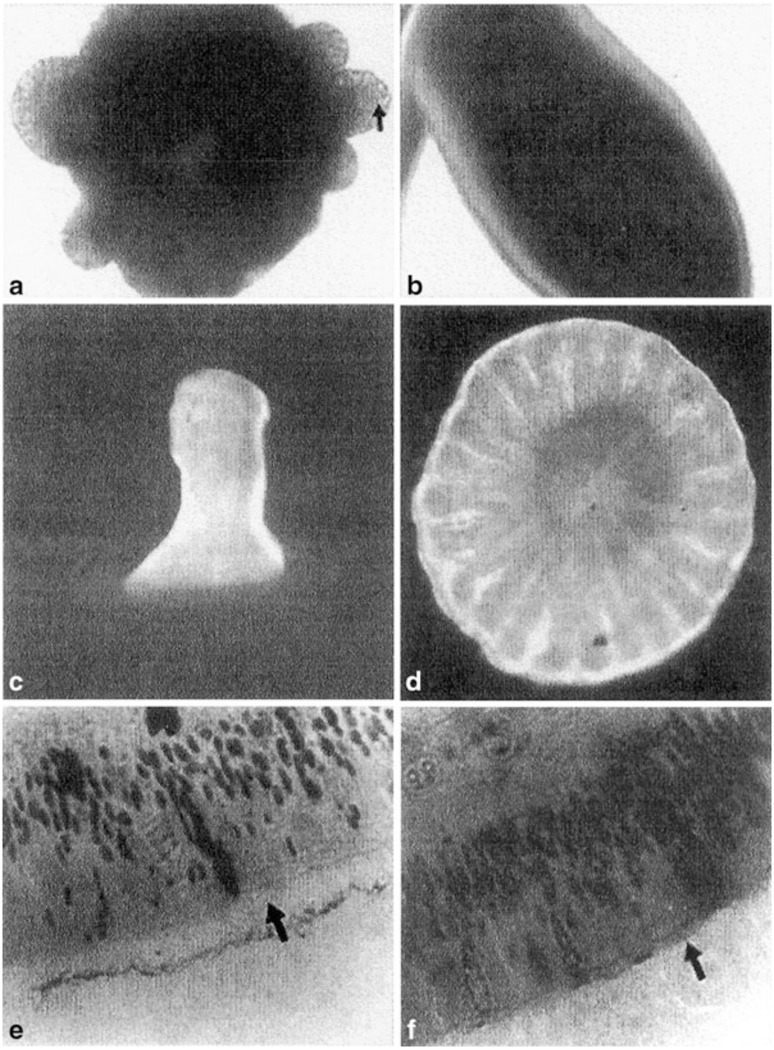


Fig. 7.7 Effects of dispersed oil on *S. pistillata* planulae. (**a**) Disintegration. Release of small spherical bodies through the ectodermal layer (*arrow*) (**b**) Control planula (**c**) deformed primary polyp with no mouth or tentacles (**d**) deformed unattached planula with 12 pairs of septa instead of 6 (**e**) and (**f**) are histological sections from treated larva (**e**) and control (**f**). *Arrows* show ectodermal layer which is damaged in (**e**) (Reprinted from Epstein et al. 2000: 501, courtesy Elsevier Publishing Co.)

7.4.3 Hypoxia

Miller and Graham (2012) made the unexpected finding that low DO had a positive effect on planula larva settlement of the jellyfish *Aurelia* sp. Greatest settlement rates occurred under lowest DO (1.3 mg l^{-1}), indicating that reduced DO promotes settlement. In another set of experiments, they found that survival of scyphistomae (the benthic attached stage) decreased only marginally under prolonged (56 days) hypoxia. Laboratory experiments showed that the normal sessile community coverage was significantly reduced under similar levels of hypoxia when compared to normoxia. Thus, not only was settlement of planulae favored at low DO, but potentially competing species were reduced. These findings support previous ideas that eutrophication and hypoxia can promote outbreaks of jellyfish.

7.4.4 Climate Change/Ocean Acidification

In a field study, artificial collectors were placed for 1 month along pH gradients near CO_2 vents in the Tyrrhenian Sea, Italy to collect newly settled forms (Cigliano et al. 2010). Seventy-nine taxa were identified from six main groups (foraminiferans, nematodes, polychaetes, molluscs, crustaceans and chaetognaths). Calcareous foraminiferans, serpulid polychaetes, gastropods and bivalves were greatly reduced as $p\text{CO}_2$ rose from normal (336–341 mg l^{-1}, pH 8.09–8.15) to high levels (886–5,148 mg l^{-1}) near vent sites (pH 7.08–7.79). The syllid polychaete *Syllis prolifera* was most abundant at the most acidic station, although many polychaetes and small crustaceans could settle and survive. A few taxa (*Amphiglena mediterranea*, *Leptochelia dubia*, *Caprella acanthifera*) were most abundant at sites with intermediate $p\text{CO}_2$ (pH 7.41–7.99), showing that increased $p\text{CO}_2$ can affect the settlement of a wide range of benthic organisms.

Corals

Nakamura et al. (2011) found that the oxygen consumption of planula larvae of the coral *Acropora digitifera* was reduced (but not significantly) with reduced pH (8.0, 7.6, and 7.3). Metamorphosis was significantly decreased under acidified conditions after both short- (2 h) and long- (7 days) term exposure. Larval metabolism and settlement, and post-settlement growth of the coral *Porites astreoides* was studied at ambient seawater, 560, and 800 μatm. Larval metabolism was depressed by 27 and 63 % at 560 and 800 μatm, respectively (Albright and Langdon 2011). Settlement was reduced by 45 % at 560 μatm and 60 % at 800 μatm, but via indirect pathways, i.e. by altering the substrate community composition and reducing settlement cues. Post-settlement growth decreased by 16 and 35 % at 560 and 800 μatm, respectively. Similarly, $p\text{CO}_2$ concentrations of 800 and 1,300 μatm significantly

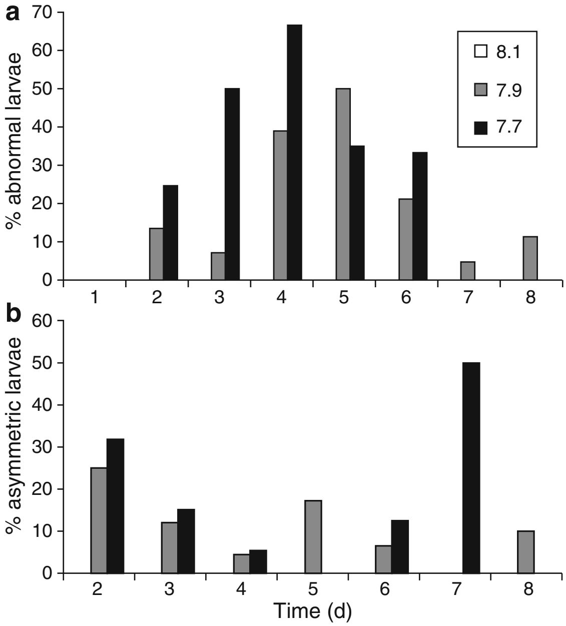


Fig. 7.8 Percentage abnormal (**a**) and asymmetric (**b**) *O. fragilis* larvae at pH 8.1, 7.9, and 7.7. Note absence of abnormalities at pH 8.1 (controls) (Reprinted from Dupont et al. 2008: 293, courtesy Inter-Research)

reduced *Acropora millepora* settlement and crustose coralline algae (CCA) cover by ≥45 % (Doropoulos et al. 2012) (CCA are important for inducing coral settlement). The preferred alga substrate for settlement (*Titanoderma*) of control larvae was avoided by larvae as pCO$_2$ increased, and other substrates selected. These results suggest acidification may reduce coral populations by reducing coral settlement rates, disrupting larval settlement behavior, and reducing the availability of desirable coralline algal species for coral settlement and recruitment.

Echinoderms

Larval development of the brittlestar *Ophiothrix fragilis* (a keystone species throughout the shelf seas of the eastern Atlantic) was studied by DuPont et al. (2008). A decrease of 0.2 pH units induced 100 % larval mortality within 8 days while control larvae showed 70 % survival. Low pH also resulted in smaller larval size, abnormal development and skeletogenesis including abnormalities, asymmetry, and altered skeletal proportions (Fig. 7.8). The larval development of the sea urchin *Arbacia dufresnei* from a sub-Antarctic population was studied at high (8.0), medium (7.7) and low (7.4) pH (Catarino et al. 2012). Larvae showed a developmental delay at low pH but without increases in abnormalities. Even at calcium carbonate saturation states <1, skeletal deposition occurred. Thus, some polar and sub-polar sea urchin larvae are resilient to acidification.

Larval sand dollars *Dendraster excentricus* have calcified skeletal rods supporting their bodies, and propel themselves with ciliated bands looped around projections called arms. The ciliated bands are used in food capture, and filtration rate is correlated with band length. Therefore, swimming and feeding performance are sensitive to morphological changes. When reared at elevated $p\text{CO}_2$ (1,000 mg l^{-1}), larvae developed significantly narrower bodies at the four and six-arm stages and had significantly smaller stomachs and bodies, suggesting reduced feeding ability (Chan et al. 2011).

Sea urchins (*Heliocidaris erythrogramma*) were reared by Byrne et al. (2011) in varying temperature and pH: +2–4 °C, and pH 7.6–7.8. Treatments resulted in unshelled larvae and abnormal juveniles, with the percentage of normal juveniles decreasing in response to both stressors. The number of spines decreased with increasing acidification, and the interactive effect between stressors indicated that +2 °C warming reduced the negative effects of low pH, which may be good news for the future of this species, since warming and lower pH will happen together. To investigate effects of acidification on calcification at the molecular level, Kurihara et al. (2012) evaluated the expression of biomineralization-related genes in the sea urchin *Hemicentrotus pulcherrimus* at control, 1,000, and 2,000 mg l^{-1} CO_2 from egg to pluteus larva. They found that the expression of the gene *msp130*, which is proposed to transport Ca^{2+} to the calcification site, is suppressed by increased CO_2. This suggests that OA suppression of the expression of skeletogenesis-related genes is responsible for impaired biomineralization of sea urchins.

7.5 Conclusions/Discussion

Larvae are very sensitive to contaminants, in some cases more so than embryos. The studies that have been done are numerous, and are a “mixed bag.” While many studies to date focus solely on measurements of mortality (i.e. LC50 in standard toxicity tests), other (more interesting) studies examine sublethal effects on growth, development, behavior, physiological processes, etc. Studies of sublethal effects on these processes enable one to understand the ways in which a particular stressor affects the larvae, and may give insight and understanding into possible mechanisms of larval mortality. The taxa that have been used in these studies tend to be ones for which techniques have been developed and standardized for raising them in the laboratory, but techniques can be developed for culturing additional species. This should be encouraged and would be a significant advance for the field, as responses and sensitivities vary considerably among different taxa. Settlement and metamorphosis to juvenile stages is critical in many taxa, and is often the stage most sensitive to stressors. Furthermore, some effects of larval exposures are not apparent until after larvae have metamorphosed and have become juveniles or adults. This emphasizes the point that long-term studies are far more useful and important than short term larval toxicity tests.

References

Albright R, Langdon C (2011) Ocean acidification impacts multiple early life history processes of the Caribbean coral *Porites astreoides*. Glob Change Biol 17:2478–2487

Anderson BS, Middaugh DP, Hunt JW, Turpen SL (1991) Copper toxicity to sperm, embryos and larvae of topsmelt *Atherinops affinis*, with notes on induced spawning. Mar Environ Res 31:17–35

Anderson HR, Wollenberger L, Halling-Sørensen B, Kusk KO (2001) Development of copepod nauplii to copepodites—a parameter for chronic toxicity including endocrine disruption. Environ Toxicol Chem 20:2821–2829

Arnold KE, Findlay HS, Spicer JI, Daniels CL, Boothroyd D (2009) Effect of CO2-related acidification on aspects of the larval development of the European lobster, *Homarus gammarus* (L.). Biogeosci Discuss 6:3087–3107

Arukwe A, Cangialosi MV, Letcher RJ, Rocha E, Mortensen AS (2013) Changes in morphometry and association between whole-body fatty acids and steroid hormone profiles in relation to bioaccumulation patterns in salmon larvae exposed to perfluorooctane sulfonic or perfluorooctane carboxylic acids. Aquat Toxicol 130–131:219–230, http://dx.doi.org/10.1016/j.aquatox.2012.12.026

Baker SM, Mann R (1992) Effects of hypoxia and anoxia on larval settlement, juvenile growth, and juvenile survival of the oyster *Crassostrea virginica*. Biol Bull 182:265–269

Baker SM, Mann R (1994) Feeding ability during settlement and metamorphosis in the oyster *Crassostrea virginica* (Gmelin, 1791) and the effects of hypoxia on post-settlement ingestion rates. J Exp Mar Biol Ecol 181:239–253

Barton A, Hales B, Waldbusser GG, Langdon C, Feeley RA (2012) The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: implications for near-term ocean acidification effects. Limnol Oceanogr 57:698–710

Béguer M, Pasquaud S, Noël P, Girardin M, Boët P (2008) First description of heavy skeletal deformations in *Palaemon* shrimp populations of European estuaries: the case of the Gironde (France). Hydrobiologia 607:225–229

Beiras R, His E (1994) Effects of dissolved mercury on embryogenesis, survival and metamorphosis of *Crassostrea gigas* oyster larvae. Mar Ecol Prog Ser 113:95–103

Bengtsson BE, Bengtsson Å, Himberg M (1985) Fish deformities and pollution in some Swedish waters. Ambio 14:32–35

Bergey L, Weis JS (2008) Aspects of population ecology in two populations of fiddler crabs, *Uca pugnax*. Mar Biol 154:435–442

Bookhout CG, Costlow JD Jr (1974) Crab development and effects of pollutants. Thalassia Jugoslavica 10:77–87

Bookhout CG, Monroe RJ (1977) Effects of malathion on the development of crabs. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Physiological responses of marine biota to pollutants. Academic, New York, pp 3–19

Bookhout CG, Monroe RJ, Forward RB, Costlow JD (1984) Effects of hexavalent chromium on development of crabs *Rhithropanopeus harrisii* and *Callinectes sapidus*. Water Air Soil Pollut 21:199–216

Breitholtz M, Wollenberger L (2003) Effects of three PBDEs on development, reproduction and population growth rate of the harpacticoid copepod *Nitocra spinipes*. Aquat Toxicol 64:85–96

Brereton A, Lord H, Webb JS (1973) Effect of zinc on growth and development of larvae of the Pacific oyster, *Crassostrea gigas*. Mar Biol 19:96–101

Brix KV, Gillette P, Pourmand A, Capo TR, Grosell M (2012) The effects of dietary silver on larval growth in the echinoderm *Lytechinus variegates*. Arch Environ Contam Toxicol 63:95–100

Byrne M, Ho M, Wong E, Soars NA, Selvakumaraswamy P, Shepard-Brennand H, Dworjanyn SA, Davis AR (2011) Unshelled abalone and corrupted urchins: development of marine calcifiers in a changing ocean. Proc R Soc B Biol Sci 278:2376–2383

Calabrese A, Macinnes JR, Nelson DA, Miller JE (1977) Survival and growth of bivalve larvae under heavy metal stress. Mar Biol 41:179–184

Cao L, Huang W, Shan X, Xiao Z, Wang Q, Dou S (2009) Cadmium toxicity to embryonic–larval development and survival in red sea bream *Pagrus major*. Ecotoxicol Environ Saf 72:1966–1974

Catarino A, De Ridder C, Gonzalez M, Gallardo P, Dubois P (2012) Sea urchin *Arbacia dufresnei* (Blainville 1825) larvae response to ocean acidification. Polar Biol 35:455–461

Chan HY, Xu WZ, Shin PKS, Cheung SG (2008) Prolonged exposure to low dissolved oxygen affects early development and swimming behaviour in the gastropod *Nassarius festivus* (Nassariidae). Mar Biol 153:735–743

Chan KYK, Grünbaum D, O'Donnell MJ (2011) Effects of ocean-acidification-induced morphological changes on larval swimming and feeding. J Exp Biol 214:3857–3867

Chandler GT, Scott GI (1991) Effects of sediment-bound endosulfan on survival, reproduction and larval settlement of meiobenthic polychaetes and copepods. Environ Toxicol Chem 10:375–382

Chandler GT, Shipp MR, Donelan TL (1997) Bioaccumulation, growth and larval settlement effects of sediment-associated polynuclear aromatic hydrocarbons on the estuarine polychaete, *Streblospio benedicti* (Webster). J Exp Mar Biol Ecol 213:95–110

Chiu JM, Po B, Chan CY, Lam MH, Qian PY, Wu RS (2012) Polybrominated diphenyl ethers (PBDEs) alter larval settlement of marine intertidal organisms across three phyla via reducing bacterial abundance on the biofilms. Environ Sci Technol 46:7772–7781

Cigliano M, Gambi MC, Rodolfo-Metalpa R, Patti FP, Hall-Spencer JM (2010) Effects of ocean acidification on invertebrate settlement at volcanic CO_2 vents. Mar Biol 157:2489–2502

Costlow JD (1979) Effect of Dimilin® on development of larvae of the stone crab *Menippe mercenaria* and blue crab *Callinectes sapidus*. In: Vernberg WB, Thurberg FP, Calabrese A, Vernberg FJ (eds) Marine pollution: functional responses. Academic, New York, pp 355–363

Couillard CM, Lee K, Légaré B, King TL (2005) Effect of dispersant on the composition of the water-accommodated fraction of crude oil and its toxicity to larval marine fish. Environ Toxicol Chem 24:1496–1504

Couillard CM, Lebeuf M, Legaré B, Trottier S (2008) Effects of diazinon on mummichog (*Fundulus heteroclitus*) larvae produced from eggs differentially treated with PCB126. Arch Environ Contam Toxicol 54:283–291

Crim RN, Sunday JM, Harley CD (2011) Elevated seawater CO_2 concentrations impair larval development and reduce larval survival in endangered northern abalone (*Haliotis kamtschatkana*). J Exp Mar Biol Ecol 400:272–277

Cross J, Hardy JY, Hose JE, Hershelman GP, Antrim LD, Gossett RW, Crecelius EA (1987) Contaminant concentrations and toxicity in sea surface microlayer near Los Angeles California. Mar Environ Res 23:307–323

Dethlefsen V, von Westernhagen H, Rosenthal H (1975) Cadmium uptake by marine fish larvae. Helgolander Wiss Meeresunters 27:396–407

Doropoulos C, Ward S, Diaz-Pulido G, Hoegh-Guldberg O, Mumby PJ (2012) Ocean acidification reduces coral recruitment by disrupting intimate larval-algal settlement interactions. Ecol Lett 15:338–346

Dupont S, Havenhand J, Thorndyke W, Peck L, Thorndyke M (2008) Near-future level of CO2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. Mar Ecol Prog Ser 373:285–294

Epstein N, Bak RP, Rinkevich J (2000) Toxicity of third generation dispersants and dispersed Egyptian crude oil on Red Sea coral larvae. Mar Pollut Bull 40:497–503

Faulk CK, Fuiman L, Thomas P (1999) Parental exposure to *ortho*, *para*-dichlorodiphenyltrichloroethane impairs survival skills of atlantic croaker (*Micropogonias undulatus*) larvae. Environ Toxicol Chem 18:254–262

Foekema EM, Deerenberg CM, Murk AJ (2008) Prolonged ELS test with the marine flatfish sole (*Solea solea*) shows delayed toxic effects of previous exposure to PCB 126. Aquat Toxicol 90:197–203

Foekema EM, Fischer A, Lopez Parron M, Kwadijk C, de Vries P, Murk AJ (2012) Toxic concentrations in fish early life stages peak at a critical moment. Environ Toxicol Chem 31:1381–1390

Frommel AY, Maneja R, Lowe D, Malzahn AM, Geffen AJ, Folkvord A, Piatkowski U, Reusch TBH, Clemmesen C (2012) Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. Nat Clim Change 2:42–46

Gazeau F, Quiblier C, Jansen JM, Gattuso J-P, Middelburg JJ, Heip CHR (2007) Impact of elevated CO_2 on shellfish calcification. Geophys Res Lett 34(L07603):1–5

Gazeau F, Gattuso JP, Dawber C, Pronker AE, Peene F, Peene J, Heip CHR, Middelburg JJ (2010) Effect of ocean acidification on the early life stages of the blue mussel *Mytilus edulis*. Biogeosciences 7:2051–2060

Geffard O, Geffard A, His E, Budzinski H (2003) Assessment of the bioavailability and toxicity of sediment-associated polycyclic aromatic hydrocarbons and heavy metals applied to *Crassostrea gigas* embryos and larvae. Mar Pollut Bull 46:481–490

Gorski J, Nugegoda D (2006) Sublethal toxicity of trace metals to larvae of the blacklip abalone, *Haliotis rubra*. Environ Toxicol Chem 25:1360–1367

Hanson B, Fotel FL, Jensen NJ, Wittrup L (1997) Physiological effects of the detergent linear alkylbenzene sulphonate on blue mussel larvae (*Mytilus edulis*) in laboratory and mesocosm experiments. Mar Biol 128:627–637

Hardy J, Kiesser S, Antrim L, Stubin A, Kocan R, Strand J (1987) The sea-surface microlayer of Puget Sound: Part I. Toxic effects on fish eggs and larvae. Mar Environ Res 23:227–249

Hettinger A, Sanford E, Hill TM, Russell AD, Sato KN, Hoey J, Forsch M, Page HN, Gaylord B (2012) Persistent carry-over effects of planktonic exposure to ocean acidification in the Olympia oyster. Ecology 93:2758–2768

His E, Seaman MNL, Beiras R (1997) A simplification of the bivalve embryogenesis and larval development bioassay method for water quality assessment. Water Res 31:351–355

His E, Heyvang I, Geffard O, de Montaudouin X (1999) A comparison between oyster (*Crassostrea gigas*) and sea urchin (*Paracentrotus lividus*) larval bioassays for toxicological studies. Water Res 33:1706–1718

Hoare K, Davenport J, Beaumont AR (1995) Effects of exposure and previous exposure to copper on growth of veliger larvae and survivorship of *Mytilus edulis* juveniles. Mar Ecol Prog Ser 120:163–168

Holdway DA, Barry MJ, Logan DC, Robertson D, Young V, Ahokas JT (1994) Toxicity of pulse-exposed fenvalerate and esfenvalerate to larval Australian crimson-spotted rainbow fish (*Melanotaenia fluviatilis*). Aquat Toxicol 28:169–187

Hutchinson TH, Williams TD, Eales GJ (1994) Toxicity of cadmium, hexavalent chromium and copper to marine fish larvae (*Cyprinodon variegatus*) and copepods (*Tisbe battagliai*). Mar Environ Res 38:275–290

Ingvarsdóttir A, Bjørkblom C, Ravagnan E, Godal BF, Arnberg M, Joachim DL, Sanni S (2012) Effects of different concentrations of crude oil on first feeding larvae of Atlantic herring (*Clupea harengus*). J Mar Syst 93:69–76

Ishibashi Y, Inoue K, Nakatsukasa H, Ishitani Y, Miyashita S, Murata O (2005) Ontogeny of tolerance to hypoxia and oxygen consumption of larval and juvenile red sea bream, *Pagrus major*. Aquaculture 244:331–340

Johns M, Pechenik J (1980) Influence of water-accommodated fraction of Number 2 fuel oil on energetics of Cancer irroratus larvae. Mar Biol 55:247–254

Kawaguchi M, Song J-Y, Irie K, Murakami Y, Nakayama K, Kitamura S-I (2011) Disruption of *Sema3A* expression causes abnormal neural projection in heavy oil exposed Japanese flounder larvae. Mar Pollut Bull 63:356–361

Kessabi K, Annabi A, Hassine A, Bazin I, Mnif W, Said K, Messaoudi I (2013) Possible chemical causes of skeletal deformities in natural populations of *Aphanius fasciatus* collected from the Tunisian coast. Chemosphere 90(11):2683–2689, http://dx.doi.org/10.1016/j.chemosphere.2012.11.047

Key PB, Fulton MH, Scott GI, Layman SL, Worth EF (1998) Lethal and sublethal effects of malathion on three life stages of the grass shrimp *Palaemonetes pugio*. Aquat Toxicol 40:311–322

Key PB, Chung KW, Hoguet J, Shaddrix B, Fulton MH (2008) Toxicity and physiological effects of brominated flame retardant PBDE-47 on two life stages of grass shrimp, *Palaemonetes pugio*. Sci Total Environ 399:28–32

Kurihara H, Kato S, Ishimatsu A (2007) Effects of increased seawater pCO_2 on early development of the oyster *Crassostrea gigas*. Aquat Biol 1:91–98

Kurihara H, Asai T, Kato S, Ishimatsu A (2008) Effects of elevated pCO_2 on early development in the mussel *Mytilus galloprovincialis*. Aquat Biol 4:225–233

Kurihara H, Takano Y, Kurokawa D, Akasaka K (2012) Ocean acidification reduces biomineralization-related gene expression in the sea urchin, *Hemicentrotus pulcherrimus*. Mar Biol 159:2819–2826

LaBreche TM, Dietrich AM, Gallagher DL, Shepherd N (2002) Copper toxicity to larval *Mercenaria mercenaria* (hard clam). Environ Toxicol Chem 21:760–766

Lang WH, Miller DC, Ritacco PJ, Marcy M (1981) The effects of copper and cadmium on the behavior and development of barnacle larvae. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Biological monitoring of marine pollutants. Academic, New York, pp 165–203

Laughlin R, Neff JM (1979) Interactive effects of salinity, temperature, and polycyclic aromatic hydrocarbons on survival and development rate of larvae of the mud crab, *Rhithropanopeus harrissi*. Mar Biol 53:281–291

Laughlin R, Ng J, Guard N (1981) Hormesis: a response to low environmental concentrations of petroleum hydrocarbons. Science 211:705–707

Lee WY, Winters K, Nicol JAC (1970) The biological effects of the water-soluble fractions of a no. 2 fuel oil on the planktonic shrimp, *Lucifer faxoni*. Environ Pollut 15:167–183

Liem K (1981) Larvae of air-breathing fishes as countercurrent flow devices in hypoxic environments. Science 211:1177–1179

Liu Y, Tam N, Guan Y, Yasojima M, Zhou J, Gao B (2011) Acute toxicity of nonylphenols and bisphenol A to the embryonic development of the abalone *Haliotis diversicolor supertexta*. Ecotoxicology 20:233–1245

Lopez Greco L, Bolanos J, Rodriguez EM, Hernandez G (2001) Survival and molting of the pea crab larvae *Tunicotheres moseri* Rathbun 1918 (Brachyura, Pinnotheridae) exposed to copper. Arch Environ Contam Toxicol 40:505–510

MacInnes JA, Calabrese A (1979) Combined effects of salinity, temperature, and copper on embryos and early larvae of the American oyster, *Crassostrea virginica*. Arch Environ Contam Toxicol 8:553–562

Markey KL, Baird AH, Humphrey C, Negri AP (2007) Insecticides and a fungicide affect multiple coral life stages. Mar Ecol Prog Ser 330:127–137

McCarthy I, Fuiman LA, Alvarez MC (2003) Aroclor 1254 affects growth and survival skills of Atlantic croaker *Micropogonias undulatus* larvae. Mar Ecol Prog Ser 252:295–301

McFadzen IRB (1992) Growth and survival of cryopreserved oyster and clam larvae along a pollution gradient in the German Bight. Mar Ecol Prog Ser 91:215–220

McKenney CL (2005) The influence of insect juvenile hormone agonists on metamorphosis and reproduction in estuarine crustaceans. Integr Comp Biol 45:97–105

McKenney CL, Costlow JD (1981) the effects of salinity and mercury on developing megalopae and early crab stages of the blue crab, *Callinectes sapidus*. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Biological monitoring of marine pollutants. Academic, New York, pp 241–262

McKenney CL Jr, Matthews E (1990) Influence of an insect growth regulator on the larval development of an estuarine shrimp. Environ Pollut 64:169–178

Mecklenburg TA, Rice SD, Karinen JF (1977) Molting and survival of king crab (*Paralithodes camschatica*) and coonstripe shrimp (*Pandalus hypsinotus*) larvae exposed to cook inlet crude oil water-soluble fractions. In: Wolfe DA (ed) Fate and effects of petroleum hydrocarbons in marine organisms and ecosystems. Pergamon Press, New York, pp 221–228

Mhadhbi L, Fumega J, Boumaiza M, Beiras R (2012) Acute toxicity of polybrominated diphenyl ethers (PBDEs) for turbot (*Psetta maxima*) early life stages (ELS). Environ Sci Pollut Res Int 19:708–717

Miller M-E, Graham W (2012) Environmental evidence that seasonal hypoxia enhances survival and success of jellyfish polyps in the northern Gulf of Mexico. J Exp Mar Biol Ecol 432–433:113–120

Money C, Braungardt CB, Jha AN, Worsfold PJ, Achterberg EP (2011) Metal speciation and toxicity of Tamar Estuary water to larvae of the Pacific oyster, *Crassostrea gigas*. Mar Environ Res 72:3–12

Monosson E, Fleming WJ, Sullivan CV (1994) Effects of the planar PCB 3,3′,4,4′-tetrachlorobiphenyl (TCB) on ovarian development, plasma levels of sex steroid hormones and vitellogenin, and progeny survival in the white perch (*Morone americana*). Aquat Toxicol 29:1–19

Mortimer MR, Miller GJ (1994) Susceptibility of larval and juvenile instars of the sand crab, *Portunus pelagicus* (L.) to sea water contaminated by chromium, nickel or copper. Aust J Mar Freshw Res 45:1107–1121

Mottier A, Kientz-Bouchart V, Serpentini A, Lebel JM, Jha AN, Costil K (2013) Effects of glyphosate-based herbicides on embryo-larval development and metamorphosis in the Pacific oyster, *Crassostrea gigas*. Aquat Toxicol 128–129:67–78

Munday PL, Gagliano M, Donelson JM, Dixson DL, Thorrold SR (2011a) Ocean acidification does not affect the early life history development of a tropical marine fish. Mar Ecol Prog Ser 423:211–221

Munday PL, Hernaman V, Dixson DL, Thorrold SR (2011b) Effect of ocean acidification on otolith development in larvae of a tropical marine fish. Biogeosciences 8:1631–1641

Nakamura M, Ohki S, Suzuki A, Sakai K (2011) Coral larvae under ocean acidification: survival, metabolism, and metamorphosis. PLoS One 6(1):art.e14521

Nice HE, Thorndyke MC, Morritt D, Steele S, Crane M (2000) Development of *Crassostrea gigas* larvae is affected by 4-nonylphenol. Mar Pollut Bull 40:491–496

Olsvik PA, Hansen BH, Nordtug T, Moren M, Holen E, Lie KK (2011) Transcriptional evidence for low contribution of oil droplets to acute toxicity from dispersed oil in first feeding Atlantic cod (*Gadus morhua*) larvae. Comp Biochem Physiol Part C Toxicol Pharmacol 154:333–345

Olufsen M, Arukwe A (2011) Developmental effects related to angiogenesis and osteogenic differentiation in Salmon larvae continuously exposed to dioxin-like 3,3′,4,4′-tetrachlorobiphenyl (congener 77). Aquat Toxicol 105:669–680

Osterberg JS, Darnell KM, Blickley TM, Romano JA, Rittschof D (2012) Acute toxicity and sublethal effects of common pesticides in post-larval and juvenile blue crabs, *Callinectes sapidus*. J Exp Mar Biol Ecol 424–425:5–14

Paixão JF, Nascimento IA, Pereira SA, Leite MBL, Carvalho GC, Silveira JSC Jr, Rebouças M, Matias GRA, Rodrigues ILP (2007) Estimating the gasoline components and formulations toxicity to microalgae (*Tetraselmis chuii*) and oyster (*Crassostrea rhizophorae*) embryos: an approach to minimize environmental pollution risk. Environ Res 103:365–374

Pansch C, Nasrolahi A, Appelhans YS, Wahl M (2012) Impacts of ocean warming and acidification on the larval development of the barnacle *Amphibalanus improvisus*. J Exp Mar Biol Ecol 420–421:48–55

Parker LM, Ross PM, O'Connor WA, Borysko L, Raftos DA, Pörtner HO (2012) Adult exposure influences offspring response to ocean acidification in oysters. Glob Change Biol 18:82–92

Pfeiler E (2001) Changes in hypoxia tolerance during metamorphosis of bonefish leptocephali. J Fish Biol 59:1677–1681

Reichelt-Brushett AJ, Harrison PL (2000) The effect of copper on the settlement success of larvae from the Scleractinian Coral *Acropora tenuis*. Mar Pollut Bull 41:385–391

Roesijadi G, Petrocelli S, Anderson JW, Giam S, Neff J (1976) Toxicity of polychlorinated biphenyls (Aroclor 1254) to adult, juvenile, and larval stages of the shrimp, *Palaemonetes pugio*. Bull Environ Contam Toxicol 15:297–304

Rosenberg R, Costlow JD (1976) Synergistic effects of cadmium and salinity combined with constant and cycling temperatures on the larval development of two estuarine crab species. Mar Biol 38:291–303

Ryan RL, Lachmayr KL, Jay JA, Ford TE (2001) Developmental effects of PCBs on the hard clam (*Mercenaria mercenaria*). J Toxicol Environ Health A 36:1571–1578

Simith D, Diele K, Abrunhosa FA (2013) Carry-over effects of delayed larval metamorphosis on early juvenile performance in the mangrove crab *Ucides cordatus* (Ucididae). J Exp Mar Biol Ecol 440:61–68

Snyder MJ, Mulder EP (2001) Environmental endocrine disruption in decapod crustacean larvae: hormone titers, cytochrome P450, and stress protein responses to heptachlor exposure. Aquat Toxicol 55:177–190

Soffientino B, Nacci DE, Specker JL (2010) Effects of the dioxin-like PCB 126 on larval summer flounder (*Paralichthys dentatus*). Comp Biochem Physiol C 152:9–17

Somasundaram B, King PE, Shackley SE (1984) Some morphological effects of zinc upon the yolk-sac larvae of *Clupea harengus* L. J Fish Biol 25:333–343

Spicer JI, Eriksson SP (2003) Does the development of respiratory regulation always accompany the transition from pelagic larvae to benthic fossorial postlarvae in the Norway lobster *Nephrops norvegicus* (L.)? J Exp Mar Biol Ecol 295:219–243

Talmage SC, Gobler CJ (2011) Effects of elevated temperature and carbon dioxide on the growth and survival of larvae and juveniles of three species of northwest Atlantic bivalves. PLoS One 6(10):art.e26941

Walker AN, Bush P, Puritz J, Wilson T, Chang ES, Miller T, Holloway K, Horst MN (2005) Bioaccumulation and metabolic effects of the endocrine disruptor methoprene in the lobster, *Homarus americanus*. Integr Comp Biol 45:118–126

Walther K, Anger K, Pörtner HO (2010) Effects of ocean acidification and warming on the larval development of the spider crab *Hyas araneus* from different latitudes (54° vs. 79°N). Mar Ecol Prog Ser 417:159–170

Wang WX, Widdows J (1991) Physiological responses of mussel larvae *Mytilus edulis* to environmental hypoxia and anoxia. Mar Ecol Prog Ser 70:223–236

Wang C, Zhang F (1995) Effects of environmental oxygen deficiency on embryos and larvae of bay scallop, *Argopecten irradians irradians*. Chin J Oceanol Limnol 13:362–369

Wang Q, Yang H, Liu B, Wang X (2012) Toxic effects of benzo[*a*]pyrene (Bap) and Aroclor1254 on embryogenesis, larval growth, survival and metamorphosis of the bivalve *Meretrix meretrix*. Ecotoxicology 21:1617–1624

Weltzien F, Doving KB, Carr WE (1999) Avoidance reaction of yolk-sac larvae of the inland silverside *Menidia beryllina* (Atherinidae) to hypoxia. J Exp Biol 202:2869–2876

Williams ND, Holdway DA (2000) The effects of pulse-exposed cadmium and zinc on embryo hatchability, larval development, and survival of Australian crimson spotted rainbow fish (*Melanotaenia fluviatilis*). Environ Toxicol 15:165–173

Wong CK, Cheung JK, Chu KH (1995) Effects of copper on survival, development and growth of *Metapenaeus ensis* larvae and postlarvae (Decapoda: Penaeidae). Mar Pollut Bull 31:416–419

Zippay ML, Hofmann GE (2010) Effect of pH on gene expression and thermal tolerance of early life history stages of red abalone (*Haliotis rufescens*). J Shellfish Res 29:429–439

Chapter 8
Developmental Processes Later in Life

Abstract Growth is an obvious and easily measured response, and is included in many standard toxicity tests as well as in research projects. Reduced growth is frequently traced back to reduced food intake, but even without reduced feeding, it is a logical outcome since organisms must expend energy to defend themselves against and detoxify contaminants. The more energy needed to detoxify pollutants, the less will be available for growth. In addition to overall body growth, molting, regeneration, development of calcified structures (shell and bone), carcinogenesis, and smoltification are other developmental processes that take place after larval stages. These processes are all sensitive to environmental contaminants.

Keywords Calcification • Carcinogenesis • Ecdysis • Molting • Regeneration • Shell • Smolt

8.1 Growth

Growth is the end result of a number of processes including food intake and energy metabolism which are affected negatively by many contaminants. Consequently, growth tends to be reduced in exposed organisms.

8.1.1 Fishes

Fishes continue growing as juveniles and adults, albeit more slowly over time, throughout their lives, and growth can be affected by contaminants. There have been hundreds of studies of fish growth in response to contaminants, largely in freshwater species and early life stages. Many of these studies are based on dietary exposure, and show that reduced feeding is responsible for growth effects. However,

J.S. Weis, *Physiological, Developmental and Behavioral Effects of Marine Pollution*, DOI 10.1007/978-94-007-6949-6_8,

some environmentally important pollutants, including xenoestrogens and metals have been shown to affect growth via hormone-mediated mechanisms, possibly by interfering with the GH receptor and/or GH transcription (Deane and Woo 2009). Other contaminants, such as dioxins, furans, PCBs, polycyclic aromatic hydrocarbons, pesticides, phenols, ammonia, pharmaceuticals and metals, may affect growth and other developmental processes by thyroid disruption (reviewed by Brown et al. 2004; Rolland 2000).

Woltering (1984) reviewed 173 studies to date on fish growth. The tests include exposure to metals, pesticides, unclassified organics, inorganic compounds, detergents and complex effluents. Fry survival was significantly reduced in 57 % of all tests at the lowest effect level and fry growth was reduced in 36 % of the tests. Only 60 % of the tests include exposure of adults; adult growth was seldom the most sensitive effect. Fry survival and growth were very often equally sensitive.

Metals

There have been many studies of effects of metals on juvenile fishes, of which a few will be mentioned. Baker et al. (1998) fed juvenile grey mullet (*Chelon labrosus*) a basal (4.4 mg Cu/kg dry wt) or high-Cu diet (2,400 mg Cu/kg dry wt) for 10 weeks to assess the relationship between growth and Cu-induced oxidative damage. No mortalities were seen, but growth rate and food intake were reduced by 43 and 29 %, respectively, in the high-Cu group. Aqueous exposure to Cu also affects feeding and growth. Buckley et al. (1982) exposed coho salmon (*Oncorhynchus kisutch*) to sublethal levels of aqueous copper (0.5 and 0.25 of the LC_{50}.), which caused loss of appetite and decreased growth. These both recovered when fish were returned to clean water.

Juvenile green and white sturgeon (*Acipenser medirostris* and *A. transmontanus*) were exposed to dietary meHg, at 25, 50, and 100 mg meHg kg^{-1} diet for 8 weeks. Higher mortality and lower growth rate were noted in both species in a dose-dependent manner (Lee et al. 2011). Green sturgeon exhibited earlier and more severe effects than white sturgeon. Hg accumulated to the highest concentrations in kidneys of both species.

In a field study, Cd, Cr, Cu, Fe, Pb, and Zn concentrations in the muscle, gill and liver of six fish species (*Sparus auratus*, *Atherina hepsetus*, *Mugil cephalus*, *Trigla cuculus*, *Sardina pilchardus* and *Scomberesox saurus*) from the northeast Mediterranean Sea were measured and the relationships between fish size (length and weight) and metal concentrations were investigated by linear regression analysis (Canli and Atli 2003). In most cases, relationships between metal concentrations and fish size were negative. Highly significant ($P < 0.001$) negative relationships were found between fish length and Cr concentrations in the liver of *A. hepsetus* and *M. cephalus*, and Cr concentrations in the gill of *T. cuculus*. Cr and Pb concentrations in the liver and Cu concentrations in all the tissues of *Scomberesox saurus* also showed very significant ($P < 0.001$) negative relationships.

Organics

Most studies of organic chemicals affecting marine fish growth have been done on oil and its components. Pink salmon fry (*Oncorhynchus gorbuscha*) fed oil-contaminated prey (0.6, 3.2, and 6.5 mg l^{-1}) had a reduced growth rate with increasing concentrations, which was strongly correlated with decreases in feeding rate (Schwartz 1985). Growth reduction in fry exposed to 0.7 mg l^{-1} of WSF in the water was greater than those of fry fed the oil-contaminated prey.

Following the *Exxon Valdez* spill, Heintz et al. (2000) reported delayed effects in pink salmon that had been exposed as embryos to gravel coated with weathered oil. Weathering caused the PAH composition to be dominated by heavier compounds. Survivors that appeared healthy were released to the marine environment with coded-wire tags and their survival evaluated when they returned 2 years later. Fish that had been exposed to an initial concentration of total PAH equal to 5.4 μg l^{-1} had a 15 % decrease in marine survival compared to unexposed fish. Reduced growth was seen in fish that had had embryonic exposure to 18 μg l^{-1} PAH. Thus, exposure at sensitive early life stages led to reduced growth and higher mortality later in life.

Rice et al. (2000) exposed polychaetes (*Armandia brevis*) for 28 days to sediments with benzo(a)pyrene (BaP), para-para dichlorodiphenyldichloroethylene (pp′DDE), Aroclor 1254, or field sediments collected from sites in Puget Sound, Washington, contaminated predominantly with PAHs or chlorinated compounds. Exposed worms were then fed to juvenile English sole (*Pleuronectes vetulus*) for 10 or 12 days, and fish were measured for length and weight, sacrificed, and preserved. Growth of fish was lower than reference in all but one of eight groups fed contaminated worms, but statistically significant reductions in growth were only observed in three groups, at least in part due to low statistical power. Fish from all exposed groups showed increased expression of CYP1A, and fish exposed to BaP showed evidence of hepatic PAH-DNA adducts.

Cong et al. (2009) investigated effects of the organophosphate insecticide diazinon on juvenile snakehead fish *Channa striata*, which were exposed twice to 4-day pulses of 0.016, 0.079 or 0.35 mg l^{-1} of diazinon, separated by a 2 week interval (done to imitate the exposure conditions near rice fields). Fish were then moved to clean water for recovery. Diazinon caused long term inhibition of brain ChE activity, which was still significantly depressed at the termination of the experiment, and the highest diazinon concentration caused a significant 30 % growth inhibition.

Hypoxia

Growth rates of winter flounder *Pseudopleuronectes americanus* and summer flounder *Paralichthys dentatus* were reduced as DO decreased, particularly at 50–70 % sat, and as temperature increased. Summer flounder were more tolerant than winter flounder. A significant relationship between feeding rate and growth indicated that reduced food consumption was responsible for growth limitation (Steirhoff et al. 2006).

Effects of moderate hypoxia and oscillating DO on growth of European sea bass (*Dicentrarchus labrax*) were investigated (Thetmeyer et al. 2001). Fish were exposed to one of three conditions: 40 % sat; oscillations between 40 and 86 % with a period of 770 min, and 86% sat as a control at 22 °C for 1 month. Fish in hypoxia consumed significantly less food, had reduced growth, and lower condition factor. Those in oscillating conditions were intermediate, and not statistically distinguishable from either normoxic or hypoxic groups. Growth was correlated with food intake, suggesting that reduced growth was primarily due to reduced appetite.

When juvenile turbot *Scophthalmus maximus* and sea bass *Dicentrarchus labrax* were fed to satiation, food intake and growth were depressed under hypoxia (3.2 and 4.5 mg O_2 l^{-1}). Growth was comparable between fish fed to satiation in hypoxia and those reared in normoxia but fed restricted rations (Pichavant et al. 2001). Decreased food thus appears to be a mechanism by which prolonged hypoxia reduces growth, and may be a way to reduce energy and thus oxygen demand.

Killifish (*Fundulus heteroclitus*) were subjected to severe hypoxia (1.2 mg O_2 l^{-1}), moderate hypoxia (3.0 mg O_2 l^{-1}), normoxia (7.1 mg O_2 l^{-1}) and hyperoxia (10.6 mg O_2 l^{-1}) and specific growth rate calculated weekly. Severe hypoxia reduced growth and reduced muscle protein content and RNA: DNA compared with other treatments. However, growth increased significantly during the second 2-week interval, to the rate of normoxic fish (Rees et al. 2012). Neither moderate hypoxia nor hyperoxia affected growth or biochemical variables, showing that *F. heteroclitus* tolerates hypoxia and, during prolonged severe hypoxia, compensates for the initial negative effects on growth. Its capacity to grow in low DO contributes to its persistence in highly degraded habitats.

Polluted Environment

European flounder (*Platichthys flesus*) responses to chemical stress were assessed in four estuaries with different patterns of contamination. Fish in contaminated estuaries were characterized by high levels of bioaccumulated contaminants, slow energetic metabolism and reduced growth rate (Evrard et al. 2010).

Juvenile turbot (*Scophthalmus maximus*) were exposed to sediments contaminated with metals, PAH and PCBs (Kerambrun et al. 2012a). Significant decreases in growth rates, morphometric index, RNA:DNA ratio and lipid storage index were observed with increasing levels of contamination from site A to site C (Fig. 8.1). The decrease in physiological status could be related to the accumulation of metals in gills and of PAH metabolites in bile.

Juvenile sea bass (*Dicentrarchus labrax*) and turbot (*Scophtalmus maximus*) were caged for 38 days at three locations with varying levels of contaminants (Kerambrun et al. 2012b). At the most contaminated station, all the fish died within

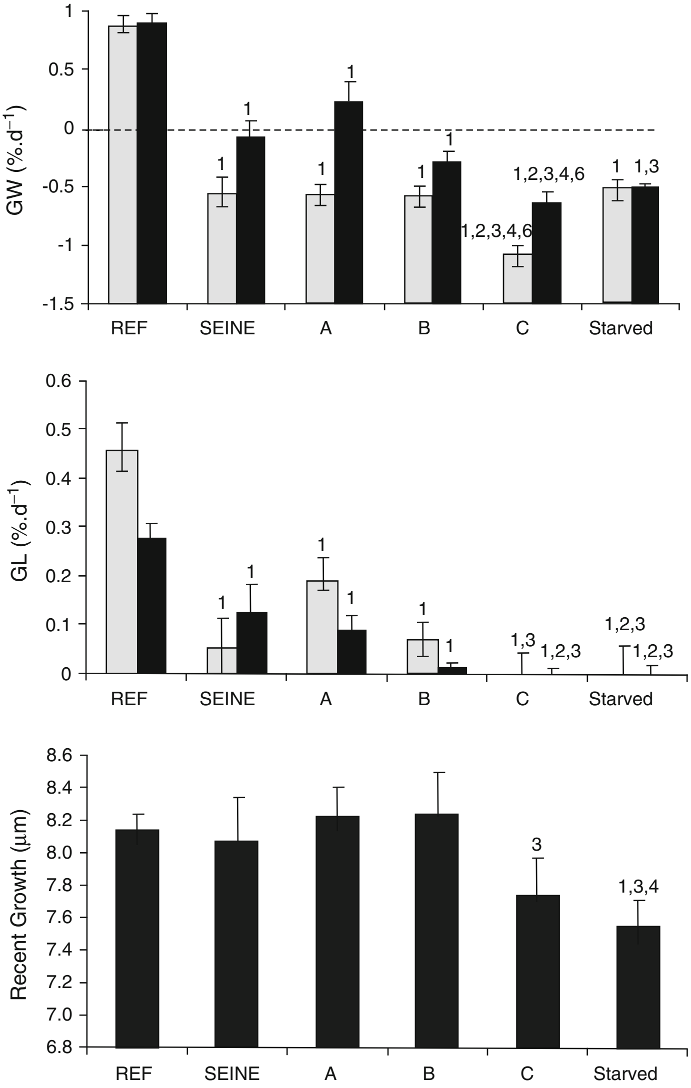


Fig. 8.1 Differences in specific growth rate in weight (*GW*), length (*GL*) and recent otolith growth (*RG*) of juvenile turbot exposed to 5 sediments (Ref. Seine, A,B,C) after 7 (*gray*) and 21 (*black*) days (M ± SE). Numbers = significant differences compared to t_0 (Reprinted from Kerambrun et al. 2012a: 136, courtesy of Elsevier Publishing Co.)

2 weeks. At the least contaminated station, fish had higher growth, RNA:DNA ratios and condition index than at the intermediate station. Lipid storage index, based on the ratio of the quantity of triacylglycerols to sterols (TAG:ST), was significantly higher in the less contaminated station. Reduced growth and condition was associated with metal accumulation in gills.

8.1.2 Mollusks

Growth of mollusks can be investigated by measuring increments in the shell, or by measuring the condition index (weight of soft tissues vs shell), which reflects growth of soft tissues. Studies are quite limited, and many of the studies on contaminant effects on mollusk growth are quite old. Studies on shell growth reflect effects on calcification, which will be discussed later in the chapter.

Metals

Juvenile and adult mollusks are sensitive to metals. Boyden et al. (1975) found that Zn at 0.25 and 0.50 mg l^{-1} reduced growth of juvenile *C. gigas* to 78 and 51 % of control values, but after a 5-day depuration period, growth rebounded. Cunningham (1976) found similar retardation of growth in juvenile *C. virginica* exposed to 0.01 and 0.10 mg l^{-1} Hg for 47 days, and similar recovery after return to clean water for about a month. Exposure to a mercury-equilibrated algal suspension containing 0.25, 0.42 and 1 Hg l^{-1} reduced growth and condition of adult limpets *Crepidula fornicata* over 16 weeks (Thain 1984).

Organics

There has been some work, much of it old, on effects of pesticides and PAHs on clam growth. Butler et al. (1968) investigated effects of the pesticide carbaryl (Sevin) and its breakdown product, 1-naphthol, on clams (*Clinocardium nuttalli*). Sevin was less toxic than 1-naphthol to juvenile clams, the respective 96-h TLM's (median tolerance limits) being 3.75 and 2.70 mg l^{-1}. Growth of clams was reduced more by 1-naphthol than by Sevin. The food consumption of clams exposed to 1.6 mg l^{-1} Sevin was markedly reduced and their food conversion efficiency was impaired.

Small (<30 mm) clams, *Protothaca staminea*, were marked and placed in the intertidal zone of Sequim Bay, Washington for 1 year to measure effects of oiled sediments on growth (Anderson et al. 1981). Growth in oil-treated sediment was significantly slower than in clean sediment; oil mixed into sediment (10 cm) produced greater tissue contamination and greater growth reduction than a layer (3 cm) of oiled sediment. Effects of residual *Exxon Valdez* oil on *P. staminea*, were studied 5–6 years after the spill using reciprocal transplants of clams between unoiled and oiled sites (Fukuyama et al. 2000). Tagged clams were transplanted in 1994 and collected in 1995 and 1996. Greatest mortality and tissue burdens of hydrocarbons were in clams from the oiled site, and the best growth was in clams moved from the oiled to the unoiled site where they could depurate.

Hypoxia

Burrell et al. (2011) examined the relationship between diel-cycling hypoxia and growth and the acquisition and progression of *Perkinsus marinus* infections in *C. virginica*. Laboratory and field experiments indicated that diel-cycling hypoxia increased acquisition and progression of infection, and decreased growth. Patterson et al. (2011) found heavier ^{15}N in tissues of oysters under lower DO conditions; this is consistent with reduced feeding and catabolism of tissues, common in stressed bivalves and in keeping with the reduced growth noted previously.

Acidification/Climate Change

Sydney rock oysters, *Saccostrea glomerata*, were deployed at sites affected and unaffected by acid sulfate soils in two Australian estuaries. After 10 weeks, oysters were transplanted within and across sites and maintained another 10 weeks. Oysters that remained 20 weeks at affected sites grew at about half the rate of oysters at reference sites (Amaral et al. 2012b) Oysters moved from acidified to reference sites grew more than oysters moved from reference to acidified sites or that stayed at reference sites. Dickinson et al. (2012) studied interactive effects of salinity and CO_2 on growth and energy homeostasis in *C. virginica*. Juveniles were exposed for 11 weeks to 30 or 15 psu salinity at current atmosphere (~400 μatm), or ~700–800 μatm. Exposure to elevated P_{CO2} and/or low salinity led to increased mortality, reduced tissue energy stores (glycogen and lipid) and negative soft tissue growth, indicating energy deficiency. In contrast, juvenile mussels, *M. galloprovincialis* under conditions of −0.3 and −0.6 pH units had increased absorption efficiency and ammonium excretion, and increased scope for growth and tissue dry weight, suggesting that this species tolerates acidification (Fernandez-Reiriz et al. 2012).

Other Pollutants

Growth of oysters (*Crassostrea virginica*) subjected to chlorine-produced oxidants was studied by Scott and Vernberg (1979). The condition index and gonadal index were reduced by 0.16 mg l^{-1}. This concentration also reduced fecal production. This implies effects on feeding, which was not directly measured. There were seasonal differences in responses, reflecting temperature interacting with toxic effects.

8.1.3 Crustaceans

Most effects on growth are related to the molt cycle, which is discussed below. However, there is some evidence of effects of selected organic contaminants on somatic growth of crustaceans.

Organics

Guo et al. (2012) investigated long-term effects of PCB 126 on the copepod *Tigriopus japonicus*. No obvious effects were observed in the first generation at concentrations $<100\ \mu g\ l^{-1}$ but in the second generation effects on reproduction were seen at levels over $1\ \mu g\ l^{-1}$ and on growth at concentrations over 0.1 μg/l. Thus, they became more sensitive in the second generation and growth was the most sensitive parameter. The authors concluded that environmental risk assessment of contaminants should be based on a long-term multigenerational exposure to provide realistic measures of pollutant effects.

8.1.4 Other Taxa

Acidification/Climate Change

Sea urchins, *Lytechinus variegatus*, were reared in ambient seawater (380 μatm) and two elevated levels of pCO_2, 560 and 800 μatm. After 89 days, urchins at ambient pCO_2 weighed 12 % more than those at 560 μatm and 28 % more than those at 800 μatm (Albright et al. 2012). Skeletons examined with scanning electron microscopy, showed degradation of spines at 800 μatm. Doropoulos et al. (2012) found that after 2 months' growth in ambient or elevated pCO_2 levels, the linear extension and calcification of coral (*Acropora millepora*) recruits decreased as CO_2 partial pressure (pCO_2) increased. When recruits were subjected to incidental fish grazing, their mortality was inversely size dependent. There was an additive effect of pCO_2 such that recruit mortality was higher under elevated pCO_2 irrespective of size. In elevated pCO_2, coral recruits needed to double their size at the highest pCO_2 to escape grazing mortality. This general trend was seen with different predators (blenny, surgeonfish, and parrotfish). In contrast, OA-like conditions can enhance the ecological success of non-calcifying anthozoans e.g. sea anemones. Increased growth (abundance and size) of the sea anemone (*Anemonia viridis*) was observed by Suggett et al. (2012) along a natural pCO_2 gradient at Vulcano, Italy. Both gross photosynthesis (*P*G) and respiration (R) increased with pCO_2 indicating that the increased growth was, at least in part, fuelled by bottom up (CO_2 stimulation) of metabolism. The increase of *P*G outweighed that of R and the genetic identity of the symbiotic microalgae (*Symbiodinium* spp.) remained unchanged. These observations of enhanced productivity with pCO_2, may increase fitness and enable non-calcifying anthozoans to thrive in future high CO_2 environments.

Reduced growth can have profound ecological consequences for calcifying organisms. Kroeker et al. (2013) investigated species interactions that can modify direct effects of acidification on individual species and showed that altered competitive dynamics between calcareous species and fleshy seaweeds drive significant ecosystem shifts. Although calcareous species recruited and grew in low pH during early successional stages, they were rapidly overgrown by fleshy seaweeds later in succession in low pH. The altered competitive dynamics between calcareous and fleshy seaweeds is probably due to decreased growth of calcareous species, increased growth of fleshy seaweeds, and/or altered grazing rates. Phase shifts towards dominance by fleshy seaweeds are common in many marine ecosystems, and these results suggest that changes in the competitive balance between these groups is a key factor leading to profound ecosystem changes as the pH decreases.

Six Caribbean coral reef sponges—*Aiolochroia crassa*, *Aplysina cauliformis*, *Aplysina fistularis*, *Ectyoplasia ferox*, *Lotrochota birotulata* and *Smenospongia conulosa*—were grown for 24 days in seawater ranging from current summer maxima (28 °C; pH = 8.1) to those predicted for 2100 (31 °C; pH = 7.8). For all species, growth and survival were similar among temperature and pH levels (Duckworth et al. 2012). Sponge attachment, which is important for reef consolidation, was unchanged by pH for all species, and highest at 31 °C for *E. ferox*, *I. birotulata* and *A. cauliformis*. These findings on adult sponges suggest that ecological roles and growth of these species will not be adversely affected by the temperature and pH predicted for the end of the century.

8.2 Regeneration and Molting

Regeneration of lost tissue is a process that, while extremely limited in birds and mammals, is common among invertebrates and fishes. The processes by which structures are regenerated resemble the cell differentiation that goes on in embryos, although a pool of stem cells must be initially developed from more differentiated tissue near the point of injury. In crustaceans, the regeneration of appendages is tied in with the molt cycle.

8.2.1 Crustaceans

In order to grow, crustaceans must molt their old exoskeleton to accommodate their larger body. Growth is thus periodic, and depends on molting of the exoskeleton. The frequency of molting and the growth increment are influenced by environmental factors and hormones, particularly ecdysteroids, which are steroids. Molt-inhibiting hormones also play a role. In preparation for molting (proecdysis) ecdysteroid levels in hemolymph increase and the tissue layer under the exoskeleton secretes materials that erode and soften the old cuticle. Calcium is dissolved and moved to interior storage places, softening the old cuticle. The epidermal tissues then produce a thin, soft new exoskeleton underneath it. Then the animal takes in a lot of water, swells up and bursts the old skeleton along specific weak points, and extricates itself from it (ecdysis). Mobility is limited right after a molt (postecdysis) because the new exoskeleton is very soft and not rigid enough to keep the limbs stiff. Animals are very vulnerable until the new exoskeleton expands and hardens. Although most crustaceans molt throughout their entire life, small individuals must molt frequently; some species have a terminal molt after reaching their maximum size. During ecdysis, postecdysis, and intermolt phases, ecdysteroid levels in hemolymph are low.

Some crustaceans can autotomize injured limbs, i.e., break them off at a preformed breakage plane where there is a membrane, thus minimizing tissue damage. Autotomy is an effective anti-predator response, and animals subsequently regenerate lost appendages, in association with the molt cycle. Regeneration begins after a period of tissue re-organization and is first noticeable as a small protrusion at the autotomy plane. The limb bud grows within a covering of cuticle. In crabs, regenerating limbs grow folded and only unfold when the animal molts. In shrimp, the regenerating limbs have joints nestled within one another, so they also do not become functional until the animal molts. Thus, regeneration is closely tied with the molt cycle. Regeneration consists of basal growth, when tissue differentiation occurs and which is independent of the molt cycle, and pro-ecdysial growth, a phase of rapid growth which is dependent on molting hormones. There may be a plateau between basal and proecdysial growth. Multiple autotomy, removal of many limbs, stimulates accelerated regeneration and molting. Many chemicals alter the rate of limb regeneration and/or molting. The two processes may be affected independently, but it is often not possible to distinguish effects on regeneration *per se* from those on the molt cycle, since they are usually coupled and both processes are affected simultaneously. Some toxicants produce morphological alterations in the regenerated limbs; these may be relatively minor, such as reduced number of pigment cells, setae, or tubercles, or may be major, such as abnormal bending or defects in exoskeleton formation. By molting, crustaceans may be able to reduce their body burdens of contaminants that are contained within the exoskeleton (Keteles and Fleeger 2001; Bergey and Weis 2007).

Metals

The most common effect of metals is retardation of regeneration accompanied by a delay in ecdysis; in some cases regeneration is affected without altering the timing of the molt. A series of studies of metals on regeneration in fiddler crabs was conducted in the 1970s and 1980s by Weis and colleagues; these are reviewed in Weis et al. (1992). Delayed regeneration and molting were observed in *Uca pugilator* after exposure to $HgCl_2$, meHg, Cd, and Zn at 0.5–1.0 mg l^{-1}. Retardation of regeneration was accompanied with delayed ecdysis so when molting took place limbs were fully formed. MeHg at 0.1 mg l^{-1} inhibited melanin pigment development in the regenerated limbs and reduced the number of tubercles on regenerated first walking legs of males. While Hg and Cd individually retarded limb regeneration, the presence of meHg reduced the particularly toxic effects of Cd at low salinity. Zn and Hg together were additive, while Zn and Cd interacted antagonistically. *Uca pugnax* from a contaminated site were less affected by meHg

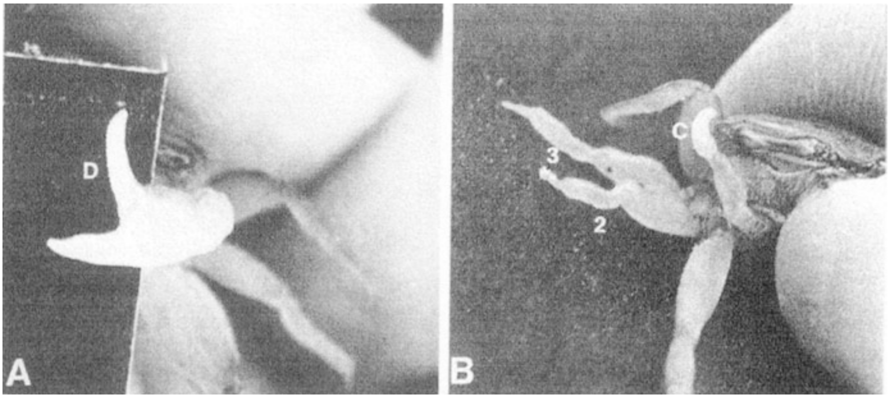


Fig. 8.2 (**a**) Malformed chela in fiddler crab after regenerating in TBT. Chela has deformity in the dactyl (*D*). (**b**) Curled, stunted chela (*C*) and malformed walking legs 2 and 3 after regeneration in TBT (Reprinted from Weis et al. 1987b: 323, courtesy Springer Publishing Co.)

than those from a relatively clean site, i.e., limb regeneration was not as greatly retarded, indicating that they had acquired some tolerance. However, short-term pre-exposure to low concentrations of meHg did not enhance tolerance to higher concentrations.

Juvenile tiger shrimp, *Penaeus monodon*, shortened the time to first molt and decreased molting frequency after exposure to 0.9 mg l^{-1} Cr (Chen and Lin 2001). They had a reduced feeding rate. Grass shrimp, *P. pugio*, telson regeneration was retarded by exposure to Hg or meHg (10 μg l^{-1}) and they had a reduced intermolt period (Kraus and Weis 1988). However, these effects were not seen in shrimp from a contaminated site. Hexavalent Cr inhibited limb regeneration in grass shrimp (Rao et al. 1985). The antifouling agent tributyltin (TBT) retarded limb regeneration and molting in *U. pugilator* at 0.5 μg l^{-1}, and produced anatomical abnormalities in regenerated chelae of males, in which the regenerated dactyl curved upward, away from the pollex, instead of downward towards it (Fig. 8.2) (Weis et al. 1987b). In *P. pugio*, 0.5 μg l^{-1} TBT slowed regeneration rate, but did not produce abnormalities (Khan et al. 1993).

Organic Contaminants

Aromatic Hydrocarbons

Exposure of juvenile blue crabs to 1 mg l^{-1} benzene or dimethylnaphthalene increased the length of the intermolt cycle, decreased the increment per molt, and retarded limb regeneration (Cantelmo et al. 1981). Affected crabs had slower limb regeneration, a longer plateau stage, and a longer time for regenerated limbs to develop pigmentation. Wang and Stickle (1987) found that the WSF (1.5 or 2.5 mg l^{-1}) of South Louisiana crude oil inhibited growth and molting in blue crabs, reduced the increment at molt, and prolonged the intermolt period.

Pesticides, PCBs and Dioxins

The PCB mixture Aroclor 1242 (8 mg l^{-1}) inhibited limb regeneration in *U. pugilator*, with greater inhibition at high and low salinities than at intermediate salinities (Fingerman and Fingerman 1978). Chlorophenols and dithiocarbamates at 0.1–1.0 mg l^{-1} inhibited regeneration in *P. pugio*, generally without affecting the timing of molting. Retarded limb regeneration and molting in *P. pugio* was seen in response to dithiocarbamates and pentachlorophenol, with the early stages of regeneration more sensitive than later ones (Rao et al. 1979). Other chlorophenols (2,3,4,5-tetrachlorophenol and 2,3,4,6-tetrachlorophenol at 0.3 and 0.7 mg l^{-1} respectively) inhibited limb regeneration but did not alter the duration of the molt cycle, suggesting a direct effect on limb growth rather than one on hormones (Rao et al. 1981).

Current insect growth-regulating pesticides are very toxic to growth processes in crustaceans. The chitin-synthesis inhibitor, diflubenzuron (Dimilin®) interferes with chitin deposition and produces disturbances in cuticular structure. Treated larvae appear healthy until they molt, at which time they may be unable to shed the old cuticle and die. Exposure to 0.5, 5.0 and 50 μg l^{-1} produced a dose-dependent retardation of regeneration and molting in *U. pugilator*; crabs that molted in high concentrations had high mortality (Weis et al. 1987a). Regenerated limbs had blackened areas in which the cuticle had not developed properly. Dimilin also retarded the molt cycle in juvenile fiddler crabs (Cunningham and Myers 1987) and grass shrimp, and 0.11 μg l^{-1} inhibited limb regeneration (Touart and Rao 1987). This suggests that in addition to affecting cuticle synthesis, it affects molting hormones, mitosis and differentiation of limb buds. Stueckle et al. (2008) found that methoprene, a juvenile hormone mimic, retarded regeneration in *U. pugnax*, with greater effects on males, which took longer than females at 0.1 μg l^{-1} and exhibited a greater frequency of abnormalities at 1.0 μg l^{-1}. They had a greater frequency of abnormal limbs that failed to regenerate or had a bent or bulging merus, carpus or propodus, or a hook-shaped dactyl. The frequency of abnormal limbs was greatest at intermediate concentrations, producing inverted U-shaped curves, reminiscent of other endocrine disrupting effects (Fig. 8.3).

However, not all toxic chemicals retard limb regeneration and molting. DDT (10 μg l^{-1}) accelerated limb regeneration in *U. pugilator* (Weis and Mantel 1976). In the case of crabs with multiple autotomy, the time to ecdysis was shortened as well. These responses may have been due to heightened excitation of the nervous system and secretion of neuroendocrine factors promoting molting.

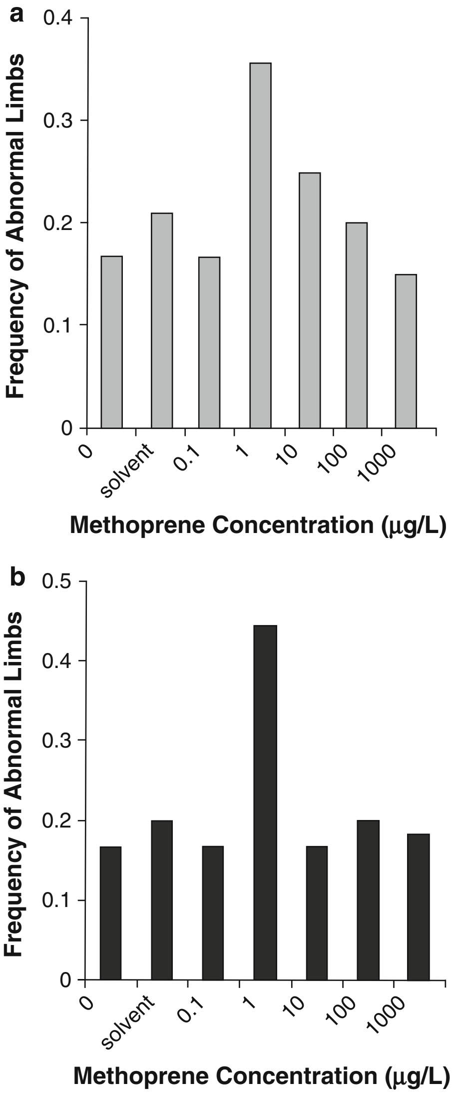


Fig. 8.3 Malformed regenerated limbs in methoprene-exposed *U. pugnax*. (**a**-females, **b**-males) Methoprene-exposed crabs showed tendency to abnormal limb formation, especially at 1 µg l^{-1} (Reprinted from Stueckle et al. 2008: 372, courtesy Elsevier Publishing Co.)

Hypoxia

Shrimps (*Penaeus semisulcatus*) kept for 17 days at an oxygen level of 2 mg l^{-1} did not molt, and high mortality was observed. When the DO was increased to 5 mg l^{-1} the mortalities ceased and many molts took place (Clark 1986).

8.2.2 Fishes

Teleost fins are composed of connective tissue and bony rays, and can regenerate after amputation. Cells at the cut end dedifferentiate to form a blastema, a mass of undifferentiated stem cells, which then grows and develops new bony rays and connective tissue. Teleosts are also capable of regenerating scales and other structures, including components of the nervous system.

Metals

The rate of caudal fin regeneration in *Fundulus heteroclitus* was retarded by 0.1 mg l^{-1} Cd (Weis and Weis 1976) and 0.001 mg l^{-1} meHg (Weis and Weis 1978), which also retarded regeneration in *Mugil cephalus*. However, the two metals interacted in an antagonistic fashion so that fish exposed to combinations of meHg and Cd regenerated at rates comparable to controls. In addition, fish pre-exposed to 0.05 mg l^{-1} Cd acclimated to the metal and their regeneration in 0.1 mg l^{-1} was not retarded, but was faster than controls, evidence of hormesis (Weis and Weis 1986). In contrast to Hg and Cd, Zn at 1.0, 3.0 and 10.0 mg l^{-1} accelerated regeneration in a dose-dependent fashion, and could counteract the inhibitory effects of meHg (Weis and Weis 1980).

Exposure to Cd and Zn at 0.1 mg l^{-1} reduced calcification and reduced Ca/P ratios in regenerating scales of *F. heteroclitus* (Sauer 1987).

Organics

The pesticides DDT, malathion, parathion, and carbaryl at 10 $\mu g\ l^{-1}$ retarded fin regeneration in *F. heteroclitus* (Weis and Weis 1975). The effects of DDT were less pronounced than the other three insecticides.

Polluted Environment

There have been a number of field reports of increased prevalence of a fin erosion condition (or “fin rot”) in fishes from highly contaminated sites. While these reports were more common in decades past, new reports continue to be published (Bodammer 2000; Ziskowski et al. 2008). While many of these studies find bacterial infection associated with the condition, the infection may be opportunistic and the condition may also involve poor ability to regenerate fins after injury.

8.2.3 Other Taxa

Echinoderms are capable of regenerating their arms. This ability was apparently unknown to people who tried to kill nuisance crown-of-thorns starfish by cutting them up. All they accomplished by this was to produce more nuisance starfish.

Metals

Arm regeneration in the brittle star, *Ophioderma brevispina* was inhibited by 0.1 $\mu g\ l^{-1}$ of TBT or triphenyltin (Walsh et al. 1986). Cadmium-exposed brittle stars, *Microphiopholis gracillima*, regenerated thinner arms with less soft and skeletal tissue (D'Andrea et al. 1996).

Hypoxia

Arm regeneration in the brittle star *Amphiura filiformis* was studied in normoxia >8.5 mg $O_2\,l^{-1}$ (control) and in two levels of hypoxia, 2.7 and 1.8 mg $O_2\,l^{-1}$ (29 and 19 % sat). The treatments were chosen to simulate short term hypoxia (17 days) over a 2-month period. Reduced arm growth was seen in both hypoxic treatments compared with normoxia. Slowest regeneration of arms was observed at 1.8 mg $O_2\,l^{-1}$ (65 % arm regeneration compared to controls) (Nilsson and Sköld 1996).

Acidification/Climate Change

Sea stars, *Luidia clathrata* with two arms excised, were maintained in seawater of pH 8.2 or 7.8. After 97 days, a period of time sufficient for 80 % arm regeneration, the lower pH did not significantly effect whole animal growth, arm regeneration, or biochemical composition (Schram et al. 2011). Wood et al. (2011) investigated responses of the brittlestar, *Ophiocten sericeum*, a polar species, to a temperature increase of 3.5 °C (ambient, 5–8.5 °C) and reduction in pH to 7.7 or 7.3 (ambient was 8.3). Faster arm regeneration was stimulated by higher temperature but was counteracted by low pH; at pH 7.3 in high temperature, the maintenance of calcified structures reduced the rate of regeneration, possibly due to use of energy reserves.

8.3 Shell/Bone Formation

Numerous studies have been done on effects of contaminants on formation of skeletal structures. In recent years studies on calcification have been dominated by examination of effects of ocean acidification on different taxa, which vary greatly in susceptibility. A widespread analysis suggests that increased acidity is affecting the size and weight of shells and skeletons, and the trend is widespread across marine species (Watson et al. 2012). Variation in shell thickness and skeletal size was studied in clams, snails, brachiopods, and sea urchins from 12 different environments from tropics to polar regions to get a clearer understanding of similarities and differences among taxa, and to make better predictions of how animals respond to increasing acidity. The study showed that over evolutionary time, animals adapt to environments where calcium carbonate is difficult to obtain by forming lighter skeletons. Low pH makes it harder for marine animals to make shells and skeletons. As the availability of calcium carbonate decreases, skeletons get lighter and account for a smaller part of the animal's weight. The fact that same effect occurs consistently across taxa suggests the effect is widespread. The effect is greatest at low temperatures; polar species have the smallest and lightest skeletons, suggesting that they may be at greater risk as the ocean becomes more acidic.

8.3.1 Crustacea

Polluted Sites

Many crustaceans inhabiting degraded estuaries and coastal waters can develop a condition called "shell disease" or shell erosion. For example, lobsters and rock crabs (*Homarus americanus* and *Cancer irroratus*) from grossly polluted areas of the NY Bight (a sewage sludge dump site) had erosion on legs, spines and elsewhere. The condition could be produced in the lab by exposing animals to sediments from the sewage sludge or dredge spoil disposal site (Young and Pearce 1975). Shrimp, *Crangon septemspinosa*, from the vicinity of the dump site had high prevalence of eroded appendages and blackened erosion of the exoskeleton, rarely observed elsewhere (Gopalan and Young 1975); this condition was produced in the laboratory in 50 % of the individuals exposed to sea water from the site. Erosion was progressive and the eroded parts of appendages did not regenerate after molting. Shell erosion is caused by bacteria that break down chitin, with subsequent secondary infection of underlying tissue by other bacteria. These dump sites have been closed, and shell erosion disease has not been reported from the area since then.

Acidification

Ries et al. (2009) investigated responses of various marine organisms to different $p\text{CO}_2$ levels. In three crustaceans, blue crabs (*Callinectes sapidus*), lobsters (*Homarus americanus*), and shrimps (*Penaeus plebejus*) net calcification was,

Fig. 8.4 TBT-exposed oyster shell showing chambers in upper valve (Reprinted from Alzieu 1986, courtesy of IEEE)

surprisingly, greatest under the highest level of $p\mathrm{CO}_2$ (2,856 mg l^{-1}). An explanation offered for these unexpected results was that if seawater is the source of an organism's calcifying fluid, then the concentration of dissolved inorganic carbon (DIC) in this fluid will increase as $p\mathrm{CO}_2$ increases. Organisms that can maintain an elevated pH at their site of calcification despite reduced external pH will convert much of this increased DIC to carbonate. They would have a final carbonate concentration at the site of calcification that is only slightly less than (and possibly equal to or greater than) that under present-day $p\mathrm{CO}_2$ levels, depending on the efficiency of their proton-regulating mechanism. Furthermore, organisms that accrete shell that is covered and protected by an external organic layer (such as the crustacean epicuticle) can be more resilient to acidification than those whose shell is directly exposed to seawater.

8.3.2 Mollusks

Metals

The effects of Cu and Zn on the daily shell growth of *M. edulis* were measured with a laser diffraction technique. Within 3 days 10 μg l^{-1} Cu inhibited shell growth (Manley et al. 1984); effects became more severe as exposure time increased. Recovery of nearly normal shell growth occurred upon transfer to clean sea water. Effects of zinc were less severe. Oysters (*C. gigas*) are sensitive to effects of TBT at levels as low as 0.05 μg l^{-1} Oysters living near marinas developed anomalies in which the shell developed many layers with gel-like material between the layers (Fig. 8.4) (Waldock and Thain 1983). Abnormalities decreased with distance from port areas, and TBT could induce it in the laboratory. Oysters transplanted into port areas developed these abnormalities. After the use of TBT paints was banned, abnormalities decreased (Alzieu et al. 1986).

Zuykov et al. (2011) studied morphology of the inner shell surface of the mussel *Mytilus edulis* after short-term exposures to Ag as free-ion (Ag^{+}) and as nanoparticles. The nacre portion of exposed mussels (both treatments) developed doughnut shaped structures of calcium carbonate micrograins covering the surface. Formation of these structures was explained as a disturbance of shell calcification.

Organics

Mollusks in mesocosms with sediment were exposed to oil and oil + dispersant equivalent to 50 mg l^{-1} oil (Carr et al. 1987). *Mya arenaria* showed major reduction in shell growth and condition index in oil and in oil + dispersant treatments.

Acidification

Pteropods are shelled pelagic mollusks that play an important role in planktonic food webs. The impact of pH was investigated on *Limacina helicina*, a key species of Arctic ecosystems. Pteropods were kept under pH conditions corresponding to $p\mathrm{CO}_2$ levels of 350 and 760 μatm. The pteropods had a 28 % decrease in calcification at the higher $p\mathrm{CO}_2$ (expected for the year 2100), supporting concerns for their future in high CO_2, as well as for species dependent on them for food (Comeau et al. 2009). A more comprehensive study (Comeau et al. 2010) examined $p\mathrm{CO}_2$ levels of 280, 380, 550, 760 and 1,020 μatm with ambient (control) and elevated (+4 °C) temperatures. Respiration was unaffected by $p\mathrm{CO}_2$ at control temperature, but significantly increased as a function of the $p\mathrm{CO}_2$ level at elevated temperature. Precipitation of CaCO_3 declined as a function of $p\mathrm{CO}_2$ at both temperatures. The decrease in CaCO_3 precipitation was highly correlated to the aragonite saturation state. Even though the pteropods could precipitate CaCO_3 at low aragonite saturation state, their shell production is very sensitive to decreased pH. Comeau et al. (2012) found evidence for shell dissolution in this species at pH of 7.9. Bednarsek et al. (2012) found shell dissolution of this species is already happening. They examined *L. helicina antarctica* collected from the top 200 m of the water column, where aragonite saturation levels were around 1, and where upwelled deep water mixes with surface water with anthropogenic CO_2. They compared the shell structure under a scanning electron microscope with samples from aragonite-supersaturated regions, and found severe shell dissolution in the undersaturated region. Laboratory incubations for 8 days in aragonite saturation levels of 0.94–1.12 produced equivalent levels of shell dissolution. Benthic mollusks are also affected. Gazeau et al. (2007) demonstrated that calcification rates of *M. edulis* and *C. gigas* decline linearly with increasing $p\mathrm{CO}_2$. Mussel and oyster calcification was projected to decrease by 25 and 10 %, respectively, by the end of the century. Gaylord et al. (2011) found that acidification degrades the mechanical integrity of larval shells of the mussel *M. californianus*, on rocky shores in the northeastern Pacific. Larvae cultured in seawater with CO_2 of 540 or 970 mg l^{-1} produced weaker, thinner and smaller shells and lower tissue mass than those raised under current conditions. *M. edulis* adults exposed to elevated $p\mathrm{CO}_2$ decreased shell growth and exhibited internal shell corrosion of the nacreous layer at high $p\mathrm{CO}_2$ (240 and 405 Pa) (Fig. 8.5) (Melzner et al. 2012). Thus the inner shell surface is vulnerable in mussels, especially under conditions of low food.

Nienhuis et al. (2010) tested the effects of increased $p\mathrm{CO}_2$ (forecast to occur in 100 and 200 years) on shell deposition and dissolution in the rocky intertidal

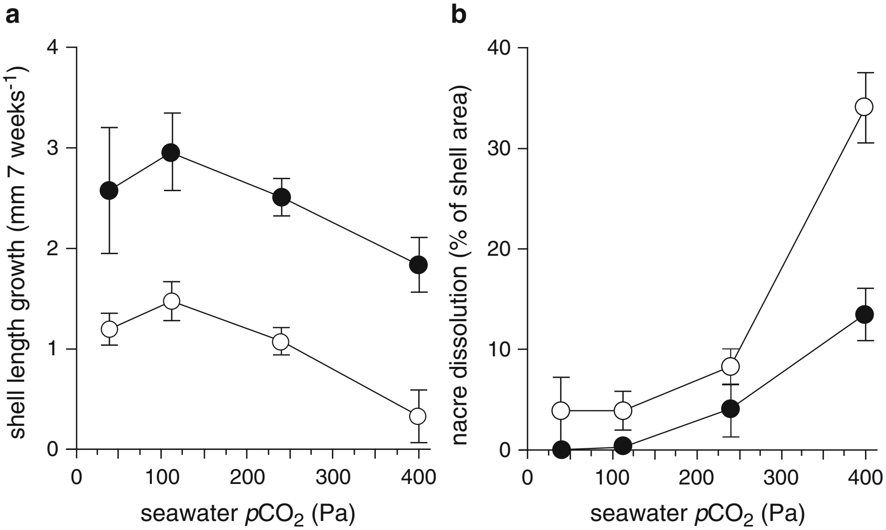


Fig. 8.5 (**a**) Mussel Shell length growth during 7 weeks (**b**) Internal shell surface dissolution. Low food (*white*) and high food (*black*) groups, mean ± SEM (Reprinted from Melzner et al. 2012 (open access))

snail, *Nucella lamellosa*. Shell weight gain decreased linearly with increasing $p\text{CO}_2$ levels. However, this trend was paralleled by weight loss of empty shells, suggesting that the declines in shell weight gain in live snails were due to dissolution of existing shell, rather than reduced production of new shell material. Acidification may therefore have a greater effect on shell dissolution than deposition, at least in this species. Range et al. (2012) found some similar results with mussels. The carbonate chemistry of Rio Formosa water was manipulated by diffusing CO_2, to attain two reduced pH levels, by –0.3 and –0.6 pH units, relative to control seawater. After 84 days, no differences were in seen in growth (somatic or shell) of juvenile mussels *M. galloprovincialis*. The naturally elevated total alkalinity of the seawater prevented under-saturation of $\text{CaCO}_{3.}$ However, calcification was reduced in elevated CO_2, but most of the loss of shell probably occurred as post-deposition dissolution in the internal aragonite nacre layer. The results show that, even under extreme levels of acidification, juvenile *M. galloprovincialis* continued to calcify and grow in this coastal lagoon. Melatunan et al. (2013) studied effects pH levels of 8.0 and 7.7 and temperatures of 15 or 20 °C on shell growth of *Littorina littorea*. Snails in acidified seawater, elevated temperature, or both had reduced shell growth. The increase in shell length was lower for individuals kept in low pH + high temperature, and the shell thickness increase at the growing edge was lower under low pH and combined conditions. ATP was positively correlated with shell thickening and weight, indicating that effects of low pH and elevated temperature may occur in part through metabolic disruption.

Shellfish with weakened shells can become more susceptible to predation and bioerosion. Amaral et al. (2012a) investigated the susceptibility of oysters from acidified areas (receiving runoff from acid sulphate soils) and reference areas to predation by the gastropod *Morula marginalba*. Shells were significantly weaker at acidified sites than at reference sites (Amaral et al. 2012b), and more oysters were consumed because *M. marginalba* needed less time to drill through the weaker shells. The boring sponge *Cliona celata* was grown on scallop (*Argopecten irradians*) shells in seawater at current pH and that predicted for 2100 (7.8) (Duckworth and Peterson 2013). Lower pH greatly affected shell boring. At $\text{pH} = 7.8$, sponges bored twice the number of holes and removed twice as much shell weight than at $\text{pH} = 8.1$, showing that OA may increase boring rates of *C. celata* in shellfish with weaker shells. Green crabs *Carcinus maenas* and periwinkles *Littorina littorea* were put under ambient conditions or warming and acidification, both separately and in combination, for 5 months and predators, prey, and their interactions were examined (Landes and Zimmer 2012). Acidification reduced the claw strength in *C. maenas* and weakened the shells of *L. littorea*. Predator–prey interactions were not changed, indicating that both species were affected equally. One would not expect that to be the case in most predator/prey interactions, however.

In the Pacific Coast of North America, because of patterns of ocean circulation, shellfish are already suffering from changes in ocean chemistry. Colder, more acidic waters well up from the depths and move inshore in bays and estuaries of Oregon, Washington, and British Columbia, causing damage to oysters (Feely et al. 2008). Wild oysters in many sites have failed to reproduce successfully because acidic waters have prevented larvae from forming shells. Oyster hatcheries have adapted to the acidity by buffering the water in which they grow their larvae, by providing the oysters with sodium bicarbonate.

In contrast to the response of most mollusks, calcification rates in the cephalopod *Sepia officinalis* increased during long-term exposure to elevated *p*CO2 (Gutowska et al. 2010). The potential negative impact of increased calcification in the cuttlebone of *S. officinalis* was discussed with regard to its function as a lightweight and porous buoyancy regulation device. The response of the nautilus, a cephalopod with an outer shell, is very different. In *Argonauta nodosa*, females construct a very thin (225 μm) shell that lacks an outer protective layer and that is used as a brood chamber for developing embryos. Wolfe et al. (2012) immersed shell fragments at different temperatures and pH. Shells incubated in pH 7.8 (projected for 2070) for 14 days had reduced weight due to dissolution, with increased dissolution in warmer and lower pH treatments. Unless living animals respond with increased mineralization, the brood chamber will be susceptible to dissolution under ocean acidification, which could compromise survival of the species, according to the authors.

The diversity of responses among species prevents clear predictions about ecosystem level impacts of acidification. Kroeker et al. (2011) used shallow water CO_2 vents as a model system to examine ecosystem responses to acidification in rocky reefs. They found fewer taxa, reduced taxonomic evenness, and lower biomass in low pH zones. However, the number of individuals did not differ, suggesting that there is compensation via population increases of acid-tolerant taxa. The trophic structure shifted to fewer trophic groups and dominance by generalists, suggesting a simplification of food webs.

Polluted Sites

Blue mussels, *M. edulis*, were exposed to dredged material from a polluted site, Black Rock Harbor, Connecticut (US) at 0–10 mg l^{-1} of suspended sediment (Nelson 1987). At >1.5 mg l^{-1} mussels showed reduced scope for growth and shell growth. Exposure in the field to lower concentrations of dredged material produced no noticeable effects.

8.3.3 Fishes

There have been far fewer papers studying effects of contaminants on bone development in fishes. Of those studies that have been done, many are on freshwater species.

Metals

Lead has particular toxicity causing weakened bones in fishes, but this has been seen primarily in fresh water systems where the pH is low, allowing more Pb to be dissolved and bioavailable to fish (Hamilton and Haines 1989).

Organics

After a spill of the pesticide kepone in the James River Virginia, effects on the skeletal system were seen in spot (*Leiostomus xanthurus*), croaker (*Micropogonias undulatus*), and black drum (*Pogonias cromis*), which had shortened vertebral columns and “broken back” syndrome due to vertebral fractures (Davis 1997). This could be demonstrated in the laboratory (Couch et al. 1979) with kepone, as well as other compounds such as trifluralin, dibutylphthalate, toxaphene, PCBs, and toxaphene (Karen et al. 1998, 2001).

Striped bass (*Morone saxatilis*) from estuaries along the East Coast were examined for body burdens of selected contaminants and bone strength (vertebral mechanical properties) (Mehrle et al. 1982). Fish from the Hudson River, the most polluted site, had the weakest vertebrae. PCBs were the most prevalent contaminant. Authors proposed a mode of action involving competition for vitamin C between collagen in bone and MFOs (mixed-function oxidases) that detoxify the contaminants. The competition for vitamin C causes a decrease in bone vitamin C and reduces collagen content, with an increase in bone minerals and the ratio of minerals:collagen that renders the backbone more fragile. Sea bass (*Dicentrarchus labrax*) were exposed to the soluble fraction of light cycle oil (1,136 ng l^{-1}) for 7 days which did not affect the frequency and severity of vertebral abnormalities, but decreased mineralization of vertebrae in a reversible way, indicating that it is an early stress indicator (Danion et al. 2011).

8.3.4 Other Taxa

Foraminifera

Foraminifera are single-celled protists with a calcified spiral shell. Prazeres et al. (2011) investigated effects of Zn in the symbiont-bearing foraminifer *Amphistegina lessonii*. Forams were acutely (48 h) exposed to dissolved Zn (9.5–93.4 μg Zn l^{-1}). Many individuals showed white spots and/or dark-brown areas in the test after 24 and 48 h, with a positive correlation between this endpoint and dissolved Zn. Increases in lipid peroxidation and metallothionein-like protein were observed, particularly in pale/partly-bleached individuals. Denoyelle et al. (2012) developed a chronic bioassay by incubating forams for 30 days in seawater with Cd, fuel Oil #2, or drilling muds. Responses included pseudopodal activity and number of newly built chambers. Experiments were conducted in a solution of calcein in seawater, so that foraminiferal tests with newly added chambers could be seen by an epifluorescence microscope. The forams had a strong response to incubation with high concentrations of all tested pollutants.

Not surprisingly, acidification has negative effects on foram shells. McIntyre-Wressnig et al. (2013) assessed effects of elevated $p\text{CO}_2$ on the survival, fitness, shell microfabric and growth of *Amphistegina gibbosa*, a symbiont-bearing, benthic coral-reef species that precipitates low-Mg calcite tests. Specimens were cultured in controlled $p\text{CO}_2$ (ambient, 1,000 ppm by volume [ppmv], and 2,000 ppmv) for 6 weeks. Fitness and survival were not affected. While test growth was not affected by elevated $p\text{CO}_2$, areas of dissolution were observed in small, well defined patches distributed over the whole test surface. Similar dissolution was observed in offspring produced in the 2,000 ppmv $p\text{CO}_2$ treatments.

Echinoderms

Ossicles of the sea urchin *Echinus acutus* and the sea star *Asterias rubens* were studied in stations along a metal contamination gradient. Ossicles with major mechanical functions – sea urchin spines and sea star ambulacral plates – were analyzed for metals and mechanical properties. Sea star plates were more contaminated by Cd, Pb, and Zn than sea urchin spines, and their stiffness and toughness decreased in the most contaminated station (Moureaux et al. 2011). This was attributed either to the incorporation of metals in the calcite lattice and/or to deleterious effects of metals during skeletogenesis.

Ries et al. (2009) tested various marine organisms under different $p\mathrm{CO}_2$ levels and found that in purple sea urchins (*Eucidaris tribuloides*) net calcification increased relative to the control under intermediate $p\mathrm{CO}_2$ levels (605 and 903 mg l^{-1}), and then declined at the highest *p*CO2 level (2,856 mg l^{-1}), and spines deteriorated. Urchins accrete shell that is covered by an external organic layer, which may give them more resilience to elevated $p\mathrm{CO}_2$ than species producing shell that is directly exposed to ambient water. Wood et al. (2008) found that the brittle star, *Amphiura filiformis*, increased its rate of metabolism and ability to calcify to compensate for increased seawater acidity. However, the up-regulation of metabolism and calcification, potentially ameliorating some of the effects of increased acidity, came at a substantial cost, namely muscle wastage.

Corals

Organics

Jackson et al. (1989) and Guzmán et al. (1994) studied effects of a Panamanian oil spill on injury, growth, and regeneration of corals over 5 years. The spill initially caused bleaching, tissue swelling, mucus production, and dead areas, even in subtidal reefs that had not been in contact with the oil. Corals from heavily oiled reefs had higher levels of injury, faster regeneration but slower growth. Hydrocarbons in reef sediments were correlated with the degree of injury and negatively correlated with growth (Fig. 8.6). The probable cause of persistently high injury was chronic exposure to sediments mixed with partially degraded oil that were exported from mangroves onto reefs. Injury resulted in a reallocation of resources to regeneration and decreased investment in fecundity and growth. There was no evidence of coral recovery 5 years after the spill.

Shafir et al. (2007) used a “nubbin assay” on coral fragments to evaluate short- and long-term impacts of dispersed oil from six commercial dispersants, the dispersants themselves, and water-soluble-fractions (WSFs) of Egyptian crude oil, on two branching corals, *Stylophora pistillata* and *Pocillopora damicornis*. Survival and growth of nubbins were recorded for up to 50 days after a single, 24 h exposure to various concentrations. Manufacturer-recommended dispersant concentrations were highly toxic and caused mortality for all nubbins. Dispersed oil and the dispersants were significantly more toxic than crude oil WSFs. As corals are particularly susceptible to dispersants and dispersed oil, authors felt their results rule out the use of dispersants near coral reefs.

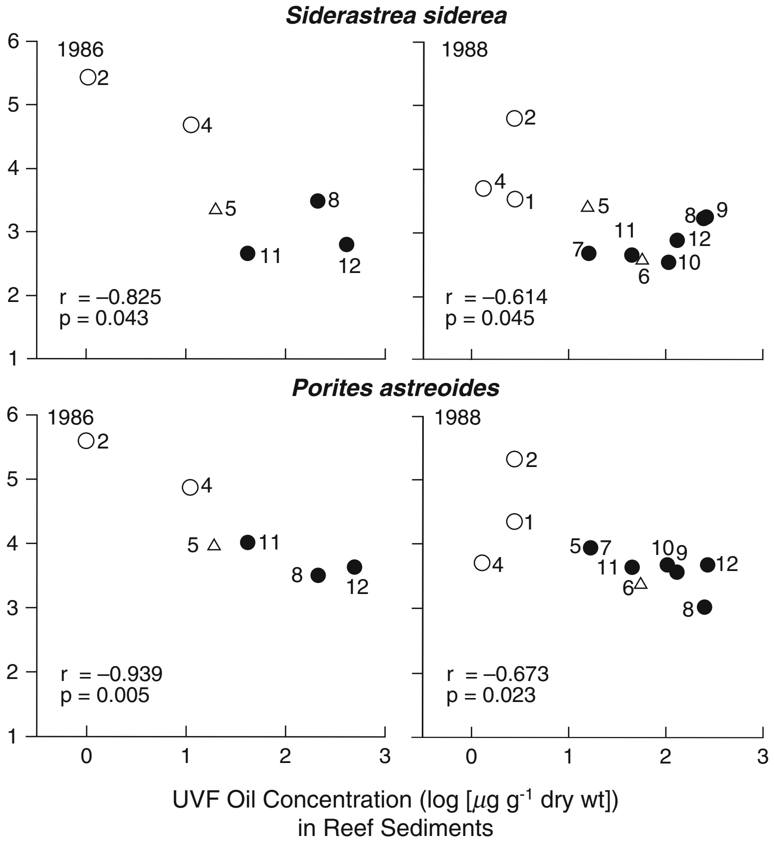


Fig. 8.6 *Siderastrea siderea* and *Porites astreoides*. Relation between growth rates and oil in reef sediments in 1986 and 1988. Symbols connote specific reefs (Reprinted from Guzman et al. 1994: 239, courtesy Inter-Research)

Ocean Acidification/Climate Change

Corals are particularly sensitive to effects of climate change and acidification, and it is predicted by some that within decades the rates of reef erosion will exceed rates of accretion throughout much of the tropics and subtropics. In addition to acidification reducing calcification, rising temperatures trigger bleaching, the loss of symbiotic microalgae from the coral, doubly stressing the corals. Loss of corals reduces habitat for associated reef species and the whole reef community can be degraded. However some reef species may benefit from weakened coral skeletons; bioeroding sponges such as *Cliona orientalis* normally erode coral skeletons. Wisshak et al. (2012) found a clear relationship between the pH of the seawater and the bioerosion rate of the sponges. Thus, with increasing ocean acidification corals will be exposed to a double stress: calcification will be more difficult, and existing skeletons will be weakened more by bioerosion.

Pandolfi et al. (2011) reviewed historical data on past climate change and found that of the five major episodes of severe “reef crises,” four coincided with periods of ocean acidification. However, there is considerable variation among different types of corals, with some being much more resistant than others. An inventory of coral growth from Pacific Panama shows that declines have occurred in some, but not all species (Manzello 2010). Growth declined significantly in the most important reef builder of the eastern tropical Pacific, *Pocillopora damicornis*, by nearly one-third from 1974 to 2006. The rate of decline in skeletal extension for *P. damicornis* from Pacific Panama (0.9 % year^{-1}) was comparable to *Porites* in the Indo-Pacific over the past 20–30 years (0.89–1.23 % year^{-1}). Branching pocilloporid corals show increased tolerance to thermal stress, but appear to be very susceptible to OA. In contrast, the massive pavonid corals are relatively tolerant to both thermal stress and to OA.

Ries et al., (2010) investigated impacts of OA on the temperate coral *Oculina arbuscula* by rearing colonies for 60 days in chambers bubbled with air-CO_2 gas mixtures of varying pCO_2. Measurement of calcification and linear extension revealed that skeletal accretion was minimally impaired; the corals continued accreting new skeletal material, although at reduced rates. Correlation between rates of linear extension and calcification suggests that reduced calcification resulted from reduced aragonite accretion, rather than from dissolution. Accretion of pure aragonite under each condition discounts the possibility that corals will begin producing calcite, a less soluble form of $CaCO_3$, as oceans acidify. The corals’ nonlinear responses and their ability to accrete new skeletal material suggest that they can control the biomineralization process.

Calcification was measured in the Mediterranean cold-water scleractinian coral (CWC) *Madrepora oculata* in sea water reduced or enriched in pCO_2 (Maier et al. 2012). Calcification rates were the same for ambient and elevated pCO_2 (404 and 867 μatm) with 0.06 ± 0.06 % day^{-1}, while calcification was 0.12 ± 0.06 % day^{-1} when pCO_2 was reduced to its pre-industrial level (285 μatm), suggesting that present-day CWC calcification in the Mediterranean Sea has already drastically declined by 50 % due to OA.

Physiological data and models of coral calcification indicate that corals utilize a combination of seawater bicarbonate and respiratory CO_2 for calcification, rather than seawater carbonate. However, a number of investigators attribute effects of experimental acidification by CO_2 or hydrochloric acid additions to a reduction in seawater carbonate ion concentration and thus aragonite saturation state (Jury et al. 2010). Thus, there is a discrepancy between the physiological and geochemical views of coral biomineralization. Furthermore, not all calcifiers respond negatively to decreased pH or saturation state. *Madracis* corals responded strongly to variation in bicarbonate concentration, but not consistently to carbonate concentration, aragonite saturation state, or pH. Corals calcified at normal or elevated rates under low pH

(7.6–7.8) when the seawater bicarbonate was above 1,800 μm. Conversely, corals incubated at normal pH had low calcification rates if the bicarbonate concentration was lowered.

There may be differences in the energetic costs of calcification among different corals. Pandolfi et al. (2011) stated that corals probably utilize all forms of seawater carbon, using enzyme and proton pumps to convert CO_2 and HCO_3^- to carbonate ions for rapid calcification, but this process is energetically costly; well-nourished corals are better equipped to do this in a high CO_2 environment. However, poorly nourished or energetically depleted corals (including bleached ones) are more sensitive to acidification. Evolutionary adaptation and phenotypic plasticity in thermal tolerance may also buffer populations of corals, as will be discussed further in Chap. 11.

The role of zooxanthellae was further investigated by Holcomb et al. (2012) in *Astrangia poculata*. Elevated $p\mathrm{CO}_2$ appeared to have a similar effect on calcification whether or not zooxanthellae were present at 16 °C. However, at 24 °C, corals spawned and there was a sex difference in response. Females exposed to elevated $p\mathrm{CO}_2$ had calcification rates 39 % lower than females at ambient $p\mathrm{CO}_2$, while males showed a non-significant decline. The increased sensitivity of females may reflect a greater investment of energy in reproduction. Thus, sex and spawning affect the sensitivity to acidification.

Thresher et al. (2011) examined the distribution and skeletal characteristics of coral taxa along a natural deep-sea concentration gradient on seamounts of SW Australia. Carbonate undersaturation had little effect on the depth distribution, growth or skeletal composition of live scleractinians or gorgonians, with corals growing, often abundantly, in waters as much as 20–30 % under-saturated. Evidence for an effect of acidification on the accumulation of reef structure was not clear. Abundant old scleractinian skeletons were present well below the aragonite saturation horizon, although this might have been the result of ferromanganese deposition on exposed skeletons.

The exact responses of coral reefs to pH changes are uncertain. Naturally low pH (6.70–7.30) groundwater normally discharges at localized submarine springs at Puerto Morelos, Mexico, and gives insight into potential long term responses of coral ecosystems to low pH. Crook et al. (2012) found species richness and colony size declined with increasing proximity to low-pH water. Iguchi et al. (2012) found that the massive coral, *Porites australiensis* decreased calcification and fluorescence yield (reflecting photosynthesis) in acidified seawater, but that zooxanthellar density was unchanged, unlike in *Acropora* species. Thus responses are quite variable among different corals.

A compilation of studies by Edmunds et al. (2012) showed great variation in calcification as a function of pH, $[HCO_3^-]$, and $[CO_3^{2-}]$, and concluded that studies of the effects of OA on corals need to pay closer attention to reducing variance in experimental outcomes to achieve stronger synthetic capacity; coral genera respond in dissimilar ways to pH, $[HCO_3^-]$, and $[CO_3^{2-}]$; and calcification of massive *Porites* spp. is relatively resistant to short exposures of increased $p\mathrm{CO}_2$, similar to that expected within 100 years.

Coccolithophores/Calcareous Algae

Coccolithophores are phytoplankton with calcified tests, and are susceptible to OA. A significant effect of $p\mathrm{CO}_2$ and temperature on calcification was found by De Bodt et al. (2010) on *Emiliania huxleyi*. Coccosphere particles were smaller at higher temperature and CO_2. The number of well-formed coccoliths decreased with increasing $p\mathrm{CO}_2$ but temperature did not affect morphology. In natural ecosystems, there is a shift in species composition from strongly to weakly calcified species and strains. As OA increases, species that have to invest more energy to form their calcite skeleton may be displaced (Beaufort et al. 2011). However, in the coastal zone of Chile, where currently acidic conditions prevail (pH 7.6–7.9 instead of 8.1 on average), highly calcified individuals were found; a strain of *E. huxleyi* has evolved there that is resistant to acidification. Furthermore, Smith et al. (2012) studied coccolithophore morphology in the Bay of Biscay and found seasonality in the morphotypes of *E. huxleyi*, the most abundant species. While pH and CaCO_3 saturation are lowest in winter, the *E. huxleyi* population shifts from $<10\ \%$ (summer) to $>90\ \%$ (winter) of the heavily calcified form. The finding that the most heavily calcified form dominates under acidic conditions is contrary to the earlier predictions and raises questions about responses of coccolithophores to high $p\mathrm{CO}_2$.

Larger calcareous algae may also be affected by ocean acidification. Price et al. (2011) investigated potential effects on growth, calcification and photophysiology of two species of reef macroalgae, and found *Halimeda opuntia* had net dissolution and 15 % reduction in photosynthetic capacity, while *H. taenicola* did not calcify but did not alter its photophysiology. The different responses of the two species may be due to anatomical and physiological differences and could represent a future shift in their relative dominance. Ries et al. (2009) found that calcifying red algae (*Neogoniolithon*) grew better at 600 mg l^{-1} than at 300 mg $\mathrm{l}^{-1}\mathrm{CO}_2$, but exhibited reduced calcification at higher $p\mathrm{CO}_2$ levels. Johnson and Carpenter (2012) investigated effects of elevated $p\mathrm{CO}_2$ and temperature on calcification of *Hydrolithon onkodes*, a coralline alga, and subsequent effects on susceptibility to grazing by sea urchins. *H. onkodes* was exposed for 21 days to a combination of $p\mathrm{CO}_2$ (420, 530, 830 μatm) and temperature (26, 29 °C). They found increased calcification in moderately elevated $p\mathrm{CO}_2$, similar to Ries et al. (2009), and reduced calcification at higher $p\ \mathrm{CO}_2$. There was a trend for highest calcification at ambient temperature. When *H. onkodes* was exposed to the sea urchin *Echinothrix diadema*, grazing removed 60 % more algae grown at high temperature and high $p\mathrm{CO}_2$ than at ambient temperature and low $p\mathrm{CO}_2$. The increased susceptibility to grazing was considered early evidence of the potential for cascading effects of OA and temperature on coral reef ecosystems. However, the possibility that feeding by *E. diadema* might also be impaired after spending 21 days in these conditions did not seem to be considered.

Since calcifying algae are impaired by acidification, they can be outcompeted by non-calcifying species. Hofmann et al. (2012) examined *Corallina officinalis* (calcifying) and *Chondrus crispus* (noncalcifying) and found an interactive effect of CO_2 concentration and exposure time on growth rates of *C. officinalis*. Community structure changed, as *Chondrus crispus* cover increased in all treatments (385, 665, and 1,486 μatm $p\mathrm{CO}_2$) while *C. officinalis* cover decreased in both elevated-$p\mathrm{CO}_2$ treatments.

8.4 Carcinogenesis

Cancer is a developmental process gone awry – it is uncontrolled growth of cells. Normal cells multiply when the body needs them, stop dividing when growth is complete, and die when no longer needed. Cancer occurs when cells divide and grow uncontrollably, forming malignant tumors or neoplasms, which may invade nearby parts of the body. The cancer may also spread to more distant parts of the body via cells that break off and enter the blood to be transported to other organs. Not all tumors are cancerous, however. Benign tumors do not grow uncontrollably, do not invade neighboring tissues, and do not spread through the body. The development of cancer is often associated with exposure to certain chemicals, termed carcinogens, in both humans, and marine animals.

8.4.1 Mollusks

A number of laboratory and field studies have associated environmental chemicals with neoplasms in shellfish (Yevich and Barszcz 1977). The soft shell clam *Mya arenaria* from chronically oil-polluted sites had elevated levels of gonadal and hematopoeitic neoplasms. Similar findings were seen in soft shell clams from an oil spill site in Massachusetts (Brown et al. 1977), in *Macoma balthica* from areas of Chesapeake Bay (Christensen et al. 1974), and others (Sindermann 1979). Oysters (*C. virginica*) developed neoplasms when exposed in the laboratory or field to contaminated sediment from Black Rock Harbor (BRH), Bridgeport, Connecticut (Gardiner et al. 1991). Neoplasia was seen after 30- and 60-days laboratory exposure to a 20 mg l^{-1} suspension of BRH sediment with postexposure for 0, 30, or 60 days. Composite tumor incidence was 13.6 %. Tumors were most prevalent in the renal epithelium, followed by gill, gonad, gastrointestinal, heart, and neural tissue; tumors did not regress when oysters were placed in clean sediments. Gill neoplasms developed in oysters deployed for 30 days at BRH and 36 days at a BRH dredge material disposal area, and kidney and gastrointestinal neoplasms developed in oysters caged 40 days in Boston Harbor. Exposed oysters accumulated high concentrations of PCBs, PAHs, and chlorinated pesticides. Gonad tumors and siphon anomalies were seen in *Mya arenaria* at sites in New England with widespread use of the herbicides Tordon 101, 2,4-D,2,4,5-T, and other agrochemicals (Gardner et al. 1991).

The general hypothesis has been that cancers are caused by chemical pollutants. However, when the occurrence of mollusk neoplasms was reviewed by Mix (1988), who examined neoplastic diseases in shellfish from around the world, results supporting this hypothesis were not prevalent in the literature. Some studies found shellfish with no neoplasms in highly polluted environments and others found neoplastic diseases in pristine areas. Mix concluded that more research is needed to better understand the association between chemicals and neoplasia in shellfish. However, there has been little research since then to lead to this understanding. Wolowicz et al. (2005) thought pollution by carcinogens was implicated in the Gulf of Gdańsk, southern Baltic Sea. A higher prevalence of mollusk tumors was observed in infaunal deposit/suspension feeders compared to epifaunal suspension-feeders, providing new ecological insights into the genesis of the neoplasia. They discussed a relationship between sediment factors and the incidence of neoplasia across a range of environmental conditions.

8.4.2 Fishes

There have been numerous reports of liver tumors in fishes from contaminated sites around the world. Fish in the laboratory can develop tumors after treatment with a variety of chemical carcinogens (Couch and Harshbarger 1985). The field was reviewed by Bauman (1992) who concluded that some types of tumors, but only neoplasms that have chemicals as a portion of their etiology, would be useful in assessing ecosystem health. Lesions which may fit these criteria include liver neoplasms and skin lesions in a variety of primarily benthic fishes, and neural lesions in various drum species and butterflyfish. There is a correlation with habitat degradation and length of time of residence in a contaminated site. Bottom-dwelling fishes in contact with contaminated sediments appear to be most vulnerable. Winter flounder (*P. americanus*) from degraded east coast estuaries had tumors (Murchelano and Wolke 1985). Winter flounder fed BRH-contaminated (see above) blue mussels contained xenobiotic chemicals analyzed in mussels (PCBs, PAHs, chlorinated pesticides), and developed renal and pancreatic neoplasms and hepatic neoplastic precursor lesions, demonstrating trophic transfer of sediment-bound carcinogens up the food chain (Gardiner et al. 1991). Hepatic lesions in fish from Boston Harbor were correlated with chlorinated hydrocarbons in the liver (Moore et al. 1996). They have been decreasing over time along with the level of pollution in Boston Harbor (Fig. 8.7).

Tumors in West Coast flatfish (English sole, *Parophrys vetulus*) were also associated with contaminants (Malins et al. 1984, 1988); Sole with the highest frequencies of liver neoplasms were from the urban Duwamish Waterway (16 %) and Everett Harbor (12 %), while frequencies in sole from other areas ranged from 0 to 5.5 %. Sediment PAH was significantly correlated with tumors. Metals correlated with total (including non-neoplastic) hepatic lesions. Myers et al. (1990)

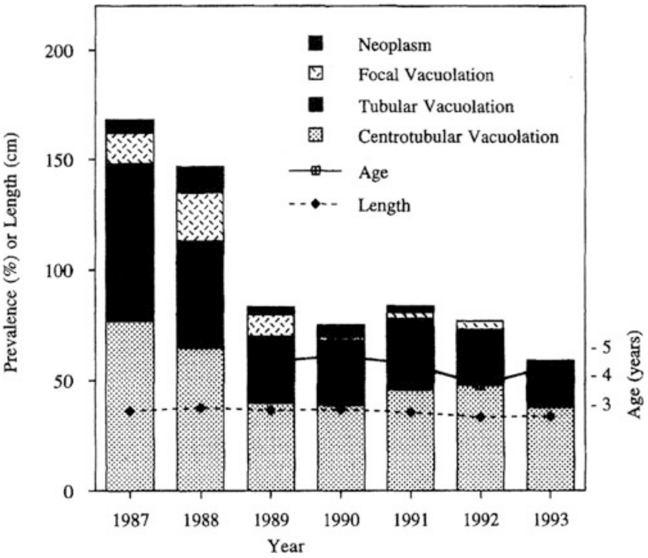


Fig. 8.7 Cumulative graph of winter flounder lesion prevalence 1987–1993 from Deer Island, Boston Harbor. Mean length and age for each year are shown as line plots to indicate between-year comparability of samples (Reprinted from Moore et al. 1996: 464, courtesy Elsevier Publishing Co.)

reported that liver lesions of English sole in Puget Sound represent morphologically identifiable steps leading to the development of neoplasms, similar to carcinogenesis in rodents. The view that these lesions are caused by exposure to chemicals in Puget Sound is based on statistical associations between levels of aromatic hydrocarbons (AH) in sediment and prevalence of lesions, the contribution of AHs in accounting for the variability in neoplasm prevalence in a regression model, correlations between lesions and levels of metabolites of aromatic compounds in bile, and experimental induction of lesions in fish injected with extracts of Puget Sound sediments. These induced lesions were structurally identical to those seen in wild fish from the same site. Since remediation (sediment capping) of highly contaminated areas, there has been a trend of decreasing lesions in English sole (Myers et al. 2008). Lesion risk has been consistently low (<0.20), showing that sediment capping was effective in reducing AH exposure and cancers in resident flatfish.

The Atlantic tomcod, *Microgadus tomcod*, is a bottom-dwelling anadromous species in estuaries including the Hudson River (HR), which contains high concentrations of sediment-bound contaminants including PCBs and PAHs. The HR population was reported to have a high level of liver tumors (up to 90 % in 2-year old fish) compared to only 5 % in fish from other estuaries (Dey et al. 1993). These tomcod have elevated levels of CYP1A mRNA, hydrophobic DNA adducts, and elevated PCBs in their tissues, suggesting that DNA damage by carcinogens

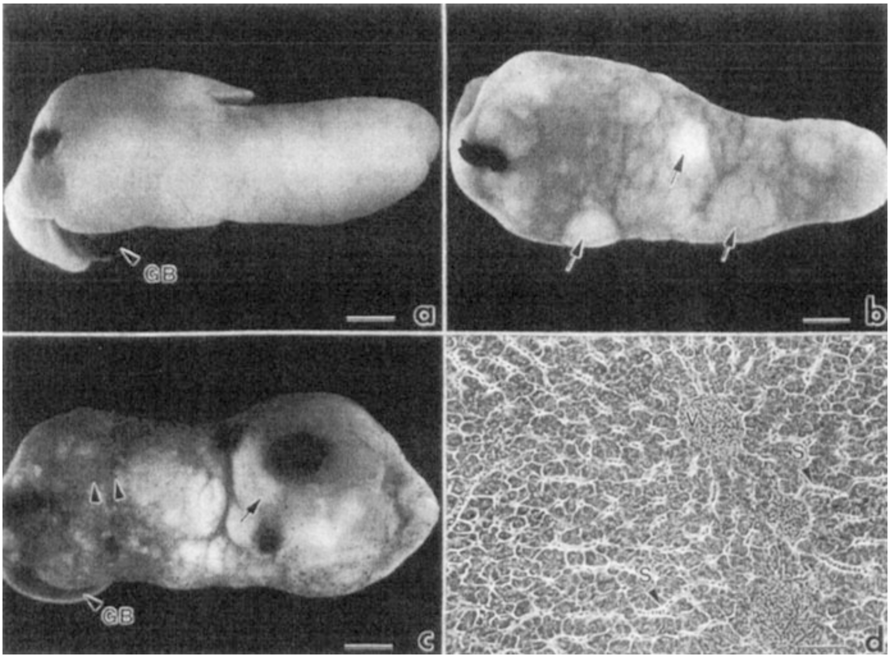


Fig. 8.8 Gross liver pathology and normal histology from mummichog from (**a**) reference site (*GB* gall bladder), (**b**) liver of fish from Station 3 exhibiting tan focal lesions (**c**) liver of fish from Station 3 exhibiting many focal lesions and one 8 mm diameter hepatocellular carcinoma (*arrow*). (**d**) normal hepatocellular structure of fish from reference site (Reprinted from Vogelbein et al. 1990: 5980, courtesy American Association for Cancer Research)

is occurring (Wirgin et al. 1994). Even the highly tolerant killifish (mummichog) *Fundulus heteroclitus*, develops liver tumors if the environment is toxic enough. Fish from a creosote-contaminated site in the Elizabeth River, VA (sediments at 2,200 mg kg^{-1} dry wt PAHs from a wood treatment facility) had liver tumors (Figs. 8.8 and 8.9) in about one third of the population (Vogelbein et al. 1990). There has been a recent report of skin cancer in fish from Australian waters in the area with the "ozone hole" and thus high exposure to UV light. Sweet et al. (2012) found extensive melanosis and melanoma in wild populations of the commercially-important coral trout, *Plectropomus leopardus*. The syndrome was similar to previous studies associated with UV induced melanomas in the platyfish, *Xiphophorus*. Relatively high prevalence rates (15 %) were found at sites in the Great Barrier Reef. Authors concluded that in the absence of microbial pathogens and given the strong similarities to UV-induced melanomas in platyfish, the likely cause was environmental exposure to UV radiation. Further studies are needed to establish the distribution of the syndrome.

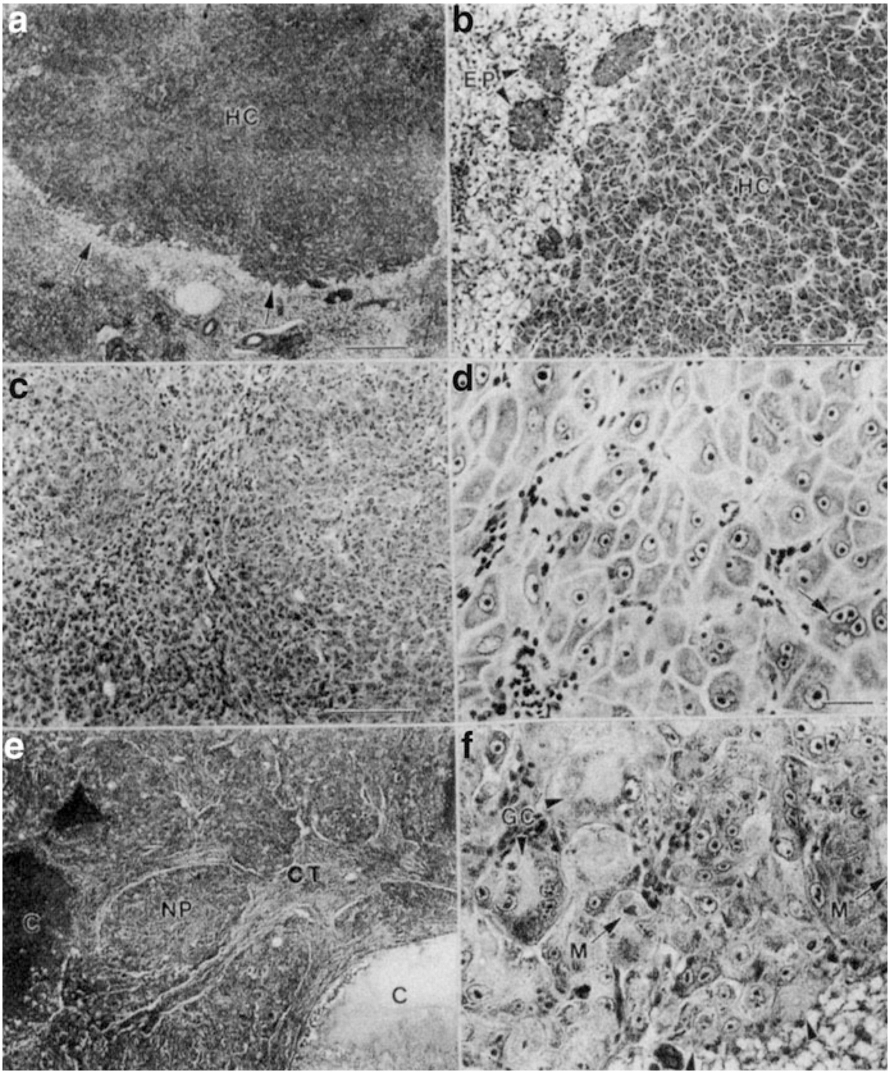


Fig. 8.9 Hepatocellular carcinomas in mummichogs from creosote-contaminated site. (**a**) carcinoma with invasive border, (**b**) higher magnification of carcinoma (**c**) less well differentiated hepatocellular carcinoma (**d**) less well differentiated hepatocellular carcinoma with epithelial-appearing tumor cells (**e**) poorly differentiated hepatocellular carcinoma (**f**) poorly differentiated anaplastic hepatocellular carcinoma with epithelial-appearing cells (Reprinted from Vogelbein et al. 1990: 5983, courtesy American Association for Cancer Research)

8.5 Smoltification

In salmonid fishes that breed in streams, larvae hatch out and live in freshwater for a period of time and then undergo further developmental processes to prepare them for their seaward migration and life in salt water. Newly hatched larvae (alevins) remain in the gravel and utilize the yolk sac as they grow into the fry stage, at which point they emerge from the gravel and feed on insects and zooplankton. As they grow to about 10 cm long, they utilize deeper water and become known as parr, which can remain in freshwater for several years. Then they become smolts, when they begin to prepare for downstream migration. An essential part of this development involves osmoregulation and development of gill enzymes carbonic anhydrase (CA) and Na^+K^+-ATPase. The regulation of smoltification requires environmental factors such as photoperiod and temperature, and endogenous factors, namely hormones. Thyroid hormones and growth hormone peak during smoltification. Treatment with either hormone can stimulate some of the changes associated with smoltification. Thus, these changes are susceptible to some endocrine disruptors. Smolts have a limited period of readiness ("physiological smolt window") for entering salt water, and the timing of sea water entry can be adversely affected by pollution.

8.5.1 Metals

Unlike seawater, freshwater pH is normally close to neutral, around 6.5–7.0. In freshwater systems affected by acid rain, pH decreases and aluminum is released from sediments to which it is usually tightly bound and unavailable. Under these conditions Al becomes toxic. Staurnes et al. (1993) exposed smolting Atlantic salmon (*Salmo salar*) to acid water (pH 5 or pH 5 plus 50 μg Al l^{-1}), which resulted in osmoregulatory failure and mortality. Al strongly enhanced toxicity. Sensitivity to low pH or low pH + Al exposure greatly increased when fish had developed to seawater tolerant smolts. In Al-exposed fish, gill CA activity decreased. Gill Na^+K^+-ATPase activity in control fish peaked in mid-May simultaneously with development of seawater tolerance. Fish from both exposed groups had low seawater tolerance and reduced Na^+, K^+-ATPase activity, which declined to 60 % in acid-exposed fish and even lower in Al-exposed fish. Kroglund et al. (2007) exposed 1-year old hatchery reared *S. salar* smolts to water of pH 5.8 from 3 to 60 days. Fish exposed to Lake Imsa water (pH > 6.5) acted as controls. Control fish had gill-Al concentrations of 5–10 μg Al g^{-1} gill dry weight (dw), while Al-exposed fish had gill-Al concentrations >20 μg Al g^{-1} gill dw prior to seawater release. Gill Na^+, K^+-ATPase activity was depressed in all groups having >25 μg Al g^{-1} gill dw. Following exposure, the smolts were released to monitor downstream migration and ocean return rates. Return rates were reduced by 20–50 % in all Al-exposed groups.

8.5.2 Pesticides and PCBs

Mortensen and Arukwe (2006) studied effects of 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene (DDE) on the thyroid and steroid-metabolizing system in Atlantic salmon parr. Fish were exposed for 5 days to waterborne DDE and thyroxine (T_4), both singly and in combination. Thyroid-stimulating hormone (TSHβ), T_4 deiodinase (T_4ORD), thyroid receptors (TRα and TRβ), and insulin-like growth factor type 1 receptor (IGF-1R) were analyzed. Results indicated that DDE alters thyroid hormone–dependent genes and hepatic CYP3A and PXR levels, demonstrating possible physiological and endocrine consequences from exposure to endocrine-disrupting chemicals during smoltification.

Many investigators have examined effects of PCBs on smoltification. Folmar et al. (1982) injected yearling coho salmon (*Oncorhynchus kisutch*) with 150 $\mu g\ kg^{-1}$ of Aroclor 1254 just prior to smoltification. Alterations in the normal developmental patterns of T_4 were observed in PCB-injected fish. At sea-water entry, one-half of each group (experimentals and controls) were put in seawater and the other half into seawater with 700 $\mu g\ l^{-1}$ No. 2 fuel oil. Significant mortalities were observed in all treatment groups. Mortalities in the PCB-injected, fuel oil-exposed fish appeared additive compared with PCB-injected-only or fuel oil-exposed-only fish. Arctic charr (*Salvelinus alpinus*) were orally dosed with 0, 1, or 100 mg Aroclor 1,254 kg^{-1} body mass (High Dose, HD) in November (Jørgensen et al. 2004). They were then held in freshwater until they smolted in June the next year when they were transferred to seawater. HD charr had reduced plasma growth hormone, insulin-like growth factor-1, and thyroxin and triiodothyronine titers during smoltification. The hormonal alterations corresponded with impaired hyposmoregulatory ability, and reduced growth rate and survival in seawater.

Lerner et al. (2007) examined effects of aqueous Aroclor 1254 (1 $\mu g\ l^{-1}$ (PCB-1) or 10 $\mu g\ l^{-1}$ (PCB-10)) on Atlantic salmon after 21 days exposure either as yolk-sac larvae or as juveniles just prior to the parr–smolt transformation. After exposure, yolk-sac larvae were reared at ambient conditions for 1 year, until smolting the following May. Juveniles were sampled immediately after exposure. At smolting, prior exposure to PCB -1 as larvae did not affect behavior, while PCB-10 dramatically decreased preference for seawater. There were no long-term effects on osmoregulation or hormones of fish exposed as larvae. Juveniles exposed to PCB-1 or PCB-10 showed a dose-dependent reduction in preference for seawater. Fish treated with PCB-10 had a 50 % decrease in gill Na^+, K^+-ATPase. In addition, plasma T_3 was reduced 35–50 % and plasma cortisol 58 % in response to either concentration. Thus, effects vary according to developmental stage. Exposure to PCBs in freshwater can inhibit changes that occur during smolting, and reduce marine survival.

8.5.3 Contaminants of Emerging Concern (CECs)

Bangsgaard et al. (2006) exposed Atlantic salmon parr or pre-smolts to estradiol-17β (E2 conc.: nominal 500 ng l^{-1}/actual 8–16 ng l^{-1}) and *tert*-octylphenol (OP: nominal 25 μg l^{-1}/actual 4.5–6.5 μg l^{-1} and OP: nominal 100 μg l^{-1}/actual 10–30 μg l^{-1}) for 26 days in freshwater, and studied effects on parr-smolt transformation. Vitellogenesis was induced by all treatments, and elevated VTG levels were still found 4–5 months after treatment. Smolting was impaired by E2 and OP-100 as judged by reduced gill Na^+, K^+-ATPase and reduced ability to regulate plasma osmolality and muscle water content in 24-h seawater (SW) challenge tests during smolting. Downstream migration was monitored by implanting passive integrated transponder tags into smolts and placing them in a stream raceway. Downstream movement was initiated in all groups, but E2 and OP-100 fish migrated at lower frequency than controls, suggesting that xenoestrogens reduce physiological and behavioral components of smoltification, even when exposure occurred several months prior to smolting.

Madson et al. (2004) gave Atlantic salmon serial injections over 20 days of 2 μg/g body weight estradiol (E2), 120 μg/g nonylphenol (4-NP) in peanut oil or peanut oil as control. After the last injection, fish were individually tagged. Two days later 100 fish per group were released into a small stream. Migration was evaluated by measuring arrival time at a trap downstream. Serum VTG levels increased several-fold in both male and female E2- and 4-NP-treated fish. Overall, E2- and 4-NP-treatment impaired smolting as judged by reduced gill Na^+, K^+-ATPase activity and α-subunit Na^+, K^+-ATPase mRNA level, reduced muscle water content and increased mortality following 24 h SW-challenge. After release, control fish initiated downstream migration immediately, with 50 % of them appearing in the trap within 10 days. E2- and 4-NP-treated fish appeared in the trap, with a delay of 6 and 8 days, respectively. The total number of fish reaching the trap was control (81 %), E2 (53 %), 4-NP (12 %), indicating that short-term exposure to environmental estrogens impaired smoltification, survival, and downstream migration.

Keen et al. (2005) studied 4-week dietary exposure of coho salmon (*Oncorhynchus kisutch*) to nonylphenol (4-NP) during the parr-smolt transformation. Doses varied between 0 (control) and 2,000 mg/kg after which fish were transferred to sea water, when all groups were fed the control diet. Dietary treatment of 4-NP did not influence growth or smoltification of coho salmon, a result that conflicts to some extent with other reports (e.g. Madson et al., above) in which deleterious effects of 4-NP were linked to disruption of the endocrine system. Differences may be due to species, concentration, route of exposure, timing, or other factors. There have not been studies of other types of CECs besides environmental estrogens.

8.5.4 Acidification

Effects of low pH were discussed in relation to Al, above. There have also been studies of effects of low pH alone. Saunders et al. (1983) reared Atlantic salmon (*Salmo salar*) at pH 6.4–6.7 (control) and 4.2–4.7, from Feb. to June, to assess the effect on survival, growth, and smolting under rising (4–8.5 °C) or relatively constant (9.5–10.5 °C) temperature. Survival was lower in low pH under both temperature regimes. Neither group in low pH gained weight while both control groups did. Parr–smolt transformation, as indicated by salinity tolerance and gill Na^+, K^+-ATPase activity, was impaired by low pH. Controls increased their tolerance to 35 psu salinity between March and May but those in low pH did not. ATPase activity was greater in controls in rising than in constant temperature. Plasma chloride and sodium levels were reduced at low pH, indicating impaired ionic regulation in freshwater. Thyroid hormones (T_3 and T_4) gave no clear indication of effects, but smoltification did not proceed normally at low pH.

Haya et al. (1985) found effects of low pH on energy metabolism, an indirect way of affecting smoltification. Juvenile *S. salar* were held for 76 days at pH 4.7 during the time when the final stages of smoltification normally occur. Controls at pH 6.5 had significant increases in weight, length and liver somatic index which were not seen in fish at low pH. After 15 days, ADP and glucose levels were higher and adenylate energy charge (AEC), and glycogen lower in muscles of fish at low pH; differences were maintained until the end of the experiment. ATP and total adenylate concentrations in muscle were lower after 62 days in low pH. Fish at low pH decreased their food intake, reducing growth.

8.6 Conclusions

This chapter has focused on developmental processes that take place in juvenile or adult animals, and the research findings have shown that developmental processes remain sensitive to contaminant effects throughout life. Effects on growth *per se* have been studied for a long time, and in many cases, reductions in growth can be traced back to reduced food intake (see Chap. 2). Reduced limb regeneration in crustaceans can be in some cases attributed to endocrine disruption of molting hormones, and impaired smoltification in salmon can also be endocrine-related. Carcinogenesis was a major research area in previous years, but studies have diminished along with much of the gross contamination that produced cancers in highly polluted systems. Reports of tumors in both fish and mollusk populations at contaminated sites have decreased considerably over the past 20 years. This may reflect improving environmental conditions due to reduction of contaminants in effluents and cessation of dumping of wastes into the ocean. However, it may also be that this type of research is not as popular as it once was. Some reports suggest that exposures to some types of contaminants are increasing (Hanson et al. 2009), probably due to increased rain and runoff from land. Recently, considerable attention is being devoted to effects of ocean acidification on deposition of calcareous structures (shells, bones) in a variety of marine species, with mollusks and corals being the taxa that appear to be at greatest risk.

References

Albright R, Bland C, Gillette P, Serafy JE, Langdon C, Capo TR (2012) Juvenile growth of the tropical sea urchin *Lytechinus variegatus* exposed to near-future ocean acidification scenarios. J Exp Mar Biol Ecol 426–427:12–17

Alzieu C (1986) TBT detrimental effects on oyster culture in France – evolution since antifouling paint restriction. In: Oceans 86, IEEE proceedings, vol 4. Marine Technology Society, Washington, DC, p 1132

Alzieu C, Sanjuan J, Deltreil JP, Borel M (1986) Tin contamination in Arcachon Bay: effects on oyster shell anomalies. Mar Pollut Bull 17:494–498

Amaral V, Cabral HN, Bishop MJ (2012a) Effects of estuarine acidification on predator–prey interactions. Mar Ecol Prog Ser 445:117–127

Amaral V, Cabral HN, Bishop MJ (2012b) Moderate acidification affects growth but not survival of 6-month-old oysters. Aquat Ecol 46:119–127

Anderson JW, Riley RG, Kiesser SL, Thomas BL, Fellingham OW (1981) Natural weathering of oil in marine sediments: tissue contamination and growth of the clam, *Protothaca staminea*. In: Technical report of the symposium on ocean pollution, Halifax, Nova Scotia, Canada, 19 Oct 1981, 25 pp

Baker RT, Handy RD, Davies SJ, Snook JC (1998) Chronic dietary exposure to copper affects growth, tissue lipid peroxidation, and metal composition of the grey mullet, *Chelon labrosus*. Mar Environ Res 45:357–365

Bangsgaard K, Madsen SS, Korsgaard B (2006) Effect of waterborne exposure to 4-tert-octylphenol and 17beta-estradiol on smoltification and downstream migration in Atlantic salmon, *Salmo salar*. Aquat Toxicol 80:23–32

Bauman PC (1992) The use of tumors in wild populations of fish to assess ecosystem health. J Aquat Ecosyst Health 1:135–146

Beaufort L et al (2011) Sensitivity of coccolithophores to carbonate chemistry and ocean acidification. Nature 476:80–83

Bednaršek N, Tarling GA, Bakker DCE, Fielding S, Jones EM, Venables HJ, Ward P, Kuzirian A, Lézé B, Feely RA, Murphy EJ (2012) Extensive dissolution of live pteropods in the Southern Ocean. Nat Geosci 5:881–885. doi:10.1038/ngeo1635

Bergey L, Weis JS (2007) Molting as a mechanism of depuration of metals in the fiddler crab, *Uca pugnax*. Mar Environ Res 64:556–562

Bodammer JE (2000) Some new observations on the cytopathology of fin erosion disease in winter flounder *Pseudopleuronectes americanus*. Dis Aquat Organ 40:51–65

Boyden CR, Watling H, Thornton I (1975) Effect of zinc on settlement of the oyster, *Crassostrea gigas*. Mar Biol 31:227–234

Brown RS, Wolke RE, Saila SB, Brown CW (1977) Prevalence of neoplasia in New England populations of the soft-shell clam (*Mya arenaria*). Ann N Y Acad Sci 298:522–534

Brown SB, Adams BA, Cyr DG, Eales JG (2004) contaminant effects on the teleost fish thyroid. Environ Toxicol Chem 23:1680–1701

Buckley JT, Roch M, McCarter JA, Rendell CA, Matheson AT (1982) Chronic exposure of coho salmon to sublethal concentrations of copper—I. Effect on growth, on accumulation and distribution of copper, and on copper tolerance. Comp Biochem Physiol C 72:15–19

Burrell RD, Breitburg D, Hondorp D, Keppel A (2011) Breathless nights: Diel-Cycling hypoxia and the prevalence of *Perkinsus marinus* (dermo) infections in *Crassostrea virginica*. Presented at Coastal and Estuarine Research Federation meetings, Nov. 2011, Daytona Beach, FL

Butler JA, Millemann RE, Stewart NE (1968) Effects of the insecticide Sevin on survival and growth of the cockle clam *Clinocardium nuttalli*. J Fish Res Board Can 25:1621–1635

Canli M, Atli G (2003) The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environ Pollut 121:129–136

Cantelmo A, Lazell R, Mantel L (1981) The effects of benzene on molting and limb regeneration in juvenile *Callinectes sapidus*. Mar Biol Lett 2:333–343

Carr RS, Neff JM, Boehm PD (1987) A study of the fate and effects of chemically and physically dispersed oil on benthic marine communities using large-scale continuous flow exposure systems. In: Vernberg WB, Calabrese A, Thurberg FP, Vernberg FJ (eds) Pollution physiology of estuarine organisms. University of South Carolina Press, Columbia, pp 47–68

Chen J-C, Lin C-H (2001) Toxicity of copper sulfate for survival, growth, molting, and feeding of juveniles of the tiger shrimp *Penaeus monodon*. Aquaculture 192:55–65

Christensen DJ, Farley CA, Kern FG (1974) Epizootic neoplasms in the clam *Macoma balthica* (L) from Chesapeake Bay. J Natl Cancer Inst 52:1739–1749

Clark JV (1986) Inhibition of moulting in *Penaeus semisulcatus* (de Haan) by long-term hypoxia. Aquaculture 52:253–254

Comeau S, Gorsky G, Jeffree R, Teyssié J-L, Gattuso J-P (2009) Impact of ocean acidification on a key Arctic pelagic mollusk (*Limacina helicina*). Biogeosciences 6:1877–1882

Comeau S, Jeffree R, Teyssié J-L, Gattuso J-P (2010) Response of the Arctic Pteropod *Limacina helicina* to projected future environmental conditions. PLoS One 5(6):e11362. doi:10.1371/journal.pone.0011362

Comeau S, Alliouane S, Gattuso J-P (2012) Effects of ocean acidification on overwintering juvenile Arctic pteropods, *Limacina helicina*. Mar Ecol Prog Ser 456:279–284

Cong NV, Phuong NT, Bayley M (2009) Effects of repeated exposure of diazinon on cholinesterase activity and growth in snakehead fish (*Channa striata*). Ecotoxicol Environ Saf 72:699–703

Couch JA, Harshbarger JC (1985) Effects of carcinogenic agents on aquatic animals: an environmental and experimental overview. Environ Carcinog Rev 3:63–105

Couch JA, Winstead JT, Hanson DJ, Goodman LR (1979) Vertebral dysplasia in young fish exposed to the herbicide trifluralin. J Fish Dis 2:35–42

Crook ED, Potts D, Rebolledo-Vieyra M, Hernandez L, Paytan A (2012) Calcifying coral abundance near low-pH springs: implications for future ocean acidification. Coral Reefs 31:239–245

Cunningham PA (1976) Inhibition of shell growth in the presence of mercury and subsequent recovery of juvenile oysters. Proc Natl Shellfish Assoc 66:1–5

Cunningham PA, Myers LE (1987) Effects of diflubenzuron (Dimilin®) on survival, molting, and behavior of juvenile fiddler crabs, *Uca pugilator*. Arch Environ Contam Toxicol 16:745–752

D'Andrea AF, Stancyk SE, Chandler GT (1996) Sublethal effects of cadmium on arm regeneration in the burrowing brittlestar, *Microphiopholis gracillima*. Ecotoxicology 5:115–133

Danion M, Deschamps M-H, Thomas-Guyon H, Bado-Nilles A, Le Floch S, Quentel C, Sire J-Y (2011) Effect of an experimental oil spill on vertebral bone tissue quality in European sea bass (*Dicentrarchus labrax* L.). Ecotoxicol Environ Saf 74:1888–1895

Davis WP (1997) Evidence for developmental and skeletal responses as potential signals of endocrine disrupting compounds in fishes. In: Rolland RM, Gilbertson M, Peterson RE (eds) Chemically induced alterations in functional development and reproduction of fishes. SETAC Press, Pensacola, pp 61–72

De Bodt C, Van Oostende N, Harlay J, Sabbe K, Chou L (2010) Individual and interacting effects of *p*CO2 and temperature on *Emiliania huxleyi* calcification: study of the calcite production, the coccolith morphology and the coccosphere size. Biogeosciences 7:1401–1412

Deane EE, Woo YS (2009) Modulation of fish growth hormone levels by salinity, temperature, pollutants and aquaculture related stress: a review. Rev Fish Biol Fish 19:97–120

Denoyelle M, Geslin E, Jorissen FJ, Cazes L, Galgani F (2012) Innovative use of foraminifera in ecotoxicology: a marine chronic bioassay for testing potential toxicity of drilling muds. Ecol Indic 12:17–25

Dey WP, Peck TH, Smith CE, Kreamer G-L (1993) Epizootiology of hepatic neoplasia in Atlantic tomcod (*Microgadus tomcod*) from the Hudson River estuary. Can J Fish Aquat Sci 50:1897–1907

Dickinson GH, Ivanina AV, Matoo OB, Pörtner HO, Lannig G, Bock C, Beniash E, Sokolova IM (2012) Interactive effects of salinity and elevated CO_2 levels on juvenile eastern oysters, *Crassostrea virginica*. J Exp Biol 215:29–43

Doropoulos C, Ward S, Marshell A, Diaz-Pulido G, Mumby PJ (2012) Interactions among chronic and acute impacts on coral recruits: the importance of size-escape thresholds. Ecology 93:2131–2138

Duckworth AR, Peterson BJ (2013) Effects of seawater temperature and pH on the boring rates of the sponge *Cliona celata* in scallop shells. Mar Biol 160:27–35

Duckworth AR, West L, Vansach T, Stubler A, Hardt M (2012) Effects of water temperature and pH on growth and metabolite biosynthesis of coral reef sponges. Mar Ecol Prog Ser 462:67–77

Edmunds PJ, Brown D, Moriarty V (2012) Interactive effects of ocean acidification and temperature on two scleractinian corals from Moorea, French Polynesia. Glob Change Biol 18:2173–2183

Engel DW (1987) Metal regulation and molting in the blue crab *Callinectes sapidus*: copper, zinc, and metallothionein. Biol Bull 172:69–82

Evrard E, Devaux A, Bony S, Burgeot T, Riso R, Budzinski H, Le Du M, Quiniou L, Laroche J (2010) Responses of the European flounder *Platichthys flesus* to the chemical stress in estuaries: load of contaminants, gene expression, cellular impact and growth rate. Biomarkers 15:111–127

Feely RA, Sabine CL, Hernandez-Ayon J, Ianson D, Hales B (2008) Evidence for upwelling of corrosive "acidified" water onto the Continental Shelf. Science 320:1490–1492

Fernandez-Reiriz M, Range P, Alvarex-Salgado X, Espinosa J, Labarta U (2012) Tolerance of juvenile *Mytilus galloprovincialis* to experimental seawater acidification. Mar Ecol Prog Ser 454:65–74

Fingerman S, Fingerman M (1978) Effects of two polychlorinated biphenyls (Aroclors 1242 and 1254) on limb regeneration in the fiddler crab *Uca pugilator* at different times of the year. Vie Milieu 28–29:69–75

Folmar LC, Dickhoff WW, Zaugg WE, Hodgins HO (1982) The effects of Aroclor 1254 and No. 2 fuel oils on smoltification and sea-water adaptation of coho salmon (*Oncorhynchus kisutch*). Aquat Toxicol 2:291–300

Fukuyama AK, Shigenaka G, Hoff RZ (2000) Effects of residual *Exxon Valdez* oil on intertidal *Protothaca staminea*: mortality, growth, and bioaccumulation of hydrocarbons in transplanted clams. Mar Pollut Bull 40:1042–1050

Gardiner G, Yevich P, Harshbarger J, Malcolm AR (1991) Carcinogenicity of Black Rock Harbor sediment to Eastern Oyster and trophic transfer of Black Rock Harbor carcinogens from the blue mussel to the winter flounder. Environ Health Perspect 90:53–66

Gardner G, Yevich P, Hurst J, Thayer P, Benyi S, Harshbarger JC, Pruell RJ (1991) Germinomas and teratoid siphon anomalies in softshell clams, *Mya arenaria*, environmentally exposed to herbicides. Environ Health Perspect 90:43–51

Gaylord B, Hill TM, Sanford E, Lenz EA, Jacobs LA, Sato KN, Russell AD, Hettinger A (2011) Functional impacts of ocean acidification in an ecologically critical foundation species. J Exp Biol 214:2586–2594

Gazeau F, Quiblier C, Jansen JM, Gattuso J-P, Middelburg JJ, Heip CHR (2007) Impact of elevated CO2 on shellfish calcification. Geophys Res Lett 34:L07603. doi:10.1029/2006GL028554

Gopalan UK, Young JS (1975) Incidence of shell disease in shrimps in the New York Bight. Mar Pollut Bull 6:149–153

Guo F, Wang L, Wang W-X (2012) Acute and chronic toxicity of polychlorinated biphenyl 126 to *Tigriopus japonicus*: effects on survival, growth, reproduction and intrinsic rate of population growth. Environ Toxicol Chem 31:639–645

Gutowska MA, Melzner F, Pörtner HO, Meier S (2010) Cuttlebone calcification increases during exposure to elevated seawater *p*CO2 in the cephalopod *Sepia officinalis*. Mar Biol 157:1653–1663

Guzmán HM, Burns KA, Jackson JB (1994) Injury, regeneration and growth of Caribbean reef corals after a major oil spill in Panama. Mar Ecol Prog Ser 105:231–241

Hamilton SJ, Haines TA (1989) Bone characteristics and metal concentrations in white suckers (*Catostomus commersoni*) from one neutral and three acidified lakes in Maine. Can J Fish Aquat Sci 46:440–446

Hanson N, Förlin L, Larsson A (2009) Evaluation of long-term biomarker data from perch (*Perca fluviatilis*) in the Baltic Sea suggests increasing exposure to environmental pollutants. Environ Toxicol Chem 28:364–373

Haya K, Waiwood BA, Van Eeckhaute L (1985) Disruption of energy metabolism and smoltification during exposure of juvenile Atlantic salmon *Salmo salar* to low pH. Comp Biochem Physiol C 82:323–330

Heintz RA, Rice SD, Wertheimer AC, Bradshaw RF, Thrower FP, Joyce JE, Short JW (2000) Delayed effects on growth and marine survival of pink salmon *Oncorhynchus gorbuscha* after exposure to crude oil during embryonic development. Mar Ecol Prog Ser 208:205–216

Hofmann LC, Straub S, Bischof K (2012) Competition between calcifying and noncalcifying temperate marine macroalgae under elevated CO_2 levels. Mar Ecol Prog Ser 464:89–105

Holcomb M, Cohen AL, McCorkle DC (2012) An investigation of the calcification response of the scleractinian coral *Astrangia poculata* to elevated $\boldsymbol{p}CO_2$ and the effects of nutrients, zooxanthellae and gender. Biogeosciences 9:29–39

Iguchi A, Ozake S, Nakamura T, Inoue M, Tanaka Y, Suzuki A, Kawahata H, Sakai K (2012) Effects of acidified seawater on coral calcification and symbiotic algae on the massive coral *Porites australiensis*. Mar Environ Res 73:32–36

Jackson JB et al (1989) Ecological effects of a major oil spill on Panamanian coastal marine communities. Science 241:37–44

Johnson MD, Carpenter RC (2012) Ocean acidification and warming decrease calcification in the crustose coralline alga *Hydrolithon onkodes* and increase susceptibility to grazing. J Exp Mar Biol Ecol 434–435:94–101

Jørgensen EH, Aas-Hansen O, Maule AG, Strand JE, Vijayan MM (2004) PCB impairs smoltification and seawater performance in anadromous Arctic charr (*Salvelinus alpinus*). Comp Biochem Physiol C Toxicol Pharmacol 138:203–212

Jury CP, Whitehead RF, Szmant AM (2010) Effects of variations in carbonate chemistry on the calcification rates of *Madracis aure tenra* (= *Madracis mirabilis sensu* Wells, 1973): bicarbonate concentrations best predict calcification rates. Global Change Biol 16:1632–1644

Karen DJ, Draughn R, Fulton M, Ross PE (1998) Bone strength and acetylcholinesterase inhibition as endpoints in chlorpyrifos toxicity to *Fundulus heteroclitus*. Pestic Biochem Physiol 60:167–175

Karen DJ, Ross PE, Klaine SJ (2001) Further considerations of the skeletal system as a marker of episodic chlorpyrifos exposure. Aquat Toxicol 52:285–296

Keen PL, Higgs DA, Hall KJ, Ikonomou M (2005) Effects of dietary exposure of 4-nonylphenol on growth and smoltification of juvenile coho salmon (*Oncorhynchus kisutch*). Sci Total Environ 349:81–94

Kerambrun E, Henry F, Perrichon P, Courcot L, Meziane T, Spilmont N, Amara R (2012a) Growth and condition indices of juvenile turbot, *Scophthalmus maximus*, exposed to contaminated sediments: effects of metallic and organic compounds. Aquat Toxicol 108:130–140

Kerambrun E, Henry F, Courcot L, Gevaert F, Amara R (2012b) Biological responses of caged juvenile sea bass (*Dicentrarchus labrax*) and turbot (*Scophtalmus maximus*) in a polluted harbour. Ecol Indic 19:161–171

Keteles KA, Fleeger JW (2001) The contribution of ecdysis to the fate of copper, zinc, and cadmium in grass shrimp, *Palaemonetes pugio* Holthuis. Mar Pollut Bull 42:1397–1402

Khan AT, Weis JS, Saharig CE, Polo AE (1993) Effect of tributyltin on mortality and telson regeneration of grass shrimp, *Palaemonetes pugio*. Bull Environ Contam Toxicol 50:152–157

Kraus ML, Weis JS (1988) Differences in the effects of mercury on telson regeneration in two populations of grass shrimp *Palaemonetes pugio*. Arch Environ Contam Toxicol 17:115–120

Kroeker KJ, Micheli F, Gambi MC, Martz TR (2011) Divergent ecosystem responses within a benthic marine community to ocean acidification. Proc Natl Acad Sci 108:14515–14520

Kroeker KJ, Micheli F, Gambi MC (2013) Ocean acidification causes ecosystem shifts via altered competitive interactions. Nat Clim Change 3:156–159

Kroglund F, Finstad B, Stefansson SO, Nilsen TO, Kristensen T, Rosseland BO, Teien HC, Salbu B (2007) Exposure to moderate acid water and aluminum reduces Atlantic salmon post-smolt survival. Aquaculture 273:360–373

Landes A, Zimmer M (2012) Acidification and warming affect both a calcifying predator and prey, but not their interaction. Mar Ecol Prog Ser 450:1–10

Lee J-W, De Riu N, Lee S, Bai SC, Moniello G, Hung SS (2011) Effects of dietary methylmercury on growth performance and tissue burden in juvenile green (*Acipenser medirostris*) and white sturgeon (*A. transmontanus*). Aquat Toxicol 105:227–234

Lerner DT, Björnsson BT, McCormick SD (2007) Effects of aqueous exposure to polychlorinated biphenyls (Aroclor 1254) on physiology and behavior of smolt development of Atlantic salmon. Aquat Toxicol 81:329–336

Madsen S, Skovbølling S, Nielsen C, Korsgaard B (2004) 17-β Estradiol and 4-nonylphenol delay smolt development and downstream migration in Atlantic salmon, *Salmo salar*. Aquat Toxicol 68:109–120

Maier C, Watremez P, Taviani M, Weinbauer MG, Gattuso JP (2012) Calcification rates and the effect of ocean acidification on Mediterranean cold-water corals. Proc R Soc B Biol Sci 279:1716–1723

Malins DC et al (1984) Chemical pollutants in sediments and diseases of bottom-dwelling fish in Puget Sound, Washington. Environ Sci Technol 13:705–713

Malins DC et al (1988) Neoplastic and other diseases in fish in relation to toxic chemicals: an overview. Aquat Toxicol 11:43–67

Manley AR, Gruffydd LD, Almada-Villela PC (1984) The effect of copper and zinc on the shell growth of *Mytilus edulis* measured by a laser diffraction technique. J Mar Biol Assoc UK 64:417–427

Manzello DP (2010) Coral growth with thermal stress and ocean acidification: lessons from the eastern tropical Pacific. Coral Reefs 29:749–758

McIntyre- Wressnig A, Bernhard JM, McCorkle DC, Hallock P (2013) Non-lethal effects of ocean acidification on the symbiont-bearing benthic foraminifer *Amphistegina gibbosa*. Mar Ecol Prog Ser 472:45–60

Mehrle PA, Haines TA, Hamilton S, Ludke JL, Mayer FL, Ribick MA (1982) Relationship between body contaminants and bone development in east-coast striped bass. Trans Am Fish Soc 111:123–130

Melatunan S, Calosi P, Rundle SD, Widdicombe S, Moody AJ (2013) Effects of ocean acidification and elevated temperature on shell plasticity and its energetic basis in an intertidal gastropod. Mar Ecol Prog Ser 472:155–168

Melzner F, Stange P, Trubenbach K, Thomsen J, Casties I, Panknin U, Gorb S, Guowska MA (2012) Food supply and seawater pCO2 impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. PLoS One 6(9):art.e24223.2011

Mix MC (1988) Shellfish diseases in relation to toxic chemicals. Aquat Toxicol 11:29–42

Moore MJ, Shea D, Hillman RE, Stegeman JJ (1996) Trends in hepatic tumours and hydropic vacuolization, fin erosion, organic chemicals, and stable isotope ratios in winter flounder from Massachusetts, U.S.A. Mar Pollut Bull 32:458–470

Mortensen AS, Arukwe A (2006) The persistent DDT metabolite,1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene, alters thyroid hormone-dependent genes, hepatic cytochrome P4503A, and pregnane X receptor gene expressions in Atlantic salmon (*Salmo salar*) Parr. Environ Toxicol Chem 25:1607–1615

Moureaux C, Simon J, Mannaerts G, Catarino AI, Pernet P, Dubois P (2011) Effects of field contamination by metals (Cd, Cu, Pb, Zn) on biometry and mechanics of echinoderm ossicles. Aquat Toxicol 105:698–707

Murchelano RA, Wolke RE (1985) Epizootic carcinoma in the winter flounder, *Pseudopleuronictes americanus*. Science 228:587–589

Myers M, Landahl JT, Krahn MM, Johnson LL, McCain BB (1990) Overview of studies on liver carcinogenesis in English sole from Puget Sound; evidence for a xenobiotic chemical etiology I: pathology and epizootiology. Sci Total Environ 94:33–50

Myers MS, Anulacion BF, French BL, Reichert WL, Laetz CA, Buzitis J, Olson OP, Sol SY, Collier TK (2008) Improved flatfish health following remediation of a PAH-contaminated site in Eagle Harbor, Washington. Aquat Toxicol 88:277–288

Nelson WC (1987) A comparison of the physiological condition of the blue mussel, *Mytilus edulis*, after laboratory and field exposure to dredged material. In: Vernberg WB, Calabrese A, Thurberg FP, Vernberg FJ (eds) Pollution physiology of estuarine organisms. University of South Carolina Press, Columbia, pp 185–205

Nienhuis S, Palmer AR, Harley CDG (2010) Elevated CO2 affects shell dissolution rate but not calcification rate in a marine snail. Proc R Soc Lond B 277:2553–2558

Nilsson HC, Sköld M (1996) Arm regeneration and spawning in the brittle star *Amphiura filiformis* (O.F. Müller) during hypoxia. J Exp Mar Biol Ecol 199:193–206

Pandolfi JM, Connolly SR, Marshall DJ, Cohen AL (2011) Projecting coral reef futures under global warming and ocean acidification. Science 333:418–422

Patterson HK, Carmichael RH, Boettcher A (2011) Stressed out oysters: measuring sublethal responses using stable isotopes and protein regulation. Presented at Coastal and Estuarine Research Federation meetings, Nov. 2011, Daytona Beach, FL

Pichavant K, Person-Le-Ruyet J, Le Bayon N, Severe A, Le Roux A, Boeuf G (2001) Comparative effects of long-term hypoxia on growth, feeding and oxygen consumption in juvenile turbot and European sea bass. J Fish Biol 59:875–883

Prazeres M, Martins SE, Bianchini A (2011) Biomarkers response to zinc exposure in the symbiont-bearing foraminifer *Amphistegina lessonii* (Amphisteginidae, Foraminifera). J Exp Mar Biol Ecol 407:116–121

Price NN, Hamilton SL, Tootell JS, Smith JE (2011) Species-specific consequences of ocean acidification for the calcareous tropical green algae *Halimeda*. Mar Ecol Prog Ser 440:67–78

Range P, Piló D, Ben Hamadou R, Chícharo MA, Matias D, Joaquim S, Oliveira AP, Chícharo L (2012) Seawater acidification by CO_2 in a coastal lagoon environment: effects on life history traits of juvenile mussels *Mytilus galloprovincialis*. J Exp Mar Biol Ecol 424–425:89–98

Rao KR, Fox FR, Conklin PJ, Cantelmo AC, Brannon AC (1979) Physiological and biochemical investigation of the toxicity of pentachlorophenol to crustaceans. In: Vernberg WB, Thurberg FP, Calabrese A, Vernberg FJ (eds) Marine pollution: functional responses. Academic Press, New York, pp 307–340

Rao KR, Fox FR, Conklin PJ, Cantelmo AC (1981) Comparative toxicology and pharmacology of chlorophenols: studies on the grass shrimp *Palaemonetes pugio*. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Biological monitoring of marine pollutants. Academic Press, New York, pp 37–72

Rao KR, Conklin PJ, Doughtie DG (1985) Physiological and histopathological evaluation of toxicity of hexavalent chromium to the grass shrimp *Palaemonetes pugio* Holthuis. In: Vernberg FJ, Thurberg FP, Calabrese A, Vernberg WB (eds) Marine pollution and physiology: recent advances. University of South Carolina Press, Columbia, pp 131–183

Rees BB, Targett TE, Ciotti BJ, Tolman CA, Akkina SS, Gallaty AM (2012) Temporal dynamics in growth and white skeletal muscle composition of the mummichog *Fundulus heteroclitus* during chronic hypoxia and hyperoxia. J Fish Biol 81:148–164

Rice CA, Myers MS, Willis ML, French BL, Casillas E (2000) From sediment bioassay to fish biomarker – connecting the dots using simple trophic relationships. Mar Environ Res 50:527–533

Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO2-induced ocean acidification. Geology 37:1131–1134

Ries JB, Cohen AL, McCorkle DC (2010) A nonlinear calcification response to CO2-induced ocean acidification by the coral *Oculina arbuscula*. Coral Reefs 29:661–674

Rolland R (2000) A review of chemically-induced alterations in thyroid and vitamin A status from field studies of wildlife and fish. J Wildl Dis 36:615–635

Sauer GR (1987) The effect of cadmium and zinc on calcium uptake and scale regeneration in *Fundulus heteroclitus*. In: Vernberg WB, Calabrese A, Thurberg FP, Vernberg FJ (eds) Pollution physiology of estuarine organisms. University of South Carolina Press, Columbia, pp 373–399

Saunders R, Henderson EB, Harmon PR, Johnston CE, Eales JG (1983) Effects of low environmental pH on smolting of Atlantic Salmon (*Salmo salar*). Can J Fish Aquat Sci 40:1203–1211

Schram J, McClintock JB, Angus RA, Lawrence JM (2011) Regenerative capacity and biochemical composition of the sea star *Luidia clathrata* (Say) (Echinodermata: Asteroidea) under conditions of near-future ocean acidification. J Exp Mar Biol Ecol 407:266–274

Schwartz JP (1985) Effect of oil-contaminated prey on the feeding and growth rate of pink salmon fry (*Oncorhynchus gorbuscha*). In: Vernberg FJ, Thurberg FP, Calabrese A, Vernberg WB (eds) Marine pollution and physiology: recent advances. University of South Carolina Press, Columbia, pp 459–476

Scott GI, Vernberg WB (1979) Seasonal effects of chlorine produced oxidants on the growth, survival, and physiology of the American oyster, *Crassostrea virginica* (Gmelin). In: Vernberg WB, Thurberg FP, Calabrese A, Vernberg FJ (eds) Marine pollution: functional responses. Academic, New York, pp 415–435

Shafir S, Van Rijn J, Rinkevich B (2007) Short and long term toxicity of crude oil and oil dispersants to two representative coral species. Environ Sci Technol 41:5571–5574

Sindermann CJ (1979) Pollution-associated diseases and abnormalities of fish and shellfish: a review. Fish Bull 76:717–749

Smith HEK et al (2012) Predominance of heavily calcified coccolithophores at low $CaCO_3$ saturation during winter in the Bay of Biscay. Proc Natl Acad Sci USA 109:8845–8849

Staurnes M, Blix P, Reite OB (1993) Effects of acid water and aluminum on parr–smolt transformation and seawater tolerance in Atlantic Salmon, *Salmo salar*. Can J Fish Aquat Sci 50:1816–1827

Steirhoff KL, Targett T, Miller K (2006) Ecophysiological responses of juvenile summer and winter flounder to hypoxia: experimental and modeling analyses of effects on estuarine nursery quality. Mar Ecol Prog Ser 325:255–266

Stueckle T, Likens J, Foran CM (2008) Limb regeneration and molting processes under chronic methoprene exposure in the mud fiddler crab *Uca pugnax*. Comp Biochem Physiol C 147:366–377

Suggett DJ, Hall-Spencer JM, Rodolfo-Metalpa R, Boatman TG, Payton R, Tye Pettay D, Johnson VR, Warner ME, Lawson T (2012) Sea anemones may thrive in a high CO_2 world. Glob Change Biol 18:3015–3025

Sweet M, Kirkham N, Bendall M, Currey L, Bythell J, Heupel M (2012) Evidence of melanoma in wild marine fish populations. PLoS One 7(8):e41989. doi:10.1371/journal.pone.0041989

Thain JE (1984) Effects of mercury on the prosobranch mollusk *Crepidula fornicata*: acute lethal toxicity and effects on growth and reproduction of chronic exposure. Mar Environ Res 12:285–309

Thetmeyer H, Waller U, Black KD, Inselmann S, Rosenthal H (2001) Growth of European sea bass (*Dicentrarchus labrax* L.) under hypoxic and oscillating oxygen conditions. Aquaculture 174:355–367

Thresher RE, Tilbrook B, Fallon S, Wilson NC, Adkins J (2011) Effects of chronic low carbonate saturation levels on the distribution, growth and skeletal chemistry of deep-sea corals and other seamount megabenthos. Mar Ecol Prog Ser 442:87–99

Touart L, Rao KR (1987) Influence of diflubenzuron on survival, molting, and limb regeneration in the grass shrimp *Palaemonetes pugio*. In: Vernberg WB, Calabrese A, Thurberg FP, Vernberg FJ (eds) Pollution physiology of estuarine organisms. University of South Carolina Press, Columbia, pp 333–349

Vogelbein WK, Fournie JW, van Veld PA, Huggett RJ (1990) Hepatic neoplasms in the mummichog *Fundulus heteroclitus* from a creosote-contaminated site. Cancer Res 50:5978–5986

Waldock MJ, Thain JE (1983) Shell thickening in *Crassostrea gigas*: organotin antifouling or sediment induced. Mar Pollut Bull 14:411–415

Walsh GE, McLaughlin L, Louie MK, Deans CH, Lores EM (1986) Inhibition of arm regeneration by *Ophioderma brevispina* (Echinodermata, Ophiuroidea) by tributyltin oxide and triphenyltin oxide. Ecotoxicol Environ Saf 12:95–100

Wang SY, Stickle WB (1987) Bioenergetics, growth and molting of the blue crab, *Callinectes sapidus*, exposed to the water-soluble fraction of Couth Louisiana crude oil. In: Vernberg WB, Calabrese A, Thurberg FP, Vernberg FJ (eds) Pollution physiology of estuarine organisms. University of South Carolina Press, Columbia, pp 107–126

Watson SA, Peck LS, Tyler PA, Southgate PC, Tan KS, Day RW, Morley SA (2012) Marine invertebrate skeleton size varies with latitude, temperature, and carbonate saturation: implications for global change and ocean acidification. Glob Change Biol 18:3026–3038

Weis JS, Mantel LH (1976) DDT as an accelerator of limb regeneration and molting in fiddler crabs. Estuar Coast Mar Sci 4:461–466

Weis JS, Weis P (1975) Retardation of fin regeneration in *Fundulus* by several insecticides. Trans Am Fish Soc 104:135–137

Weis P, Weis JS (1976) Effects of heavy metals on fin regeneration in the killifish, *Fundulus heteroclitus*. Bull Environ Contam Toxicol 16:197–202

Weis P, Weis JS (1978) Methylmercury inhibition of fin regeneration in fishes and its interaction with salinity and cadmium. Estuar Coast Mar Sci 6:327–334

Weis P, Weis JS (1980) Effect of zinc on fin regeneration in the mummichog, *Fundulus heteroclitus*, and its interaction with methylmercury. Fish Bull 78:163–166

Weis P, Weis JS (1986) Cadmium acclimation and hormesis in Fundulus heteroclitus during fin regeneration. Environ Res 39:356–363

Weis JS, Cohen R, Kwiatkowski J (1987a) Effects of diflubenzuron on limb regeneration and molting in the fiddler crab, *Uca pugilator*. Aquat Toxicol 10:279–290

Weis JS, Gottlieb J, Kwiatkowski J (1987b) Tributyltin retards regeneration and produces deformities of limbs in the fiddler crab *Uca pugilator*. Arch Environ Contam Toxicol 16:321–326

Weis JS, Cristini A, Rao KR (1992) Effects of pollutants on molting and regeneration in Crustacea. Am Zool 32:495–500

Wirgin I, Grunwald C, Courtenay S, Kreamer G-L, Reichert WL, Stein JE (1994) A biomarker approach to assessing xenobiotic exposure in Atlantic tomcod from the North American Atlantic Coast. Environ Health Perspect 102:764–770

Wisshak M, Schönberg CH, Form A, Freiwald A (2012) Ocean acidification accelerates reef bioerosion. PLoS One 7(9):e45124. doi:10.1371/journal.pone.0045124

Wolfe K, Smith AM, Trimby P, Byrne M (2012) Vulnerability of the paper nautilus (*Argonauta nodosa*) shell to a climate change ocean: potential for extinction by dissolution. Biol Bull 223:236–244

Wolowicz M, Smolarz K, Sokołowski A (2005) Neoplasia in estuarine bivalves: effect of feeding behaviour and pollution in the Gulf of Gdansk (Baltic Sea, Poland). In: The comparative roles of suspension-feeders in ecosystems. NATO Sci Ser IV Earth Environ Sci 47:165–182

Woltering D (1984) The growth response in fish chronic and early life stage toxicity tests: a critical review. Aquat Toxicol 5:1–21

Wood HL, Spicer JL, Widdicombe S (2008) Ocean acidification may increase calcification rates, but at a cost. Proc R Soc B 275:1767–1773

Wood HL, Spicer JI, Kendall MA, Lowe DM, Widdicombe S (2011) Ocean warming and acidification; implications for the Arctic brittlestar *Ophiocten sericeum*. Polar Biol 34:1033–1044

Yevich P, Barszcz CA (1977) Neoplasia in soft-shell clams (*Mya arenaria*) collected from oil-impacted sites. Ann N Y Acad Sci 298:409–426

Young JS, Pearce JB (1975) Shell disease in crabs and lobsters from New York Bight. Mar Pollut Bull 6:101–105

Ziskowski J, Mercaldo-Allen R, Pereira JJ, Kuropat C, Goldberg R (2008) The effects of fin rot disease and sampling method on blood chemistry and hematocrit measurements of winter flounder, *Pseudopleuronectes americanus* from New Haven Harbor (1987–1990). Mar Pollut Bull 56:740–750

Zuykov M, Pelletier E, Belzile C, Demers S (2011) Alteration of shell nacre micromorphology in blue mussel *Mytilus edulis* after exposure to free-ionic silver and silver nanoparticles. Chemosphere 84:701–706

Part III
Behavior

Chapter 9
Behavior

Abstract Behavior is a particularly sensitive measure of an organism's response to stresses, including environmental contaminants. Noticeable changes in behavior can be found at low concentrations of chemicals, often lower than concentrations affecting biochemical biomarkers. Since behavior is a link between physiological and ecological processes, it is a particularly important type of response. In addition to being sensitive, behavioral changes are likely to occur in nature and can have ecological effects at the population and community level. While much early research focused on avoidance, tremors, or coughs, complex behaviors such as predator/prey interactions, burrowing, reproductive, and social behaviors are much more relevant to ecological impacts.

Keywords Activity • Aggression • Burrowing • Migration • Neurotransmitter • Predator/prey • Schooling • Sense organs • Thyroid

9.1 General Activity

Reduced activity is very common in response to a variety of stresses. Contaminants may be neurotoxic, or may increase the metabolic burden as an organism attempts to detoxify or excrete the toxicant, depleting its energy reserves. Toxicants may reduce oxygen-carrying capacity of gills, which could also lead to reduced activity, which may be temporary or long lasting. Some toxicants produce hyperactivity. Both decreased and increased activity levels may lead to increased predation risk, either by not escaping quickly enough or being more conspicuous and attracting a predator's attention.

J.S. Weis, *Physiological, Developmental and Behavioral Effects of Marine Pollution*, DOI 10.1007/978-94-007-6949-6_9,

9.1.1 Crustaceans

Many behavioral observations have been done on both larval and adult crustaceans, including amphipods, mysids, copepods, and decapods over many years.

Metals

Studies of deleterious effects of low concentrations of metals on behavior of larval crustaceans go back to the early 1970s, with the Vernberg group leading the way. Fiddler crab (*Uca pugilator*) zoeae exposed to 0.018 mg l^{-1} $HgCl_2$ had reduced activity and erratic spiral swimming, swimming on their sides or darting up from the bottom then settling slowly back down (DeCoursey and Vernberg 1972). Metabolic rate (O_2 consumption) was also depressed. Mud crab, *Eurypanopeus depressus* zoeae were exposed to 10 μg Cd l^{-1} or 1.8 μg Hg^{-1}. Cd increased swimming rates of the later stages, while Hg depressed swimming rates of the early stages (Mirkes et al. 1978). Nauplius larvae of barnacles *Balanus improvisus* increased swimming speeds at 20–80 μg Cu l^{-1}, but after 72 h speed was depressed in all concentrations (Lang et al. 1981). This may be an example of hormesis. Phototactic behavior (swimming toward the light) was also altered at higher concentrations. Similarly, exposure to Cd at 50–100 μg l^{-1} increased swimming speed initially but subsequently depressed it at 100 μg l^{-1}. Sullivan et al. (1983) found that swimming speed of nauplii of the copepod *Eurytemora affinis* was affected by Cu $>$10 μg l^{-1}; they reduced their escape responses, and were more rapidly captured by larval striped bass.

Adult crustaceans have also been the subject of many behavioral investigations. Roast et al. (2001) studied effects of a 7 day exposure to 0.5 and 1.0 μg l^{-1} Cd^{2+} on swimming of the mysid, *Neomysis integer* in a flume. The lower concentration caused fewer mysids to move into the current (normal behavior) at low current speeds. Some animals maintained their position while others were swept away by the current. At the higher concentration more mysids spent more time up in the water column, which could displace them from their optimum habitat in an estuary. Reduced locomotion was seen in grass shrimp exposed to 0.56 but not to 0.3 mg l^{-1} Cd (Hutcheson et al. 1985). Wallace and Estephan (2004) found that while control amphipods (*Gammarus lawrencianus*) were active 61 % of the time, horizontal swimming decreased to only 0.3 % of the time in 125 and 500 μg Cd l^{-1}. Vertical swimming was also impaired, with significant decreases at 12 and 62 μg l^{-1}. Vertical swimming was more sensitive, presumably because of greater energetic costs to swim upwards.

When grass shrimp (*P. pugio*) were in reduced DO (4.6 mg l^{-1}) they reduced their respiration rate; they reduced their activity level at 1.8 mg l^{-1}, which is an adaptive response (Hutcheson et al. 1985). When Cd was present, respiration was reduced even further and locomotion was reduced at 0.56 mg l^{-1} Cd. Sandhopper (*Talitrus saltator*) activity varied in a dose-dependent manner in Cu and Hg (dosed sand with Hg 0.33 and 0.66 mg kg^{-1}, Cu 10 and 20 mg kg^{-1} and Cd 0.5 and 1.0 mg kg^{-1}) (Ugolini et al. 2012). In Cd circadian activity patterns (with controls more active in the dark) disappeared. Pre-exposure to metals induced a decrease of movement. The locomotor activity of sandhoppers maintained for 48 h in contaminated seawater (with different concentrations of Hg, Cu and Cd) and subsequently tested in uncontaminated sand was significantly affected by pre-exposure to trace metals. Animals avoided sand with high concentrations of Hg and Cu.

Organics

Oil

Both hyper- and hypoactivity of amphipods were seen after oil exposure. Kienle and Gehrhardt (2008) investigated short-term effects of the water accommodated fraction (WAF) of weathered crude oil on behavior of the amphipod *Corophium volutator*. Exposure to 25 and 50 % WAF caused hyperactivity, while 100 % WAF led to hypoactivity. In a sediment exposure with 100 % WAF, there was a tendency toward hyperactivity. In a pulse experiment, hyperactivity appeared after 130 min exposure to 50 % WAF. It is possible that non-polar narcosis was involved in these responses.

Pesticides

Organophosphates inhibit acetylcholinesterase (AchE), which normally inactivates the neurotransmitter acetylcholine, and thus may be expected to affect movement. Two organophosphates, methyl parathion at 0.5 μg l^{-1} and phorate at 0.1 μg l^{-1} reduced the swimming stamina of the mysid *Mysidopsis bahia* (Cripe et al. 1981). Fiddler crab *Uca minax* zoea exposed to carbaryl at 1.0, 0.5 and 0.1 mg l^{-1} had a rapid loss of positive phototaxis (normal movement towards light) after 2 h, followed by abnormal swimming, described as twitching of the body with rapid vibrations of the maxillipeds (Capaldo 1987). Larvae then ceased horizontal swimming which preceded death. It appears that in this study the behavioral changes are not true behavioral effects but “death throes.” Since organophosphates and carbamates inhibit AChE, its inhibition and changes in feeding and locomotion were investigated in the amphipod *Gammarus fossarum* during 96 h exposure to chlorpyrifos and methomyl (Xuereb et al. 2009). Feeding and locomotor impairment were generally correlated to degree of AChE inhibition (which was seen at 0.36 nM for chlorpyrifos), suggesting that this underlies behavioral effects. However, in the shrimp *Palaemon serratus*, swimming velocity was significantly reduced by deltamethrin, with a lowest observed effect (LOEC) of 0.6 ng l^{-1}, but AChE activity was increased (Oliveira et al. 2012). On the other hand, lactate dehydrogenase (LDH) activity increased in muscle of exposed prawns, showing that they required additional energy, but were probably using it for detoxification rather than locomotion, since

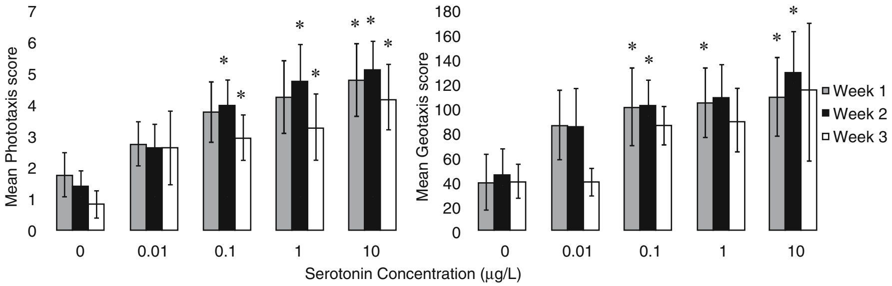


Fig. 9.1 Mean average phototaxis and geotaxis of *E. marinus* exposed to serotonin over 3 weeks. Error bars = 1 SD. * Significantly different from control (Reprinted from Guler and Ford 2010: 400, reprinted courtesy of Elsevier Publishing Co)

swimming speed was reduced. Decreased swimming speed was associated with allocation of energy for detoxification instead of swimming.

Effects of fenitrothion on swimming velocity were determined in prawns (*P. serratus*) exposed to 39–625 ng l^{-1} (Oliviera et al. 2013). A bioassay was also developed to assess prawns' ability to avoid the toxicant. Prawns exhibited a significant inhibition of swimming velocity at concentrations of 313 ng l^{-1} and avoided fenitrothion at 78 ng l^{-1}, but not at 156 ng l^{-1}. Impairment of locomotion and avoidance behavior may lead to alterations at the population level.

Photobehavior of grass shrimp (*P. pugio*) larvae was altered after they had been exposed as embryos to 0.3 μg l^{-1} of dimilin (diflubenzuron) (Wilson et al. 1985). Exposed shrimp had strongly negative responses to high and low light intensity, while controls had positive phototaxis at high light intensity. The depression of positive phototaxis and increase in negative phototaxis was greatest at 0.5 μg l^{-1}. Reversals in phototactic responses were observed when embryos were exposed at different stages of development. Altered phototaxis could alter the maintenance of proper depth in the water column, which is important for larval retention in an estuary. The swimming pattern and velocity of larval grass shrimp were also altered by embryonic dimilin exposure (Wilson et al. 1987). Low concentrations (0.3 μg l^{-1}) increased swimming speed, while higher concentrations decreased it; this may be a hormetic response. Larvae with higher exposure tended to be found lower in the water column than controls.

CECs

Effects of serotonin and the antidepressant fluoxetine at 10 ng l^{-1} to 10 μg l^{-1} were studied on the amphipod *Echinogammarus marinus* (Guler and Ford 2010). Phototaxis and geotaxis (responding to gravity, i.e. moving downward) increased in a concentration-dependent manner with serotonin (Fig. 9.1), while fluoxetine had its greatest effect at 100 ng l^{-1}, which caused animals to spend more time higher in the water column in the light, where they might be more susceptible to predation.

9.1.2 Mollusks

Since benthic bivalve mollusks tend to have limited mobility, most studies have been performed on larvae or on valve closing speeds of scallops. It is disappointing that there are so few studies on cephalopods such as squids, which are active swimmers or octopuses which are a frequent subject of study in behavior labs.

Metals

Valve closing speed of juvenile Catarina scallop (*Argopecten ventricosus*) exposed to Cd (0.02, 0.1, 0.2 mg l^{-1}), Cr (0.1, 0.5, 1.0 mg l^{-1}) or Pb (0.2, 0.4 mg l^{-1}) and mixtures was studied by Sobrino-Figueroa and Cáceres-Martínez (2009). The average closing speed was under 1 s in controls, but 2–3.6 s in Cd, 1.4–3.4 s in Cr, 3–12 s in Pb, and 12–15 s in the mixtures. Thus all metals retarded closing. In combination, effects of the metals appeared to be additive.

A dosage-sensitive relationship was seen for the loss and subsequent recovery of locomotor response and chromatophore expansion in octopuses (*Octopus joubini*, *O. maya and O. bimaculoides*) after exposure to mercuric chloride (Adams et al. 1988). For each species the 3-hour LC_{50} was 1,000 mg l^{-1}.

Organics

All species of octopus tested (*Octopus joubini*, *O. maya and O. bimaculoides*) showed a dosage-sensitive relationship for the loss and subsequent recovery of locomotor response and of chromatophore expansion after exposure to ethylene dibromide (EDB). For each species the 12-hour LC_{50} was 100 mg l^{-1} (Adams et al. 1988).

Contaminants of Emerging Concern

Fong and Molnar (2013) investigated effects of four antidepressants {fluoxetine ("Prozac"), fluvoxamine ("Luvox"), venlafaxine ("Effexor"), and citalopram ("Celexa")} on adhesion to the substrate in five marine snails (*Chlorostoma funebralis*, *Nucella ostrina*, *Urosalpinx cinerea*, *Tegula fasciatus*, and *Lithopoma americanum*) representing three different gastropod families. All antidepressants induced foot detachment in all snails in a dose-dependent manner. The lowest observed effect concentrations were seen in *Lithopoma* in 43.4 μg l^{-1} fluvoxamine and *Chlorostoma* in 157 μg l^{-1} venlafaxine. Latency to detachment was also dose dependent, with the fastest times to detach in *Chlorostoma* and *Lithopoma* (7.33 and 13.16 min in 3.13 mg l^{-1} venlafaxine).

Hypoxia

While normal levels of dissolved oxygen are ~8 mg l^{-1}, organisms can resist somewhat lower levels. A general response to hypoxia is reduced respiration and activity. Liu et al. (2011) found that larval respiration rates of the gastropods *Nassarius siquijorensis* and *N. conoidalis* were reduced at 4.5 mg O_2 l^{-1} and swimming speed was reduced in 10-day old larvae exposed to <2.0 mg O_2 l^{-1} for *N. siquijorensis* and <1.0 mg O_2 l^{-1} for *N. conoidalis*, suggesting that the latter species is more tolerant of low oxygen conditions.

Polluted Environment

A scallop "coughs" to expel feces and water from its central cavity; the friction between the two valves makes a sharp crack as the valves quickly close. By using submersible acoustic sensors (hydrophones), DiIorio et al. (2012) could record the scallops' (*Pecten maximus*) outbursts from up to 10 m away. They thought that the sounds can serve as an early warning system for water quality.

9.1.3 Fishes

Swimming activity of fishes is impaired by exposure to many contaminants. Swimming behavior is often used as an indicator of sublethal toxicity in fish (Little and Finger 1990). Studies have been done measuring both swimming speed, percentage of time swimming, and swimming stamina.

Metals

MeHg (10 μg l^{-1}) decreased activity and swimming performance in mummichogs (Weis and Khan 1990; Zhou and Weis 1998), gobies *Pomatoschistus microps* (Viera et al. 2009), and Atlantic croaker. Alvarez et al. (2006) fed adult croakers (*M. undulatus*) meHg-contaminated food for 1 month, induced spawning, and analyzed swimming speed and startle response of larvae. Maternally-transferred meHg impaired these behaviors, which are considered survival skills (Figs. 9.2 and 9.3).

Cu can either increase or decrease activity, depending on the species and concentration. Swimming velocity of silversides (*Menidia menidia*) increased after a short exposure to 100 μg l^{-1} (Koltes 1985), but 50 μg l^{-1} reduced swimming in the goby, *Pomatoschistus microps* (Viera et al. 2009). Scarfe et al. (1982) found that 72 h exposure to 0.1 mg ml^{-1} Cu decreased activity in Atlantic croaker (*M. undulatus*) and pinfish (*Lagodon rhomboids*) and increased activity of sheepshead (*Archosargus probatocephalus*) and sea catfish (*Ariopsis felus*).

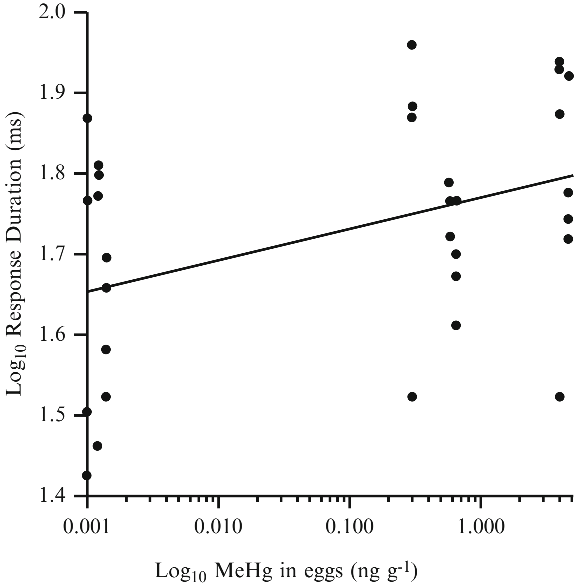


Fig. 9.2 Effect of maternally derived meHg on response duration to a vibratory startle stimulus in Atlantic croaker larvae. Behaviors were log transformed to attain normality. Data points are mean value for each spawn and developmental age (Reprinted from Alvarez et al. 2006: 334, courtesy Elsevier Publishing Co)

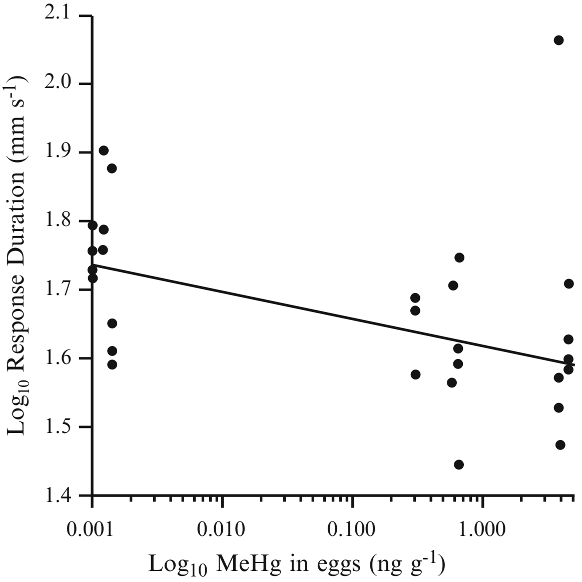


Fig. 9.3 Effect of maternally derived meHg on response speed to a vibratory startle stimulus in Atlantic croaker larvae. Behaviors were log transformed to attain normality. Data points are mean value for each spawn and developmental age (Reprinted from Alvarez et al. 2006: 334, courtesy Elsevier Publishing Co)

Newly hatched *F. heteroclitus* larvae were exposed to 0.1, 0.3, or 1.0 mg l^{-1} Pb. A reduction in spontaneous activity and swimming stamina was seen after 1 week, but when larvae were returned to clean sea water for 4 weeks, behaviors were no longer statistically different from controls, showing that effects were reversible (Weis and Weis 1998).

Organics

Oil

Juvenile coho salmon (*Oncorhynchus kisutch*) exposed for 48 h to the water soluble fraction (WSF, at 75 % of the LC50 value) of Cook Inlet crude oil showed reduced swimming activity, which was dependent on the concentration and time of exposure (Thomas et al. 1987). When fish were transferred to clean water, control activity levels were restored in 8 h. Authors suggested that oil increases metabolism, which reduces the energy available for swimming. PAHs (8 μg l^{-1} BaP), decreased locomotion in seabass, *Dicentrarchus labrax* (Gravato and Guilhermino 2009). Although they occur as mixtures, little information exists about the joint action of PAHs. In 4-day tests with juvenile gilthead seabream (*Sparus aurata*) Goncalves et al. (2008) performed concentration-response analyses for fluorene (FE), phenanthrene (PHE), and pyrene (PY). The single compounds and the mixture produced dose-related changes in activity. For lethargy and swimming, PY was the most potent (0.031 μM for swimming, 0.039 μM for lethargy) and FE the least (0.29 μM for swimming, 0.26 μM for lethargy). Effects were additive (). Changes in the number of lethargic fish were the most sensitive parameter.

To examine toxicological effects of heavy oil (HO), Kawaguchi et al. (2012) performed embryonic exposure experiments and morphological and behavioral analyses in pufferfish (*Takifugu rubripes*) larvae. Fertilized eggs were exposed to 50 mg l^{-1} of HO for 8 days then transferred to clean seawater before hatching. The hatched larvae were observed for their swimming behavior and morphology. Exposed larvae appeared normal but exhibited an abnormal swimming pattern and disorganized midbrain, which controls movement. These results suggest that HO-exposed fishes develop abnormal brain structure, which alters normal swimming behavior.

Chlorinated Organic Chemicals

Fingerman and Russell (1980) examined activity and neurotransmitters in Gulf killifish *Fundulus grandis* after exposure to PCBs (Aroclor mixture 1,242 at 0.0004 %). After a 24-h exposure, significant increases in locomotor activity were seen and norepinephrine (NE) and dopamine (DA) were lower than controls. Average NE in controls was 2.5 μg g^{-1} which decreased to 0.6 in treated fish. Average DA in controls was 0.9 μg g^{-1}, which decreased to 0.4 in treated fish. Similar increases in activity were seen in *F. heteroclitus* exposed to PCBs as embryos (Couillard et al. 2011).

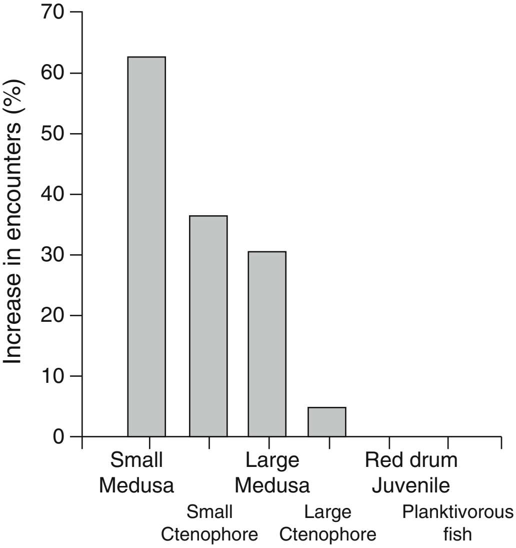


Fig. 9.4 Increase in encounter rates relative to controls between red drum larvae and their predators as a result of 4 days atrazine exposure (Reprinted from Alvarez and Fuiman 2005: 237, courtesy Elsevier Publishing Co)

Pesticides

Among the pesticides reducing swimming activity are fenitrothion and carbofuran in juvenile European seabass (*Dicentrarchus labrax*) (Almeida et al. 2010). After 96 h exposure to >31 μg l^{-1} carbofuran, decreased swimming velocity was seen (Hernández-Moreno et al. 2011) suggesting that the pesticide interfered with neuronal function. Correlations between swimming velocity and cholinesterase activity suggest that this enzyme may be related to the behavioral changes. Parental exposure to DDT caused reduced activity in Atlantic croaker fry (Faulk et al. 1999). Chlorpyrifos at 0.05 μg l^{-1} reduced swimming activity in coho salmon (*O. kisutch*) (Sandahl et al. 2005). However, some pesticides increase activity. Atrazine at 40, and 80 μg l^{-1} for 4 days increased velocity and the percentage of the time red drum (*Sciaenops ocellatus*) larvae were active (Alvarez and Fuiman 2005), thus increasing potential encounter rates with predators (Fig. 9.4); carbaryl (24 h in 100 μg l^{-1}) increased swimming activity of Atlantic silverside (*Menidia menidia*) (Weis and Weis 1974).

Emerging Contaminants

Newborn pipefish altered their distribution when exposed to exogenous estrogens (Sárria et al. 2011b). Environmentally relevant concentrations of EE_2 (nominal concentrations of 8, 12 and 36 ng l^{-1}) caused newborn *Syngnathus abaster* to shift their vertical distribution towards the surface in a dose-dependent manner. Bell (2004) exposed three-spined stickleback (*Gasterosteus aculeatus*) to environmentally relevant levels of EE. Exposure to 100 ng l^{-1} ethinyl estradiol increased activity and foraging under predation risk, which increases susceptibility to predation. Exposed fish had increased mortality later in life.

Hypoxia

A common behavioral response to hypoxia is avoidance. Craig (2012) investigated behavior of fishes in the Gulf of Mexico related to the large hypoxic region. On average, DO avoidance thresholds were low (1–3 mg l^{-1}) suggesting fish avoid only the lowest, lethal DO levels. They aggregated fairly close to the margins of the hypoxic zone, indicating that effects of hypoxia are probably most intense within a relatively narrow region. Avoidance thresholds were similar in brown shrimp and several finfish species.

Polluted Environments

Killifish, *F. heteroclitus*, from a contaminated estuary (industrialized Piles Creek in New Jersey, U.S.) had lower activity rates than fish from reference sites (Smith and Weis 1997). Young-of-the-year bluefish *Pomatomus saltatrix*, that were fed contaminated food (killifish and menhaden with high levels of Hg and PCBs collected from contaminated Hackensack Meadowlands) for a few months swam more slowly than fish fed the same prey collected from a cleaner estuary (Candelmo et al. 2010).

9.1.4 Other Taxa

Motility of coral planula larvae is sensitive to metals (Reichelt-Brushett and Harrison 2004). Larval motility was significantly affected by Cu and Pb immediately after dosing at low levels; the EC_{50} values for motility of *Goniastrea aspera* larvae (e.g. for Cu 12 h, 21 μg l^{-1}; 24 h, 16 μg l^{-1}; 48 h, 22 μg l^{-1}) were much lower than the LC_{50} values.

9.2 Burrowing Behavior

Reduced burrowing in contaminated sediments may be an avoidance response, which is protective, or can reflect behavioral toxicology. Not all studies distinguish between these different mechanisms. To do so, animals should be provided with clean as well as contaminated sediments to burrow in. In either case, an animal on the surface is more vulnerable to predation than one burrowed under the surface of the substrate.

9.2.1 Crustaceans

Metals

Burrowing by the isopod, *Saduria entomon*, decreased in metal-contaminated sediments (10 $\mu g\ g^{-1}$ dry sediment Cu, 35 $\mu g\ g^{-1}$ Cd, or 299 $\mu g\ g^{-1}$ Fe), but this was an example of avoidance, as animals burrowed normally in clean sediments. Pre-exposure at levels similar to those in contaminated estuaries of the Baltic Sea, impaired the ability of the animals to avoid contaminated sediments and reduced feeding (Pynnönen 1996). The amphipod, *Rhepoxinius* avoided burrowing in sediments with levels of sewage, Zn, or Cd typical of waste-water discharge sites (Oakden et al. 1984).

Organics

After a moderate oil spill at West Falmouth, Massachusetts in 1969, fiddler crabs, *Uca pugnax* dug abnormal burrows which did not go deep enough for them to avoid the freezing layer in the winter, resulting in excessive overwinter mortality. Effects were still seen after 7 years (Krebs and Burns 1977). Scientists revisited this marsh almost 40 years later and found that a substantial amount of moderately degraded oil remained 8–20 cm below the marsh surface (Culbertson et al. 2007) and fiddler crabs that burrow at depths of 5–25 cm were still chronically exposed to the oil. These crabs dug shallower burrows to avoid oiled layers, (Fig. 9.5) and also showed delayed escape responses and lowered feeding rates. The oil residues were therefore still affecting *U. pugnax*.

Burrowing of *Uca pugilator* was reduced by exposure to the insecticide diflubenzuron (Dimilin) and to tributyltin, a constituent in antifouling paints (Weis and Perlmutter 1987a, b). Although crabs did not avoid substrate contaminated with DFB or TBT, crabs that had been exposed to >0.5 $\mu g\ l^{-1}$ DFB or TBT for 1 week dug fewer burrows in clean sediment than control crabs.

Hypoxia

Behavioral reactions of *Crangon crangon* were studied in shrimp exposed to various degrees of hypoxia at different temperatures. At 20 °C, the normally buried *C. crangon* emerge from the sand at 40–50 % sat; at 9 °C this emersion response occurs at 20 % sat (Hagerman and Szaniawska 1986). Thus, they withstand lower DO at lower temperature.

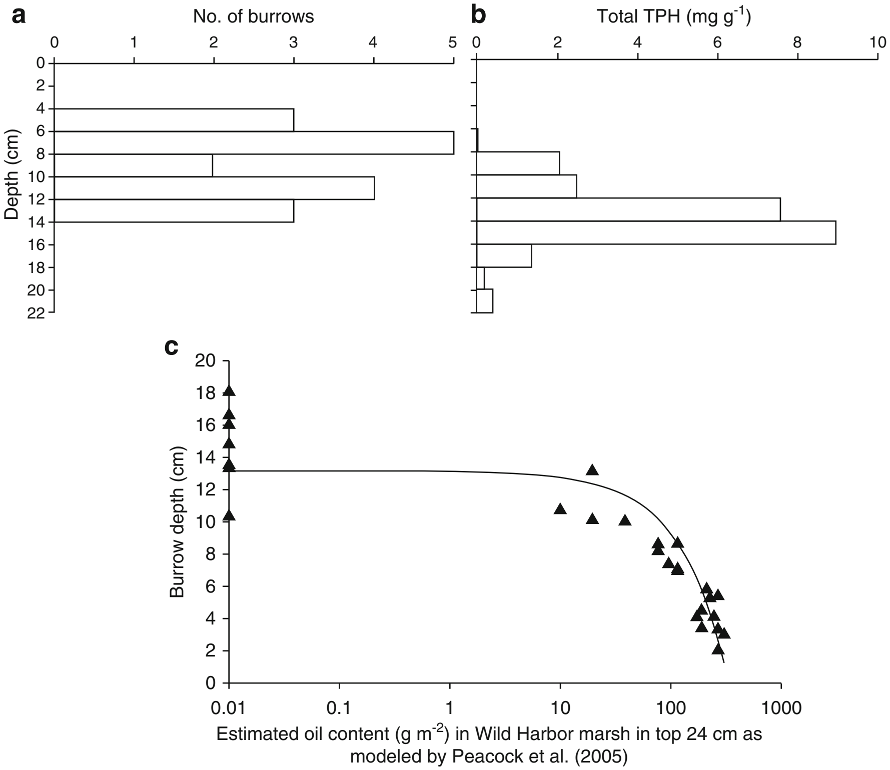


Fig. 9.5 (**a**) number of *U. pugnax* burrows at different depths, (**b**) concentration of total PAH at different depths in sediments from oiled area, (**c**) burrow depth compared to estimated PAH content (g oil m^{-2}) using modeled data (Reprinted from Culbertson et al. 2007: 957, courtesy Elsevier Publishing Co)

9.2.2 Mollusks

Metals

Burrowing (or burial) by the bivalve *Scrobicularia plana* in clean sediments was reduced by 4 days exposure to 25–150 μg Cu l^{-1} (Bonnard et al. 2009). At the end of exposure, the burrowing kinetics in clean sediment were determined after 1 and 2 days. Even at the lowest tested concentrations, Cu reduced burying. Other reports document reduced burying in 5.8 μg g^{-1} Cu-contaminated sediments (avoidance) by the clams *Protothaca staminea* (Phelps et al. 1985) and *Macomona liliana* (Roper and Hickey 1994), as well as the gastropod *Polinices sordidus* (Hughes et al. 1987). Cd also reduces burying in short-neck clam *Ruditapes philippinarum.* Clams took the longest time to burrow in sediments from sites with the highest concentrations of Cd, Cr, Cu, Ni and Zn (Shin et al. 2002) There was reduced burrowing when sediment Cd concentrations were 1 mg kg^{-1} (dw) or greater. *Macomona liliana* juveniles were exposed to Cu- and Zn-dosed sediments and sediments collected from contaminated sites. The number burying by 10 min was reduced at 25 mg Cu kg^{-1} (dw). After a 96-h exposure, clams crawled away from sediment with 10 mg Cu kg^{-1} (dw), and when a weak current was provided they left this sediment by drifting (Roper et al. 1995). Zn-dosed sediment slowed burial at 80 mg Zn kg^{-1} (dw), and stimulated crawling and drifting at 40 mg Zn kg^{-1} (dry wt). Some field-collected contaminated sediments slowed burial and stimulated drifting, which could affect the distribution of these clams in natural habitats.

Contaminants of Emerging Concern

Buffet et al. (2012) investigated effects of Zn nanoparticles in the clam *Scrobicularia plana*. They chose 3 mg kg^{-1} sediment ZnO NPs since this was considered a realistic prediction of the environmental concentration in sediments.^{67}ZnO NPs were synthesized in diethylene glycol (DEG). ^{67}Zn accumulation was seen. As with the clams (above), burrowing behavior and feeding rate were impaired in clams exposed to ^{67}ZnO NPs but also in control clams exposed to DEG alone. Therefore there is no strong evidence for a severe nanoparticle effect since effects were observed in response to DEG alone.

Polluted Environment

Tanouka et al. (2013) collected *S. plana* from a moderately polluted estuary (Loire) and reference sites, and found increased GST activity (which defends against organic compounds) and impaired burrowing behavior, condition index, and gonado-somatic index. This integrated biomarker approach indicated higher levels of stress in clams from the Loire estuary compared to those from the reference site.

9.2.3 Other Taxa

Metals

Polychaetes reduce burrowing in contaminated sediments and reduce burrowing in clean sediments after contaminant exposure. Exposure to 25 μg Cu l^{-1} reduced burrowing in *Nereis diversicolor* (Bonnard et al. 2009). Behavioral impairments were not related to AChE inhibition, but may have been due to metabolic or physiological disturbances.

Organics

Exposure of the polychaete *Glycera dibranchiata* to oil-contaminated sediments at 120, 1,067, and 2,879 μg g^{-1} (Studholme et al. 1987) impaired burrowing in clean sediments. At the two higher concentrations, worms emerged from their burrows. They recovered after being placed in clean sediment, suggesting that effects may have been due to a narcotic effect of aromatic fractions. Reduced burrowing may be responsible for increased predation due to greater accessibility of benthic infauna to predators.

Contaminants of Emerging Concern

Buffet et al. (2012) investigated of Zn nanoparticles in the polychaete *Nereis diversicolor*. They used 3 mg kg^{-1} sediment ZnO NPs since this was considered a realistic prediction of the environmental concentration in sediments.^{67}ZnO NPs were synthesized in diethylene glycol (DEG). ^{67}Zn accumulation was seen. As with the clams (above), burrowing behavior and feeding rate were impaired in worms exposed to ^{67}ZnO NPs but also in worms exposed to DEG alone. Therefore there was no evidence for a nanoparticle effect since effects were also observed with DEG alone.

Polluted Environment

Nereis diversicolor originating from a polluted estuary (Loire estuary) and a relatively clean site (Bay of Bourgneuf) were compared (Kalman et al. 2009). At the individual level no significant differences in burrowing speed were observed when worms were exposed to their sediment of origin. No link between AChE activity and burrowing was found in worms from either site. Cross-tests revealed that differences in locomotion were not due to neurological dysfunction but to avoidance of contaminated sediments.

Polychaetes, *Perinereis gualpensis* were moved from a reference site (Raqui estuary, Chile) to a location with high sediment Hg (Lenga estuary: 1.78–9.89 mg kg^{-1}). Individuals were placed in polluted and non-polluted sediments for 21 days and sampled every 7 days with cages deployed at three different depths (Díaz-Jaramillo et al. 2013). Tissue Hg was measured in conjunction with stress responses. Translocated worms accumulated Hg rapidly. Glutathione S-transferase (GST) activities measured from posterior body regions were twofold higher than controls after 21 days of exposure. Differences in burrowing behavior were observed; while controls had more homogenous vertical distributions, in Lenga, worms tended to remain in upper layers. Authors thought these studies demonstrate that under natural conditions, Hg is bioavailable to polychaetes affecting both biochemical and behavioral responses after relatively short-term exposure. However, they did not discuss other potential causes such as other sediment contaminants.

9.3 Feeding and Predator Avoidance

To survive, an individual must avoid being eaten and must find and capture food. Feeding is an ecologically meaningful response because its impairment has direct effects on individuals, populations, communities and ecosystems. Complex behaviors of prey capture and predator avoidance include locomotion/activity, sensory detection of food or predators, and rapid prey capture or escape reflexes. Visual or chemical cues provide information about the presence of predators or prey. Predator avoidance ability is often impaired in response to contaminants, resulting in reduced survival in the presence of a predator. However, the ability of the predator to capture the prey may also be impaired in contaminated environments. Contaminant-induced effects on predator/prey relationships can cause trophic cascades (top-down effects) and alter community composition (Fleeger et al. 2003). For example, the pesticide methyl carbamate reduced grazing by arthropods, causing increased abundance of macroalgae (Carpenter 1986; Duffy and Hay 2000).

9.3.1 Crustaceans

Metals

Wallace et al. (2000) investigated prey capture in grass shrimp (*P. pugio*) fed Cd-contaminated prey (field-exposed oligochaetes or laboratory-exposed *Artemia salina*). Shrimp fed contaminated *A. salina* (with Cd body burdens of 4, 16, and 40 mg g^{-1} wet weight, respectively, which would result in shrimp ingesting approximately 0.08, 0.32, or 0.80 mg Cd day^{-1}, if they consumed all of the food) had impaired ability to capture prey; after feeding on oligochaetes effects were not as severe. Cd-exposed shrimp produced a low molecular weight Cd-binding metallothionein protein; their prey capture decreased with increased Cd bound to high molecular weight proteins, i.e., enzymes. Inorganic Hg and meHg (0.01 mg l^{-1}) affected predator avoidance of *P. pugio* (Kraus and Kraus 1986). While exposure made shrimp from a reference site more vulnerable to *F. heteroclitus* predation, shrimp from a contaminated site (Piles Creek in New Jersey US) were unaffected by $HgCl_2$ and were less affected by meHg than the reference population, suggesting tolerance, which will be discussed at length in Chap. 11. Brief exposure of the copepods *Notodiaptomus conifer* and *Argyrodiaptomus falcifer* to Cu and Cr (15 min) affected their escape behavior in an unexpected fashion. Exposed copepods had higher escape ability than controls (Gutierrez et al. 2012). This may have been hormesis, which might have turned into reduced escape ability after longer exposure times.

Organics

Cellular (cell viability and immune function), physiological (cardiac activity) and behavioral (foraging) responses were evaluated in field-collected shore crabs *Carcinus maenas* from a PAH-contaminated site (Plym) and two cleaner sites and compared with responses of crabs exposed in the laboratory to the PAH pyrene (200 mg l^{-1} for 28 days). No significant cellular or physiological impacts were seen in Plym crabs, but foraging was significantly altered. When given a cockle, Plym and the laboratory-exposed crabs took longer to handle and break into the shells. Therefore, behavioral indicators were more sensitive than the cellular and physiological responses (Dissanayake et al. 2010).

Hypoxia

Feeding and predation by the mud crab *Neopanope sayi*, and juvenile blue crab *C. sapidus*, decreased during hypoxia (1.0 and 0.5 mg O_2 l^{-1}), suggesting that short hypoxic episodes may create predation refuges for prey species (Sagasti et al. 2001). (However, as seen above, prey species often become more susceptible to predation.) Bell et al. (2003) used biotelemetry with measurements of DO to monitor feeding and movement of free-ranging *C. sapidus* in the Neuse River Estuary, NC, USA during hypoxic upwelling and subsequent relaxation events. The percent feeding declined in mild (2–4 mg l^{-1}) and severe (<2 mg l^{-1}) hypoxia. Crabs reduced the proportion of time spent feeding during hypoxia, but during relaxation events, feeding time did not increase and crabs did not reinvade deep water, as was hypothesized.

Predator–prey dynamics between the blue crab *Callinectes sapidus* and an infaunal clam prey *Mya arenaria* were examined by Taylor and Eggleston (2000) to assess the impact of hypoxia on foraging rates and prey mortality. The relationship between predator consumption rates and prey density were studied in normoxia, moderate hypoxia (3.0–4.0 mg l^{-1}) after acclimation to high DO, and moderate hypoxia after acclimation to low DO. *M. arenaria* burial depth decreased and siphon extension increased in severe hypoxia. Initiation of moderate hypoxia after normoxia altered blue crab foraging. Low DO affected the interaction between *C. sapidus* and *M. arenaria* by either hindering blue crab foraging, or alternatively, increasing clam vulnerability by altering their siphon extension and depth distribution within the sediment. Predator preference for certain prey can be modified by exposure to low DO (Munari and Mistri 2012). *Carcinus aestuarii* normally prefers *Musculista senhousia* as prey, but after hypoxia, their preference was influenced by the presence of another prey, *Tapes philippinarum*.

Climate Change/Acidification

Reduced pH affected chemosensation related to feeding in hermit crabs *Pagurus bernhardi* (De la Haye et al. 2012). Crabs in 6.8 pH water with a food odor present had less antennular flicking (the “sniffing” response), were less successful in locating the odor source, and had reduced activity compared to those at normal pH. Briffa et al. (2012) reviewed studies of elevated CO_2 and the behavior of tropical reef fishes and hermit crabs. Three main routes through which behavior might be altered were elevated metabolic load, “info-disruption” (transfer of chemical information between organisms) and avoidance of polluted sites. They stated there is clear evidence that exposure to high CO_2 disrupts the ability to find settlement sites and shelters and the ability to detect predators and food. Behavioral changes appear to occur primarily via info-disruption.

Polluted Environment

Impaired prey capture may have important implications for energy flow in impacted environments. Perez and Wallace (2004) compared prey capture (of brine shrimp, *Artemia franciscana*) by grass shrimp (*P. pugio*) from a relatively clean site, Great Kills (GK) and two polluted creeks by the Arthur Kill (NY, US). GK shrimp captured prey twice as fast than shrimp from a polluted creek near landfills. Prey capture by shrimp from a creek with historic smelting activities was intermediate. When shrimp from a reference site were exposed to sediment and water from the landfill site for 8 weeks, prey capture was reduced. Video analysis showed that prey capture was impaired because of reduced use of a lunge attack, and greater use of a less efficient grab attack. Foraging (number of total scoops and scoops on a protein-rich patch) by fiddler crabs *U. pugnax* from a polluted and a reference site was compared in the laboratory by Khoury et al. (2009). Reference site crabs had twice the number of total scoops and three times the number of scoops on the patch than crabs from the polluted site. Reichmuth et al. (2009) studied blue crabs (*C. sapidus*) from polluted Hackensack Meadowlands (HM) and a reference site, Tuckerton (TK) both in New Jersey (U.S.). HM crabs were slower to capture active prey (killifish and juvenile blue crabs) though they consumed mussels and fiddler crabs at an equivalent rate. Their stomach contents in the field contained much sediment and detritus and far less live food than TK crabs supporting the laboratory observations. Transplanting HM crabs to TK improved their prey capture; transplanting TK crabs to HM impaired their prey capture, demonstrating environmental cause for the behavior (Fig. 9.6). Surprisingly, HM juveniles had better predator avoidance ability and were more aggressive than TK crabs. However, aggressiveness did not appear to be the reason for their superior predator avoidance (Reichmuth et al. 2011).

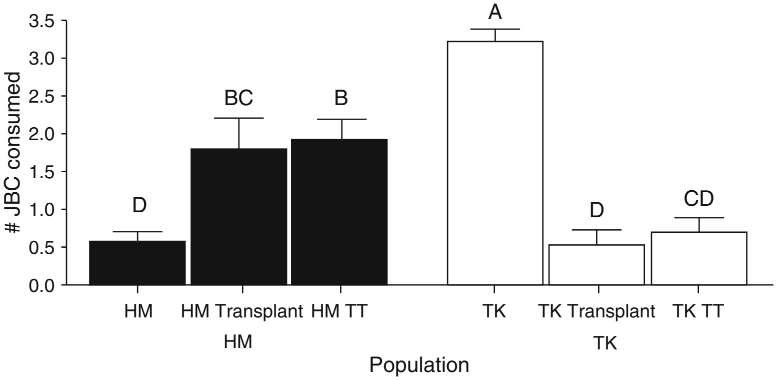


Fig. 9.6 Number of juvenile blue crab prey (*JBC*) consumed in 24 h by native adult blue crabs, transplanted crabs, lab trophic transfer [*TT*] crabs from Hackensack and Tuckerson (reference site). *Black columns* Hackensack [*HM*] crabs, *white columns* Tuckerton [*TK*] crabs. Error bars = 1 SE, letters over bars denote significantly different groups (Reprinted from Reichmuth et al. 2009: 304, courtesy Springer Publishing Co)

9.3.2 Mollusks

Effects of metals and organic chemicals on feeding are discussed in Chap. 2, Feeding and Digestion.

Hypoxia

Hypoxia can cause bivalve infauna to move up to the surface and become more susceptible to predation. Long and Seitz (2008) performed experiments varying predator access to marked *Macoma balthica* clams at deep and shallow sites in the York River, Virginia, before and during hypoxic episodes. During hypoxia, predation rates at hypoxic sites were more than double those in normoxic sites. Ambient clam densities were lower at the deep sites, and lower in August than in June. They concluded that hypoxia increased the susceptibility of benthic prey to predation. Chronic hypoxia impaired anti-predator responses in the green lipped mussel *Perna viridis* (Wang et al. 2012) by reducing the normal anti-predator responses of shell thickening, byssus thread production, and adductor muscle growth. Hypoxia affected predation by the non-native rapa whelk (*Rapana venosa*) on bivalve prey: the non-native ark shell (*Scapharca inaequivalvis*) and Manila clam (*Tapes philippinarum*), and the native cockle *Cerastoderma glaucum* (Munari and Mistri 2011). Under normoxia, *R. venosa* preferred *S. inaequivalvis*. Short-term hypoxia increased the vulnerability of *T. philippinarum*, and whelks switched their preference to this species. Altered prey preferences can affect community structure.

Climate Change/Acidification

Amaral et al. (2012) investigated the susceptibility of oysters from acidified areas (receiving runoff from acid sulphate soils) and reference areas to predation by the gastropod *Morula marginalba*. Oyster shells were weaker at acidified sites, therefore they were more vulnerable because *M. marginalba* could drill through them faster. Many other predators consume prey at rates inversely proportional to their shell strength. While not a behavioral response, this effect of acidification alters predator/prey relationships.

9.3.3 Fishes

Detection of predators and of prey is often olfactory; some fish species produce an alarm substance that warns conspecifics of danger. Impaired detection of an alarm substance would put fish at risk of predation. Visual signals are also important in detecting prey and predators. Behavioral development occurs in association with the development of the nervous system and developing fishes are generally more sensitive to contaminants than adults. Embryonic exposures to chemicals can affect subsequent predator/prey and other behavior later in life. Early life stages may be exposed to contaminants passed on from females via egg reserves as well as directly from water and food. Understanding the physiological mechanisms that underlie effects on behavior early in life has not received much attention, possibly because physiological measurements are difficult to perform on small specimens. The physiology underlying behavioral disruption in early life stages is similar to that in juveniles and adults: sensory impairment, altered neurogenesis and altered neurotransmitters (Sloman and McNeil 2012).

Metals

Copper and some other metals can suppress the olfactory response to amino acids that are detected as food odors by coho salmon (Sandahl et al. 2004). Copper also reduces the response of salmon to water-borne alarm substances, thus making them more vulnerable to predation (McIntyre et al. 2012). Injured fish release a chemical that alerts other fish. Juvenile coho salmon normally freeze in the presence of alarm substances, making it harder for predators to detect them. However, salmon in water with 5 $\mu g\ l^{-1}$ Cu failed to detect the substance and kept swimming; they were readily attacked by the predator (McIntyre et al. 2012).

Weis and Khan (1990) found that exposure of adult mummichogs (*F. heteroclitus*) to 10 $\mu g\ l^{-1}$ of either $HgCl_2$ or meHg for 1 week reduced prey capture. Sticklebacks (*Gasterosteus aculeatus*) exposed to 3 $\mu g\ l^{-1}$ TBT chose more exposed regions of the water column and had longer latency times before performing antipredator behavior in response to a simulated predator (fake heron bill) (Wibe et al. 2001), increasing their predation risk. TBT is also able to affect predatory behavior. Yu et al. (2013) examined effects of 10, 100 and 1,000 ng l^{-1} on prey capture of *Sebastiscus marmoratus*. TBT exposure depressed predatory activity after 50 days. Along with behavior changes, dopamine levels in the fish brains increased in a dose-dependent manner and 5-hydroxytryptamine and norepinephrine levels decreased in the TBT exposed group compared to the control.

Embryonic exposures of *F. heteroclitus* to 5 and 10 $\mu g\ l^{-1}$ meHg affected prey capture and predator avoidance of larvae maintained in clean water. Prey capture of early larvae was impaired, but after about 1 week after hatching prey capture was equal to controls, showing that this was a temporary effect (Weis and Weis 1995a). Exposure may have caused retardation of neurological development that was later compensated for. Larvae that had been exposed as embryos were more susceptible to predation by *P. pugio* or by adult mummichogs (Weis and Weis 1995b) and had increased activity levels, making them more susceptible (Zhou and Weis 1998). After both embryonic and larval exposure, deleterious effects were greater than embryonic exposure alone (Zhou et al. 2001).

Newly hatched *F. heteroclitus* larvae were exposed to 0, 0.1, 0.3, or 1.0 mg l^{-1} Pb and tested for prey capture (*Artemia*), and predator avoidance. Prey capture was significantly decreased after 4 weeks and susceptibility to predation by grass shrimp increased. However, when larvae were returned to clean sea water for another 4 weeks these behaviors were no longer statistically different from controls (Weis and Weis 1998).

Organics – Pesticides and PCBs

Pesticides can affect olfaction in fishes, disrupting normal predator and prey detection. Diazinon at $>1.0\ \mu g\ l^{-1}$ inhibited olfactory-mediated alarm responses in chinook salmon (*Oncorhynchus tshawytscha*) (Scholz et al. 2000). Homing behavior was impaired at 10 $\mu g\ l^{-1}$, suggesting that olfactory-mediated behaviors are sensitive, and that short-term exposure to insecticides that impair cholinesterase may cause significant behavioral deficits. After 2 h exposure to 1 and 10 $\mu g\ l^{-1}$ diazinon, chinook salmon had a reduced fright response to skin extract: instead of decreasing swimming and foraging they stayed highly active, which would increase susceptibility to predation. The fungicide iodocarb and herbicides glyphosate and atrazine suppressed the olfactory response of coho salmon, *Oncorhynchus kisutch*, to L-serene after short-term (30 min) exposure to relatively low concentrations ($\sim 1\ \mu g\ l^{-1}$) (Tierney et al. 2006).

Embryonic exposure (topical treatment) of mummichog (*F. heteroclitus*) embryos to PCB 126 (≥ 10.0 pg egg^{-1}) reduced larval prey capture of *Artemia* by 30 %, while not reducing the number of strikes, suggesting impaired coordination rather than appetite (Couillard et al. 2011). McCarthy et al. (2003) fed adult croakers (*Micropogonias undulatus*) PCBs (0.4 mg Aroclor 1,254 kg^{-1} fish day^{-1}) let them spawn, and studied larval behavior. The percentage of control larvae responding to a frightening stimulus and their burst speeds increased with age, but no such increase was seen in exposed larvae, suggesting that the transferred PCBs impair their startle response, making them more susceptible to predation.

Contaminants of Emerging Concern

The flame retardant PBDE −71 (0.001–100 $\mu g\ l^{-1}$) reduced feeding efficiency in mummichog larvae (Timme-Laragy et al. 2006). Exposed fish also performed poorly in a maze, suggesting impaired cognition. Prey capture was inhibited by the anti-depressant fluoxetine, (a selective serotonin re-uptake inhibitor, SSRI) in hybrid striped bass, with significant effects after 6 days at the lowest concentration tested, 23.2 $\mu g\ l^{-1}$ (Gaworecki and Klaine 2008). Increased time to capture prey was correlated with decreased brain serotonin, which decreased with time and concentration.

Noise pollution reduced feeding and escape behavior in the damselfish *Chromis chromis* in a Mediterranean marine reserve (Bracciali et al. 2012). Greater boat traffic volume corresponded with lower feeding frequencies. The escape reaction was longer in duration (>1 min) when boats passed nearby. Overall, the findings revealed a significant modification of the foraging behavior of *C. chromis* due to boat noise, which was only slightly buffered by no-take zones established within the reserve.

Hypoxia

When juvenile turbot *Scophthalmus maximus* and sea bass *Dicentrarchus labrax* were fed to satiation, food intake (and growth) was depressed in hypoxia (3.2 and 4.5 mg $O_2\ l^{-1}$) (Pichavant et al. 2001). Growth was comparable between fish in hypoxia that were fed to satiation and fish reared in normoxia with restricted rations. Decreased food intake could be a mechanism by which prolonged hypoxia reduces growth, and may be a way to reduce energy and thus oxygen demand. The northern, *Syngnathus fuscus*, and dusky pipefish, *S. floridae*, were held in normoxic (>5 mg l^{-1} O_2) and hypoxic (2 and 1 mg l^{-1} O_2) conditions (Ripley and Foran 2007). Both species produce high frequency, short duration clicks related to feeding activity. In hypoxia, reduced food intake corresponded with decreased sound production. Declines in both behaviors were seen after 1 day and continued while hypoxia was maintained.

Growth rates of winter flounder *Pseudopleuronectes americanus* and summer flounder *Paralichthys dentatus* were reduced as DO decreased and as temperature increased. Summer flounder were more tolerant than winter flounder (Steirhoff et al. 2006. A significant relationship between feeding rate and growth suggested reduced feeding was a major cause of growth limitation. European sea bass (*Dicentrarchus labrax* L.) were exposed to 40 % air saturation; oscillations between 40 and 86 % with a period of 770 min, and 86 % sat control) for 1 month (Thetmeyer et al.

2001). Fish in hypoxia consumed less food, had reduced growth, and a lower condition factor. Oscillating groups were intermediate. Growth was correlated with food intake, suggesting that reduced growth is primarily due to reduced appetite.

In contrast, bottom-feeding spot (*Leiostomus xanthurus*) and hogchoker (*Trinectes maculatus*) in Chesapeake Bay benefited from hypoxia, because their benthic prey were more available (Pihl et al. 1992). During or immediately after hypoxic events their gut contents contained larger, deeper-burrowing prey than during normal oxygen periods. Spot consumed a greater biomass (45–73 %) of polychaetes than other prey. Thus in areas where hypoxia is intermittent, its effect on behavior of macrobenthos may be advantageous to oxygen-tolerant bottom-feeding fish. However, in the Neuse River estuary, intermittent hypoxia had negative effects on feeding by croakers, *M. undulatus* by restricting fish to shallower oxygenated areas where prey were less abundant and by killing deeper benthic prey, thus greatly reducing their numbers (Eby et al. 2005).

Acidification

Effects of elevated $p\mathrm{CO}_2$ were tested on prey and predator by letting one predatory reef fish interact for 24 h with 8 small or large juvenile damselfishes (Ferrari et al. 2011). Both prey and predator were exposed to control (440 μatm) or elevated (700 μatm) $p\mathrm{CO}_2$. Small juveniles of all species had higher mortality from predation at high $p\mathrm{CO}_2$ because of reduced anti-predator behavior (Fig. 9.7), while larger ones were unaffected. For large prey, the pattern of prey selection by predators was reversed under elevated $p\mathrm{CO}_2$. The results demonstrate effects of CO_2 on behavior of juvenile damselfish, likely caused by altered neurological function.

Nowicki et al. (2012) found CO_2 level did not significantly affect foraging behavior in juvenile anemonefish *Amphiprion melanopus*, but there was an interaction with temperature. At high temperature (31.5 °C) and control or moderate (530 μatm) $p\mathrm{CO}_2$, food consumption and foraging activity were reduced, while high temperature and high $p\mathrm{CO}_2$ (960 μatm) caused an increase in these behaviors. Maintaining foraging activity in high temperature and CO_2 may reduce energy efficiency if the thermal optimum for food assimilation and growth has been exceeded. Elevated CO_2 and reduced pH affected olfactory preferences, activity, and feeding behavior of the brown dottyback (*Pseudochromis fuscus*) (Cripps et al. 2011). Fish were exposed to current and elevated $p\mathrm{CO}_2$ levels (~600 or ~950 μatm). Exposed fish shifted from preference to avoidance of the smell of injured prey, spending 20 % less time in a stream containing prey odor compared with controls. Activity was higher in the high $p\mathrm{CO}_2$ treatment and feeding was lower in the mid $p\mathrm{CO}_2$ treatment. Elevated activity in the high $p\mathrm{CO}_2$ treatment may compensate for reduced olfaction, as visual detection of food might improve. Juvenile damselfish *Pomacentrus amboinensis* exposed to high $p\mathrm{CO}_2$ responded differently to a potential threat, the sight of a predator, the chromis, *Acanthochromis polyacanthus*, placed in a watertight bag (Ferrari et al. 2012). Juvenile damselfish in 440 (control), 550 or 700 μatm $p\mathrm{CO}_2$ did not differ in their response to the chromis,

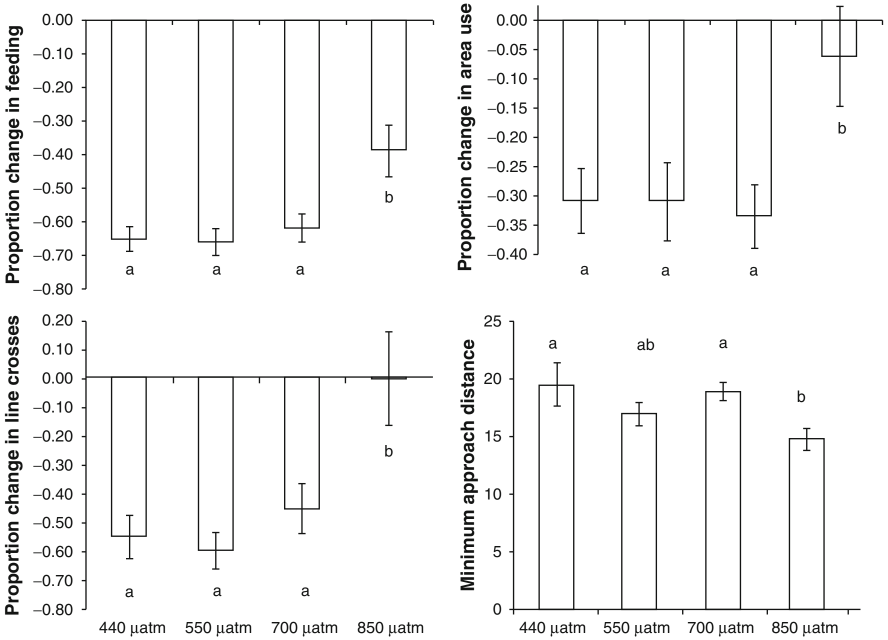


Fig. 9.7 Mean proportion change (±SE) in feeding strikes (*top left*), area use (*top right*), line crosses (*lower left*) and minimum approach distance (*bottom right*) for juvenile damselfish treated with different $p\mathrm{CO}_2$ concentrations and exposed to sight of a spiny chromis. Letters denote statistically different groups (Reprinted from Ferrari et al. 2012: 556, courtesy John Wiley & Sons)

but those in 850 μatm showed reduced antipredator responses; they failed to reduce foraging activity and area use. They moved closer to the chromis, suggesting that their response to visual cues of the predator was impaired by high $p\mathrm{CO}_2$.

Polluted Environments

Reduced feeding and condition factor were noted in *F. heteroclitus* from a heavily industrialized estuary, Piles Creek (PC) in Linden, New Jersey (U.S.). Toppin et al. (1987) showed that PC fish had reduced life span and growth; reduced feeding could be partly responsible for reduced growth. When fish from a reference site, Tuckerton (TK) were kept in aquaria with water, sediments, and food (grass shrimp) from PC, their prey capture ability declined to that of PC fish (Smith and Weis 1997). The level of Hg in their brains increased to that of the PC population. When PC fish were maintained in clean water, sediments, and food, their prey capture ability increased slightly but not significantly, and brain Hg did not decrease. The correlation of behavior with Hg does not mean that Hg causes the behavioral impairment as there are many other contaminants at the site including lead and PCBs which could contribute to behavioral deficits. PC fish collected from the field had much more sediment and detritus in their stomachs and less live prey than fish from TK, providing field validation for the poor predatory ability observed in the laboratory. PC fish also were more vulnerable to predation by blue crabs, which can contribute to their shorter life span (Smith and Weis 1997). When populations from many different sites were investigated, their prey capture ability was directly related to sediment and tissue levels of contaminants (Weis et al. 2001). Since the levels of contaminants at a site were highly correlated with each other, the role of specific contaminants could not be determined. In laboratory experiments, fish from the cleanest sites captured the most grass shrimp. Gut contents of field-collected fish revealed that fish that had the highest capture rates in the laboratory had the most grass shrimp in their diets. Goto and Wallace (2011) found that *F. heteroclitus* from chronically polluted salt marshes (Arthur Kill–AK, NY) had lower diet niche breadth than reference fish, reflecting reduced benthic macroinfaunal species diversity. AK fish had 2–3 times less food in their gut than the reference population, partly due to prey size – they ingested fewer large prey than the reference population. Gut fullness of the AK fish significantly decreased with increasing Hg body burdens. Reduced prey availability was also partly responsible for altering the feeding ecology. Young-of-the-year bluefish *Pomatomus saltatrix*, fed contaminated diets (killifish and menhaden from Hackensack Meadowlands, HM, a contaminated estuary) for 4 months showed reduced appetite and consumed less than fish that were fed diets of the same species collected from the reference site, TK (Candelmo et al. 2010). Fish fed HM food grew more slowly, probably as a result of reduced food intake.

Impaired predator avoidance (of blue crabs, *C. sapidus*) was observed in PC killifish compared to those from TK (Smith and Weis 1997). Among killifish and menhaden living in the polluted HM, higher levels of Hg and PCBs were found in fish that had been consumed by bluefish (*P. saltatrix*) than in conspecifics that had not been eaten (Weis and Candelmo 2012) suggesting that prey fish that had accumulated more neurotoxic contaminants were easier for the predator to capture. This also has the consequence of facilitating transfer of more toxicants to higher levels in the food web.

9.3.4 Other Taxa

Cu-exposed corals, *Subergorgia suberosa* were unable to catch brine shrimp effectively. The rate of successful feeding for controls was 85 %, but this was reduced to 57 % at 0.2 $\mu g\ l^{-1}$ Cu and only 24 % at 0.5 $\mu g\ l^{-1}$ (Peng et al. 2004). Other metals (Zn, Cd, Pb) did not produce this sublethal effect.

9.4 Reproductive Behavior

9.4.1 Crustaceans

Many crustaceans use pheromones to find mates and induce mating behaviors. If pollutants impair the ability to detect chemosensory cues and respond to pheromone signals (info-disruption), they could affect mating.

Metals

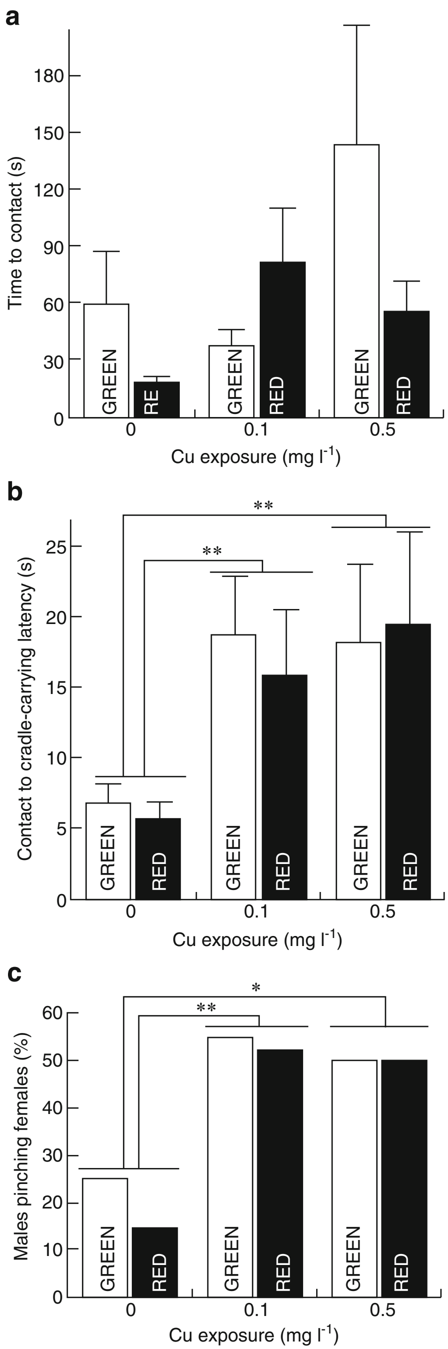
Effects of Cu (0, 0.1 or 0.5 mg Cu(II) l^{-1} for 5 days) on mating behavior of male shore (green) crabs *Carcinus maenas* were investigated by Krang and Ekerholm (2006). Cu altered the response to a pheromone stimulus (pre-molt female urine) presented alone, together with a dummy female (a sponge injected with pre-molt female urine) or with a real female. Male crabs in high Cu took over twice as long to initiate search activity after pheromone introduction and their search behavior was less directed. When offered a dummy female, they had decreased pheromone discrimination in both Cu treatments. Stroking behavior was reduced, and males from the high Cu often pinched the dummy female or real female, and took longer to establish cradle-carrying behavior, which normally precedes mating. Thus, Cu affected the ability to detect female pheromones, perform specific mating behaviors and form pairs (Fig. 9.8).

Organics

The amphipod *Corophium volutator* lives in shallow soft sediments in estuaries and coastal waters, and their reproductive season coincides with intense traffic of leisure boats, which discharge fuel into the water and sediment. Burrowed females release pheromones that guide males to them. Krang (2007) found that exposure to naphthalene, a PAH in motor fuel, disturbs this chemical communication. Males were put in Y-mazes, where they were allowed to follow female pheromones after separately exposing the males or females to naphthalene-spiked sediments (0, 0.5, and 5 $\mu g\ g^{-1}$ dw) for 3 days. Treated males' search activity was reduced by 27–45 % and they could no longer find females using olfaction. Analysis of sediments indicated that naphthalene concentrations causing this effect were 2–20 times lower than the nominal concentrations. Females continued to produce and release pheromones, despite naphthalene exposure.

Medetomidine (an antifouling compound) at 0.01 and 0.1 mg l^{-1} caused fewer males to search for females (Krang and Dahlstrom 2006).

Fig. 9.8 Behavioral response to a pre-molt female of red and green color morphs male *C. maenas* exposed to 0, 0.1 or 0.5 mg l^{-1} Cu for 5 days. (**a**) Time to contact the female, (**b**) time between contact and cradle-carrying, (**c**) proportion of males that pinched female. * and ** = significant (p 0.05 and 0.01 respectively) difference between groups below horizontal bars (Reprinted from Krang and Ekerholm 2006: 67, courtesy Elsevier Publishing Co)



9.4.2 Mollusks

Despite the extensive literature on reproductive endocrine disruption in mollusks, there do not appear to be studies on effects on reproductive behavior. This would appear to be a wide open field for investigation. However, for many mollusks that spawn, reproductive behavior *per se* is limited to simultaneous release of gametes. Some examples of contaminant effects on the timing of gamete release are discussed in Chap. 5, Reproduction. However, cephalopods have elaborate mating behavior that would be of great interest to study.

9.4.3 Fishes

Successful reproduction in fishes requires the performance of a number of different behaviors which may include spawning site selection, nest building, courtship and spawning, and may include post-spawning behaviors such as nest guarding and fanning, depending on the species, though nest building is uncommon in marine fishes. Toxicants can disrupt any of these behaviors and decrease reproductive success. However, despite considerable research on pollution and fish behavior, few articles focus on reproductive behavior (Jones and Reynolds 1997), and of these, most are on freshwater species (guppies, fathead minnows, mosquitofish, cichlids) that tend to have more complex behaviors than most marine fishes. Nest-building and courtship in male sticklebacks, which live in both fresh and salt water, have been the most studied, along with gobies.

Metals

Matta et al. (2001) found that dietary methylmercury (0.2–11 μg g^{-1}) altered male behavior in killifish (*F. heteroclitus*), increasing aggression in some fish and lethargy in others. Furthermore, their offspring were less able to reproduce successfully and had an altered sex ratio.

Organics

The organophosphate fenitrothion (FN) has structural similarities with the anti-androgen flutamide. The potential for FN to act as an anti-androgen (at 1, 50, and 200 μg l^{-1} over 26 days) was assessed in male three-spined sticklebacks, *Gasterosteus aculeatus*, by measuring kidney spiggin (the "glue" substance that holds the nest together) concentration, nest-building, and courtship behavior. FN significantly reduced spiggin production, nest-building, and courtship, especially the 'zigzag dance' and biting behavior (Sebire et al. 2009).

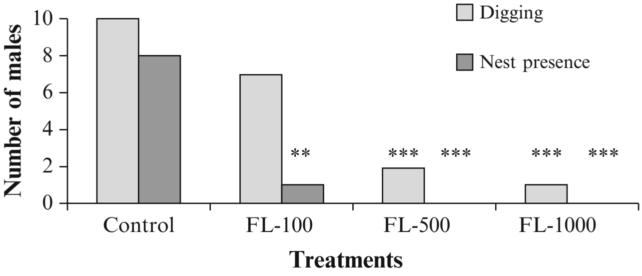


Fig. 9.9 Nest-building activity by male three-spined sticklebacks after 21-days exposure to different concentrations of flutamide. Number of males showing digging activity (*light grey*) or had a nest (*dark grey*). Significant difference from control ** $< p$ 0.01, *** $p < 0.001$ (Reprinted from Sebire et al. 2008: 42, courtesy Elsevier Publishing Co)

Contaminants of Emerging Concern (CECs)

It is not surprising that some endocrine disruptors affect courtship and mating behavior. Brian et al. (2006) examined nesting behavior of male *G. aculeatus* exposed to the synthetic estrogen, 17β-ethinylestradiol. There were immediate reductions of nest gluing frequency and time spent near the nest, but fish subsequently recovered and there was no permanent effect on nest building success. However, Wibe et al. (2002) found *G. aculeatus* exposed to17β-estradiol (2.0 μg g^{-1}) had impaired paternal care. There were no differences in number of males that built nests or in courtship displays, but exposed males started nest building later than controls, suggesting that some reproductive behaviors were altered. Sebire et al. (2008) induced breeding in male sticklebacks that were exposed to the anti-androgen flutamide (FL) at 100, 500 and 1,000 μg l^{-1} for 21 days. Exposed males had lower spiggin levels at 500 and 1,000 μg l^{-1}, built fewer nests at 100 μg l^{-1} and no nests at 500 and 1,000 μg l^{-1}(Fig. 9.9). Exposed males showed fewer zigzags towards the female.

Male *G. aculeatus* were presented with a dummy male and dummy female before and after a short-term exposure to 15 ng l^{-1} 17α-ethinyloestradiol (Dzieweczynski 2011). Courtship, aggression, and nesting behaviors were all reduced, and behavioral consistency over trials was markedly reduced after EE2 exposure.

Saaristo et al. (2009) exposed male sand gobies (*Pomatoschistus minutus*) for 1–4 weeks to 5 and 24 ng l^{-1} of 17α-ethinyl estradiol (EE2). This species has a polygynous mating system, in which mating success is skewed towards the largest males, resulting in strong sexual selection for increased male size. After exposure to EE2, male size had a reduced effect on mating success. Results suggested that exposure to EDCs can affect the mating system before physiological changes are observable.

Hypoxia

Female common gobies (*Pomatoschistus microps*) usually prefer to spawn with males that had already been chosen by females and whose nests therefore already contain eggs. However, this preference was reversed in low DO (Reynolds and Jones 1999). In 35 % sat, males showed a nearly threefold increase in ventilation of eggs and spent less time near a female. Authors thought females avoided males which would be less likely to meet the demands of care of a second clutch under low DO conditions. Naked gobies (*Gobiosoma bosc*) subjected to hypoxia in Chesapeake Bay and in the laboratory, were quite resistant in terms of reproductive behavior, and continued to guard eggs until levels approached lethal levels (<1 mg l^{-1}) (Breitburg 1992).

Polluted Environment

Sebire et al. (2011) investigated effects of 21 days exposure to a sewage plant effluent with anti-androgenic activity on reproductive physiology and behavior of *G. aculeatus*. Levels of spiggin were not affected, nor were levels of vitellogenin, but males built fewer nests and had less courtship behavior in both the 50 and 100 % effluent treatments compared with controls. Thus behavior was more sensitive than the biochemical biomarkers.

9.5 Aggression

Aggression or agonistic behaviors are common in many species, and altered social relations in response to toxicants, such as threats, chases, or bites, have been measured. Toxicants may increase or decrease agonistic behaviors.

9.5.1 Crustaceans

Organics

Dissanayake et al. (2009) exposed shore crabs, *C. maenas* to 200 μg l^{-1} of the PAH pyrene. After 14 days, crabs were used in staged agonistic interactions, where an unexposed crab was paired with an exposed crab from the same dietary regime (fully fed, starved, or diet restricted), using a cockle as the limited resource. Physiological condition had no significant effect on most behavioral measures (e.g. number of fights, fight duration), but starved pyrene-exposed crabs won the resource more of the time than starved controls. Thus, the reduced physiological condition in starved exposed crabs caused an unexpected increased competitive ability over starved unexposed crabs.

Hypoxia

Hermit crabs engage in fights over possession of the gastropod shells that they inhabit. In *Pagurus bernhardus*, attackers rap their shell against that of the defender in a series of bouts while defenders remain tightly withdrawn into their shells. At the end of a fight the attacker may evict the defender from its shell or give up without an eviction; the decision for a defender is either to maintain a grip on its shell or allow itself to be evicted. Briffa and Elwood (2000) found that the vigor of rapping and the likelihood of eviction were reduced when the attacker was subjected to low DO, but that low DO had no effect on rates of eviction when applied to defenders.

Polluted Environment

Blue crabs (*C. sapidus*) from the contaminated Hackensack Meadowlands (HM) (New Jersey US) were much more aggressive than conspecifics from a reference site (Tuckerton, TK) (Reichmuth et al. 2011). When presented with a threatening stimulus, most HM crabs attacked it while most TK crabs fled. This unexpected result is reminiscent of the results of Dissanayake above, and different from that of Khoury et al. (2009) in fiddler crabs. It has not been demonstrated whether the aggression in this case is related to contaminants in the environment or to other factors.

9.5.2 Fishes

A number of studies have found reduced aggression in various fish species exposed to metals or organic pollutants, but these have been in freshwater species.

Emerging Contaminants

Aggressive behavior is decreased in the male bluehead wrasse, *Thalassoma bifasciatum*, and in the betta when repeatedly injected with 5 $\mu g\ g^{-1}$ body weight fluoxetine (Lynn et al. 2007). Bell (2001) found that plasma gonadal steroid concentrations were related to levels of nesting and aggressive behaviors by male *G. aculeatus*. Levels of estradiol were negatively related to courtship behavior, while levels of 11-ketotestosterone were negatively related to nesting behaviors. Control males increased their aggressive response to a live male conspecific over time, while males exposed to EE2 decreased their aggressive response.

9.6 Social Behavior

9.6.1 Fishes

Schooling

Schooling behavior is a social interaction, in which fish of the same size and same species swim close together and at the same speed, in a coordinated fashion. It decreases susceptibility to predation, and can be altered by exposure to a variety of contaminants, including the pesticides DDT and carbaryl (Weis and Weis 1974). Carbaryl-exposed (100 μg l^{-1}) groups of Atlantic silversides (*Menidia menidia*) had greater distances between individuals and did not swim as parallel to one another as control groups. They also had faster swimming rates. In contrast, copper (100 μg l^{-1}) exposure of Atlantic silversides caused them to become hyperactive, but they increased rather than decreased school cohesion (Koltes 1985). Social interactions were impaired in larval *F. heteroclitus* after they had been exposed to 5 and 10 μg meHg l^{-1} as embryos (Ososkov and Weis 1996). Treated larvae collided into one another more frequently. Low concentrations of the surfactant 4-nonylphenol (1–2 μg l^{-1}) impaired schooling of juvenile banded killifish (*F. diaphanous*) – distances between fish were greater (Ward et al. 2008). Williams and Coutant (2003) found that short-term exposures to sublethal increases in water temperature during egg and larval stages affected development of schooling in the sand smelt *Atherina mochon*. Larvae tested at 10–35 dph showed retardation of schooling behavior. There was an increased number of approach–withdrawals (behavior that precedes parallel swimming; two fry approach and immediately veer away), a decrease in parallel orientation (schooling), higher latency for formation of the first schools, and shorter duration of the longest-persisting schools in exposed fish. Schooling was unstable, with wide fish-to-fish distances and an absence of synchrony. Young-of-the-year bluefish *Pomatomus saltatrix*, that were fed contaminated diets (killifish and menhaden from contaminated Hackensack Meadowlands, an estuary with multiple contaminants including Hg and PCBs) for a few months disrupted their normal schooling behavior more often than fish fed the same prey species collected from a cleaner estuary (Candelmo et al. 2010).

Communication

Some fish communicate using sounds. Vasconcelos et al. (2007) investigated effects of ship noise pollution on the detection of conspecific vocalizations by the Lusitanian toadfish, *Halobatrachus didactylus*. Ambient and ferry boat noises were recorded, as well as toadfish sounds. Hearing was measured under quiet lab conditions and in the presence of these noises at levels encountered in the field. In the presence of ship noise, auditory thresholds increased considerably because the boat noise was within the most sensitive hearing range of this species. The ship noise decreased the fish's ability to detect conspecific sounds, which are important in agonistic encounters and mate attraction.

There is concern that the noise from windmills may also decrease the effective range for sound communication of fish, but little is known to what extent it may occur. Windmill noise does not have destructive effects on hearing, even within short distances. It is estimated that fish are scared away from windmills at distances less than about 4 m, and only at high wind speeds. Thus, the acoustic impact of windmills may be one of masking communication signals rather than physiological damage or avoidance (Wahlberg and Westerberg 2005). However, data are very limited and further studies of fish behavior around windmills are needed.

9.6.2 Other Taxa

Cetaceans and Noise Pollution

Nowacek et al. (2007) reviewed responses of cetaceans to noise and found three types of responses: behavioral, acoustic and physiological. Behavioral responses include changes in surfacing, diving and swimming patterns. Acoustic responses include changes in type or timing of vocalizations. Physiological responses include auditory threshold shifts. Overall, they documented responses of cetaceans to various noise sources, but were concerned about the lack of study of effects of noise sources such as commercial sonars, depth finders and fisheries acoustics gear. Conducting experiments with cetaceans is challenging and opportunities are limited, so studies should include rigorous measurements and or modeling of exposure. Romano et al. (2004) measured blood parameters of white whale, *Delphinapterus leucas*, and bottlenose dolphin, *Tursiops truncatus*, in response to noise. Norepinephrine, epinephrine, and dopamine levels, related to stress, increased with increasing sound levels and were significantly higher after high-level sound exposures compared with low-level sound exposures or controls.

Noise from ship traffic and commercial, research and military activities has increased over the past century, and has resulted in changes in the vocalizations and behaviors of many marine mammals, such as beluga whales (*Delphinapterus leucas*) (Lesage et al. 1999), manatees (*Trichechus manatus*) (Miksis-Olds and Miller 2006) and right whales (*Eubalaena glacialis*, *E. australis*) (Parks et al. 2007). The calls of killer whales are longer in the presence of noise from boats, probably to compensate for the acoustic pollution (Foote et al. 2004), while humpback whales (*Megaptera novaeangliae*) increase the repetition of phrases in their songs when exposed to low-frequency sonar (Miller et al. 2000). Similarly, several dolphin species change their behavior and vocalization in the presence of boat sounds (Buckstaff 2004). Parks et al. (2011) documented calling behavior by individual endangered North Atlantic right whales (*Eubalaena glacialis*) in increased background noise. Right whales respond to periods of increased noise by increasing the amplitude of their calls. This may help to maintain the communication range with conspecifics during periods of increased noise. This may be interpreted as an adaptive response. However, periods of high noise are increasing and have reduced the ability of right whales to communicate with each other by about two-thirds. *E. glacialis* were studied by Hatch et al. (2012) in an ecologically relevant area (10,000 km^2 Stellwagen Bank marine sanctuary) and time period (1 month) using vessel-tracking data from the U.S. Coast Guard's Automatic Identification System to quantify acoustic signatures of large commercial vessels and calculate noise from vessels inside and outside the sanctuary. By comparing noise levels from commercial ships today with lower noise conditions a half-century ago, the authors estimate that right whales have lost about 63–67 % of their "communication space" in the sanctuary and surrounding waters. Humpback whales in this sanctuary stopped their singing during an Ocean Acoustic Waveguide Remote Sensing (OAWRS) experiment approximately 200 km away (Risch et al. 2012).

A common behavioral response is to leave the area. Olesiuk et al. (2002) assessed impacts of the sound generated by an acoustic harassment device (AHD) on the relative abundance and distribution of harbor porpoises (*Phocoena phocoena*). Abundance declined quickly when the AHD was activated. The mean number of porpoise re-sightings while tracking their movements also declined, which suggested that the few porpoises that went into the study area spent less time within it when the AHD was activated. The effect of the AHD diminished with distance but no porpoises were observed within 200 m of the AHD when it was activated.

9.7 Migration and Homing/Habitat Evaluation

9.7.1 Crustaceans

Hypoxia

Hermit crabs, *Pagurus bernhardus*, in hypoxic conditions spent less time investigating new shells before entering them, and also selected lighter shells than did crabs in normal DO levels. This shift in shell preference may reduce energy expenditure, but was at the expense of internal spaciousness of the shell, since lighter shells were smaller. These smaller shells may make residents more vulnerable to predation (Côté et al. 1998).

Acidification

Since chemoreception is a key activity by which many aquatic animals perceive their environment, disruptions of this process could have serious impacts on survival and fitness. De la Haye et al. (2011b) tested the chemosensory responses of the hermit crab *P. bernhardus* to a food odor under reduced pH (6.8). Acidifying the odor did not affect its attractiveness, but at the low pH crabs had lower antennular flicking rates, were less successful in locating the odor source, and had reduced general activity compared to those at normal pH. De la Haye et al. (2012) investigated effects of reduced pH on shell assessment and selection by *P. bernhardus*. At pH 6.8 crabs were less likely to change from a suboptimal to an optimal shell than those in normal pH, and those that did change shells took longer to do so. Thus, a reduction in pH disrupts resource assessment and decision-making of these crabs, reducing their ability to acquire a vital resource.

9.7.2 Fishes

Olfaction is important for the homing of adult salmonids from the ocean to their natal river to spawn. If the olfactory sense is impaired by toxicants, homing behavior can be affected. Smolting is the juvenile stage specialized for downstream migration, seawater entry, and marine residence; it is controlled by a number of hormones and includes numerous physiological and behavioral changes in fresh water which prepare smolts for migrating into marine waters (McCormick et al. 1998). Smolting is discussed below and further in Chap. 8.

Metals

Copper is particularly damaging to the olfactory system, which is critical for migration and homing in salmon. After exposure to 22 μg l^{-1} copper, salmonids could no longer discriminate among different sources of water (Saucier et al. 1991). When controls were given a choice between their own rearing water vs either well water or heterospecifc water, they significantly preferred their own rearing water, whereas copper-exposed groups showed no preference. The behavioral response of exposed fish indicates impairment of their olfactory discrimination ability.

Organics

The transition of salmonids to the smolting stage is controlled by the endocrine system. Growth hormone, insulin-like growth factor I (IGF-I), cortisol, and thyroxine are all involved in the development of salinity tolerance in smolting (Kavlock et al. 1996) and these hormones all increase during smolting. It is likely that exposure to endocrine-disrupting chemicals will affect smolting and affect seaward migration The pesticide atrazine (2-chloro-4-ethylamino-6-isopropylamino-*S*-triazine), which is known to modify the parr–smolt transformation and olfactory function in adult Atlantic salmon (*S. salar*) had a significant effect on migratory activity and olfactory sensitivity of smolts (Moore et al. 2007). Exposure for 81 days to 5.0 μg l^{-1}atrazine during the parr–smolt transformation significantly reduced migratory activity in an experimental stream during the period of peak migration in the wild population.

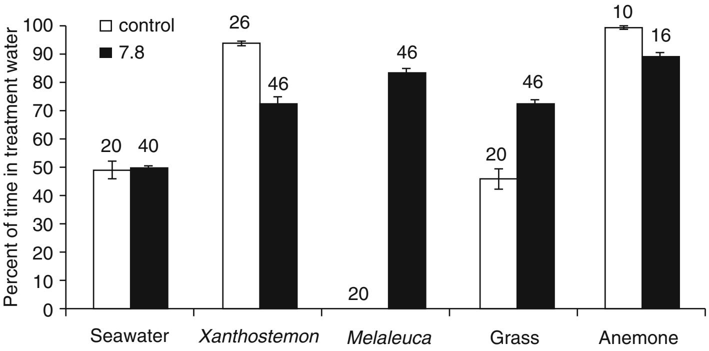


Fig. 9.10 Response of larval clownfish to olfactory cues from three tropical plants and from anemones when reared in current seawater pH (control, *open bars*) and ph 7.8 (*filled bars*). Bars = percentage of time larvae spent on the side of a 2-channel flume chamber which contained the cue. Numbers above bars = number of replicates (Reprinted from Munday et al. 2009: 1849, courtesy National Academy of Sciences)

After exposure to atrazine at (nominal) 0.5, 1.0, 2.0 and 5.0 μg l^{-1} electrophysiological responses recorded from the olfactory epithelium of salmon smolts to both L-serine and smolt urine were significantly reduced.

CECs

Exposure of migrating Atlantic salmon (*S. salar*) to 10 ng l^{-1} of the brominated flame retardant hexabromocyclododecane (HMCD) impaired olfactory responses (Lower and Moore 2007).

Exposure to loud noise can cause fish to move away from the area. The actual effect could be minor, since they may return when the noise stops. However, effects can be more extensive and result in fish leaving important feeding grounds or spawning sites. Skalski et al. (1992) found that sounds generated by acoustic geophysical survey devices resulted in reduced numbers of rockfish (*Sebastes* spp.) relative to control trials. This overall decline was also reflected in the catches of individual rockfish species – chilipepper (*S. goodei*), bocaccio (*S. paucispinis*), and greenspotted rockfish (*S. chlorostictus*).

Acidification

Larvae of clownfish (*Amphiprion percula*) reared under acidic conditions (pH 7.8) were unable to locate safe habitats (Munday et al. 2009). They had damaged sensory abilities, were unable to discern the scent and sound of predators and thus were less likely to detect and swim away from threats, lowering their chances for survival (Fig. 9.10). For example, they were more attracted to odors of terrestrial plants such as *Melaleuca*.

Many fishes also rely on hearing for orientation, habitat selection, predator avoidance and/or communication. Simpson et al. (2011) studied the influence of enriched CO_2 on directional responses of juvenile clownfish (*A. percula*) to daytime reef noise. Juveniles in ambient $p\mathrm{CO}_2$ conditions avoided reef noise, as expected, but this behavior was absent in high $p\mathrm{CO}_2$ (700 and 900 μatm $p\mathrm{CO}_2$), showing that ocean acidification affects auditory responses, with potentially detrimental impacts on early survival. Adults can also be affected. The ability of adult cardinalfish, *Cheilodipterus quinquelineatus*, to home to their diurnal resting sites after nocturnal feeding was impaired by elevated $p\mathrm{CO}_2$ (550, 700 or 950 μatm) (Devine et al. 2012). Exposed fish had reduced ability to distinguish between home vs foreign-site odors. Fish in elevated $p\mathrm{CO}_2$ showed reduced homing success when released 200 m away from home sites. Behavior at the home sites was also affected, with exposed fish having higher activity and venturing further from shelter. Thus, disruption of chemosensory mechanisms was seen in critical behaviors.

Long before awareness of ocean acidification, decreased pH in freshwater was a concern due to acid rain, and large numbers of studies of effects of this environmental problem were done in previous decades. Acidity and accompanying aluminum in freshwater can impair the development of gill Na^+, K^+-ATP-ase and thus salinity tolerance, which is essential for salmon to develop before migrating to saline waters. Exposures as short as 12 h to relatively mild acidity (pH 5.2, 31 mg l^{-1} aluminum) can impair salinity tolerance (Staurnes et al. 1996a). Atlantic salmon smolts (*S. salar*) released into an acidic river in Norway often had no adult returns and had only one-tenth the returns of fish released at the mouth of the river or in a nearby limed river (Staurnes et al. 1996b). These return rates were strongly correlated with the effect of acidity on gill Na^+, K^+-ATPase activity and osmotic balance of fish held in cages at the different sites. Therefore, the smolt stage appears to be particularly sensitive to acid rain and other pollutants. By affecting the development of salinity tolerance, migration to the sea can be impaired.

9.7.3 Other Taxa

Corals and Acidification

Coral larvae (*Acropora millepora*) normally settle on the crustose calcareous alga, *Titanoderma*, for metamorphosis. Doropoulos et al. (2012) found that as $p\mathrm{CO}_2$ increased (to 800 and 1,300 μatm), the coral larvae avoided this alga and started to settle elsewhere. *Titanoderma* also became less prevalent. Acidification therefore reduced the number of larvae settling, disrupted their normal preference for settlement, and reduced the availability of the most desirable algal substrate for their future survival.

9.8 Neurotoxicology Underlying Behavior

Effects on complex behaviors are due to underlying effects on the nervous and endocrine systems. Effects of pollutants on behavior are likely caused by interference with these systems, including neurological development and levels of neurotransmitters. Sensory receptors may be affected. Chemoreception is an important factor for responses to their environment; olfaction, vision, and hearing are important chemical senses that can be impaired by contaminants. In addition, many behaviors are affected by the endocrine system, which is very sensitive to environmental contaminants. These underlying mechanisms will be discussed only briefly.

9.8.1 Crustaceans

Neurosecretion and CNS

Various metals and organic pollutants have been found to affect the nervous system of crustaceans. Increased acetylcholinerase in thoracic ganglia of the crab *Barytelphusa guerini* was noted (Reddy and Venugopal 1993) after 4 days of Cd (0.6 mg l^{-1}) exposure, but after 15 days the enzyme was inhibited. The eyestalks of decapod crustaceans are central neuroendocrine coordinators, controlling almost all aspects of their lives including the molt cycle, color change, maturation, blood sugar, and nerve function. Cd (10 mg l^{-1}) damaged neurosecretory cells in the brain and eyestalk ganglia of *U. pugilator* (Reddy and Fingerman 1995). Impaired color change is an easily measured response to disruption of these hormones. Crabs exposed to Cd were less able to disperse pigment in their melanophores because Cd depleted neurosecretory material. Exposure to the WSF of Louisiana crude oil or naphthalene resulted in accumulation of neurosecretory material in the brain of *U. pugilator* (Deecaraman and Fingerman 1985), suggesting that these chemicals, unlike Cd, inhibit release, rather than synthesis of neurosecretory material. Similarly, exposure to PCBs inhibited release of black pigment dispersing hormone from the eyestalks, so that the crabs did not become as dark as unexposed fiddler crabs (Staub and Fingerman 1984).

Sense Organs

Crustaceans depend on chemical senses for feeding and social interactions, and their chemoreceptors are on the surface of the body and exposed directly to the environment with whatever contaminants may be present. Disrupted chemoreception can be responsible for changes in settlement of larvae, hermit crab shell acquisition, and reproductive interactions (Krang 2007; Krang and Ekerholm 2006; Krang and Dahlstrom 2006). Pesticides designed to affect the nervous system of insects are likely to have effects on the crustacean nervous system including chemoreception.

9.8.2 Fishes

Neurotransmitters

Brain neurotransmitter levels and enzyme functions are related to behaviors, so it is likely that altered neurotransmitters induced by toxicants will result in altered behaviors. One of the most common indicators of altered neural function is altered acetylcholinesterase (AchE) in the brain. AchE breaks down the transmitter acetylcholine after it diffuses across the synapse in cholinergic neurons. Organophosphate pesticides inhibit fish AchE, as this is their "mode of action," as do many carbamate pesticides (Scott and Sloman 2004). Brain cholinesterase and feeding behavior of bream (*Abramis brama*) were both affected by the organophosphate pesticide DDVP (Dichlorvos), suggesting a connection between the physiological and behavioral effects (Pavlov et al. 1992). Injection of atropine, which counteracts the effects of DDVP, restored both feeding behavior and brain AchE activity. Fish with altered behavior have been shown to have altered brain neurotransmitters. Killifish (*F. heteroclitus*) after mercury exposure or collected from polluted sites, which had reduced activity and prey capture, also had reduced serotonin in their brains (Smith et al. 1995; Zhou et al. 1999b). In contrast, Gulf killifish (*F. grandis*) showed decreased dopamine and norepinephrine after exposure to PCBs (Aroclor 242), along with greatly increased activity levels, but no effect on serotonin (Fingerman and Russell 1980). Yu et al. (2013) found that 10, 100 and 1,000 ng l^{-1} TBT reduced prey capture of *Sebastiscus marmoratus* and altered neurotransmitters. Dopamine levels in the fish brains increased in a dose-dependent manner and 5-hydroxytryptamine and norepinephrine levels decreased in TBT-exposed fish compared to controls.

Exposure to $PbNO_3$ (1.6 mg l^{-1}) reduced feeding and resting bouts of the cleaner fish *Thalassoma pavo* after 24 h of exposure, while hyperactive swimming episodes were seen (Zizza et al. 2013). The abnormal behaviors were highly correlated to up-regulated orexin receptor (ORXR) mRNA expression in the lateral thalamic nucleus and the optic tectum of the brain. These transcriptional effects were attenuated when exposed fish received either 100 ng g^{-1} of ORX-A or 0.1 $\mu g\ g^{-1}$ of γ-aminobutyric $acid_A$ receptor ($GABA_AR$) agonist muscimol (MUS). Moreover, neurodegenerative processes noted after Pb exposure were not seen after treatment with MUS, but addition of the $GABA_AR$ antagonist bicuculline (BIC; 1 $\mu g\ g^{-1}$) enhanced the behavioral and neurodegenerative effects of Pb. Thus, there are a number of different neurotransmitters that can be affected by various contaminants that are associated with altered behaviors.

Sense Organs

Olfaction

The olfactory system of fishes is open to the environment and particularly sensitive to metals including Hg (Baatrup et al. 1990), although inorganic Hg and meHg localize in different parts of the olfactory system. Many metals directly enter the olfactory system where they can disrupt normal function. By accumulating in and damaging cells of the olfactory system, toxicants can disrupt transmission of information from olfactory lobes to higher levels of the brain. Olfactory receptor neurons can be a direct transport route of contaminants to the olfactory bulbs and the brain, with resulting effects on the functioning of the nervous system. Cd appears to move along olfactory neurons by axonal transport (Scott and Sloman 2004). Some studies have shown a connection between altered behavior and altered olfactory system. Rehnberg and Schreck (1986) showed reduced avoidance of the amino acid L-serine (a potent odor to fish) by coho salmon (*Oncorhynchus kisutch*) exposed to Cu and Hg. Hg but not Cu inhibited serine binding to the olfactory epithelium. Cu produced morphological lesions in olfactory, taste, and lateral line receptor systems (Brown et al. 1982). Copper exposure of juvenile coho salmon (*Oncorhynchus kisutch*) (30 min exposure to 20 μg l^{-1} Cu) reduced the olfactory response to a natural odorant (10^{-5} M L-serine) by 82 % (McIntyre et al. 2008).

Kennedy et al. (2012) found that Cu inhibited the ability of chinook salmon to detect and avoid the odorant L-histidine amino acid in a concentration-dependent manner, and Cu toxicity (olfactory inhibition) decreased with increasing dissolved organic carbon (DOC) concentration. These finding suggest that DOC concentration should be considered when evaluating impacts of Cu on fish olfaction.

Olfactory alterations in early life history stages due to organophosphate and carbamate pesticides may be related to effects on AChE and sodium channels (Narahashi 1996).

The olfactory system of male salmonids responds to many pesticides. For example, short-term exposure of the olfactory epithelium of mature male Atlantic salmon (*S. salar*) to simazine (1.0 and 2.0 μg l^{-1}) or atrazine (1.0 μg l^{-1}) significantly reduced the olfactory response to the female priming pheromone, prostaglandin $F_{2\alpha}$. In addition, the reproductive priming effect of the pheromone on the amount of expressible milt was also reduced after exposure to the individual pesticides (Moore and Lower 2001). Exposure of mature males for 5 d to <0.004 μg l^{-1} cypermethrin significantly reduced or inhibited the olfactory response to prostaglandin $F_{2\alpha}$. In addition, exposure to cypermethrin significantly reduced their ability to respond to the priming effect of the pheromone (Moore and Waring 2001).

Impaired olfactory function may cause larval fish to be attracted to odors they normally avoid, including those from predators and unfavorable habitats. The underlying mechanism linking high CO_2 to these responses has been shown to be neurotransmitters (Nilsson et al. 2012). Abnormal olfactory preferences of fish exposed to high CO_2 can be reversed by treatment with an antagonist of the receptor for GABA-A, a major neurotransmitter receptor in the brain. This shows that high

CO_2 interferes with neurotransmitter function, which underlies many behaviors. Since these receptors are widespread in animals, rising CO_2 levels could cause sensory and behavioral impairment in a wide range of marine species.

Vision

Impaired vision can also underlie some behavioral changes. Interference with visual ability is likely to affect prey capture and predator avoidance. Cu (60–110 $\mu g\ l^{-1}$) caused pathological changes in the cornea of the developing eye of striped bass *Morone saxatilis* (Bodammer 1985), while TBT (10 and 50 ng l^{-1}) and EE_2 (3 and 9 ng l^{-1}) reduced the pupil area of pipefish, *Syngnathus abaster* larvae (Sárria et al. 2011b).

Hearing and Lateral Line

Hearing in fish can be impaired by prolonged exposure to loud noise. Marine petroleum exploration involves the repetitive use of high-energy noise sources, air-guns, which produce a short, sharp, low-frequency sound. Ears of pink snapper (*Pagrus auratus*) exposed to an operating air-gun sustained extensive damage to their sensory epithelia that was apparent as ablated hair cells (McCauley et al. 2003). Sensory cells were missing and there was considerable cell death observed. The damage was regionally severe, with no evidence of repair or replacement of damaged sensory cells up to 58 days after exposure. This study was done on fish caged near the source that could not escape. Wild fish would have left the area, mitigating the destructive effects of the noise.

The lateral line can be a target for toxicants, such as cadmium, whose effects can impair escape behavior (Faucher et al. 2006).

Endocrine System

Many studies have shown linkages between hormones and behavior. Many pollutants act as agonists or antagonists to hormones. Thyroid hormones influence many processes in fish, including neural development, metabolism, maturation (smoltification in salmonids and metamorphosis in flatfish), and behavior. Many chemicals, including chlorinated hydrocarbons, PAHs, organochlorine pesticides, chlorinated paraffins, organophosphate pesticides, carbamate pesticides, cyanide compounds, methyl bromide, phenols, ammonia, metals, acid, sex steroids, and pharmaceuticals, exert effects on the fish thyroid, which has implications for behavior. About 40 fish species have been shown to have thyroid responses to contaminants (reviewed by Brown et al. 2004). *Fundulus heteroclitus* from Piles Creek, New Jersey (contaminated with metals, PCBs and more) are sluggish with poor prey capture and predator avoidance. They have abnormal thyroid glands, with extremely enlarged and follicular cell heights, and contain elevated plasma thyroxine (T4), but not plasma or tissue T3 (Zhou et al. 1999a). Reference site fish held in conditions simulating Piles Creek also developed elevated T4.

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) affected endocrine signalling in Atlantic salmon (*Salmo salar*) embryos and larvae after exposure to 100 μg l^{-1} from egg for 52 days, followed by 1 week recovery (Spachmo and Arukwe 2012). Exposure altered expression of thyroid receptor α and β, thyroid-stimulating hormone, and T_4 outer-ring deiodinase. Turbot (*Scophthalmus maximus*) larvae exposed to WSF of crude oil had increased levels of circulating thyroxine (T_4) leading to a decrease in T_3:T_4 ratio, and reduced swimming activity (Stephens et al. 1997).

9.8.3 *Other Taxa*

Solé et al. (2011) found morphological evidence of massive acoustic trauma in four cephalopod species subjected to low-frequency controlled-exposure experiments. Exposure to low-frequency sounds resulted in permanent and substantial alterations of the sensory hair cells of the statocysts, the structures responsible for the animals' sense of balance and position.

Exposure to noise may result in physiological and endocrine responses in marine mammals with significant consequences. Rolland et al. (2012) showed that reduced ship traffic in the Bay of Fundy, Canada, after the events of 11 September 2001, resulted in a 6 dB decrease in underwater noise with a significant reduction below 150 Hz. This noise reduction was associated with decreased baseline levels of stress-related glucocorticoids in North Atlantic right whales (*Eubalaena glacialis*). This is the first evidence that exposure to low-frequency ship noise may be associated with chronic stress in whales.

9.9 Discussion and Conclusions

Behavioral ecotoxicology can link disturbances at the biochemical level (e.g., altered neurological function or thyroid hormones) to effects at the population level. Types of behaviors that have been measured include swimming activity, burrowing, and migration, which are individual behaviors, and prey capture, predator avoidance, reproductive behaviors, aggression, and social behaviors, which involve interactions among individuals. Effects on behavior may be direct, such as impairment of reproductive behavior, habitat evaluation, prey capture, and avoidance of predators. Indirect effects include alteration of activity or reproduction success due to reduced feeding and thus, less energy. The sensitivity of behavioral responses can be useful in ecological risk assessments, as behavior can be affected at levels lower than those that affect physiology.

Behavior can be altered in larvae or older stages after exposure to contaminants at the embryo stage. These delayed effects should be considered in risk assessment, though they are not generally taken into consideration. Behavior evolves in response to natural selection, maximizing an organism's fitness. Since few species show behavioral changes that are beneficial, most behavioral alterations in response to contaminants are deleterious to an organism's fitness.

References

Adams PM, Hanlon RT, Forsythe JW (1988) Toxic exposure to ethylene dibromide and mercuric chloride: effects on laboratory-reared octopuses. Neurotoxicol Teratol 10:519–523

Almeida JR, Oliveira C, Gravato C, Guilhermino L (2010) Linking behavioural alterations with biomarkers responses in the European seabass *Dicentrarchus labrax* L. exposed to the organophosphate pesticide fenitrothion. Ecotoxicology 19:1369–1381

Alvarez MC, Fuiman LA (2005) Environmental levels of atrazine and its degradation products impair survival skills and growth of red drum larvae. Aquat Toxicol 74:229–241

Alvarez MC, Murphy CA, Rose KA, McCarthy ID, Fuiman LA (2006) Maternal body burdens of methylmercury impair survival skills of offspring in Atlantic croaker (*Micropogonias undulatus*). Aquat Toxicol 80:329–337

Amaral V, Cabral HN, Bishop MJ (2012) Effects of estuarine acidification on predator–prey interactions. Mar Ecol Prog Ser 445:117–127

Baatrup E, Doving KB, Winberg S (1990) Differential effects of mercurial compounds on the electro-olfactogram (EOG) of salmon (*Salmo salar* L.). Ecotoxicol Environ Saf 20:269–276

Bell AM (2001) Effects of an endocrine disrupter on courtship and aggressive behaviour of male three-spined stickleback, *Gasterosteus aculeatus*. Anim Behav 62:775–780

Bell AM (2004) An endocrine disrupter increases growth and risky behavior in threespined stickleback (*Gasterosteus aculeatus*). Horm Behav 45:108–114

Bell GW, Eggleston DB, Wolcott TG (2003) Behavioral responses of free-ranging blue crabs to episodic hypoxia. II. Feeding. Mar Ecol Prog Ser 259:227–235

Bodammer JE (1985) Corneal damage in larvae of striped bass *Morone saxatilis* exposed to copper. Trans Am Fish Soc 114:577–583

Bonnard M, Romeo M, Amiard-Triquet C (2009) Effects of copper on the burrowing behavior of estuarine and coastal invertebrates, the polychaete *Nereis diversicolor* and the bivalve *Scrobicularia plana*. Hum Ecol Risk Assess 15:11–26

Bracciali C, Campobello D, Giacoma C, Sarà G (2012) Effects of nautical traffic and noise on foraging patterns of Mediterranean damselfish (*Chromis chromis*). PLoS One 7:art.e40582

Breitburg DL (1992) Episodic hypoxia in Chesapeake Bay: interacting effects of recruitment, behavior, and physical disturbance. Ecol Monogr 62:525–546

Brian JV, Augley JJ, Braithwaite VA (2006) Endocrine disrupting effects on the nesting behaviour of male three-spined stickleback *Gasterosteus aculeatus* L. J Fish Biol 68:1883–1890

Briffa M, Elwood RW (2000) Cumulative or sequential assessment during hermit crab shell fights: effects of oxygen on decision rules. Proc R Soc B Biol Sci 267:2445–2452

Briffa M, de la Haye K, Munday PL (2012) High CO_2 and marine animal behaviour: potential mechanisms and ecological consequences. Mar Pollut Bull 64:1519–1528

Brown SB, Evans RE, Thompson BE, Hara TJ (1982) Chemoreception and aquatic pollutants. In: Hara TJ (ed) Chemoreception in fishes. Elsevier, Amsterdam, pp 363–393

Brown S, Adams BA, Cyr DG, Eales JG (2004) Contaminant effects on the teleost fish thyroid. Environ Toxicol Chem 23:1680–1701

Buckstaff KC (2004) Effects of watercraft noise on the acoustic behaviour of bottlenose dolphins, *Tursiops truncatus*, in Sarasota Bay, Florida. Mar Mamm Sci 20:709–725

Buffett PE, Amiard-Triquet C, Dybowska A, de Faverney CR, Guibbolini M, Valsami-Jones E, Mouneyrac C (2012) Fate of isotopically labeled zinc oxide nanoparticles in sediment and effects on two endobenthic species, the clam *Scrobicularia plana* and the ragworm *Hediste diversicolor*. Ecotoxicol Environ Saf 84:191–198

Candelmo A, Deshpande A, Dockum B, Weis P, Weis JS (2010) The effect of contaminated prey on feeding, activity, and growth of young-of-the-year bluefish, *Pomatomus saltatrix*, in the laboratory. Estuar Coasts 33:1025–1038

Capaldo P (1987) Effects of carbaryl (SEVIN) on stage I zoeae of the red-joined fiddler crab, *Uca minax* (LeConte). Estuaries 10:132–135

Carpenter RC (1986) Partitioning herbivory and its effects on coral reef algal communities. Ecol Monogr 56:345–363

Côté IM, Reverday B, Cooke PK (1998) Less choosy or different preference? Impact of hypoxia on hermit crab shell assessment and selection. Anim Behav 56:867–873

Couillard CM, Légaré B, Bernier A, Dionne Z (2011) Embryonic exposure to environmentally relevant concentrations of PCB126 affect prey capture ability of *Fundulus heteroclitus* larvae. Mar Environ Res 71:257–265

Craig JK (2012) Aggregation on the edge: effects of hypoxia avoidance on the spatial distribution of brown shrimp and demersal fishes in the Northern Gulf of Mexico. Mar Ecol Prog Ser 445:75–95

Cripe GM, Nimmo DR, Hamaker TL (1981) Effects of two organophosphate pesticides on swimming stamina of the mysid *Mysidopsis bahia*. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Biological monitoring of marine pollutants. Academic Press, New York, pp 21–36

Cripps IL, Munday PL, McCormick MI (2011) Ocean acidification affects prey detection by a predatory reef fish. PLoS One 6(7):art.e22736

Culbertson JB, Valiela I, Peacock EE, Reddy CM, Carter A, VanderKruik R (2007) Long-term biological effects of petroleum residues on fiddler crabs in salt marshes. Mar Pollut Bull 54:955–962

De la Haye K, Spicer JI, Widdicombe S, Briffa M (2011a) Reduced seawater pH disrupts resource assessment and decision-making in the hermit crab *Pagurus bernhardus*. Anim Behav 82:495–501

De la Haye KL, Spicer JI, Widdicombe S, Briffa M (2012) Reduced pH sea water disrupts chemo-responsive behaviour in an intertidal crustacean. J Exp Mar Biol Ecol 412:134–140

DeCoursey P, Vernberg WB (1972) Effect of mercury on survival, metabolism and behaviour of larval *Uca pugilator*. Oikos 23:241–247

Deecaraman M, Fingerman M (1985) Changes in neurosecretory cells of the fiddler crab *Uca pugilator*, induced by exposure to the water soluble fraction of South Louisiana crude oil or its toxic aromatic components. J Reprod Biol Comp Endocrinal 5:89–96

Devine BM, Munday PL, Jones GP (2012) Homing ability of adult cardinalfish is affected by elevated carbon dioxide. Oecologia 168:269–276

Díaz-Jaramillo M, da Rocha AM, Chiang G, Buchwalter D, Monserrat JM, Barra R (2013) Biochemical and behavioral responses in the estuarine polychaete *Perinereis gualpensis* (Nereididae) after *in situ* exposure to polluted sediments. Ecotoxicol Environ Saf 89:182–188.

DiIorio L, Gervaise C, Jaud V, Robson AA, Chauvaud L (2012) Hydrophone detects cracking sounds: non-intrusive monitoring of bivalve movement. J Exp Mar Biol Ecol 432–433:9–16

Dissanayake A, Galloway TS, Jones MB (2009) Physiological condition and intraspecific agonistic behaviour in *Carcinus maenas* (Crustacea: Decapoda). J Exp Mar Biol Ecol 375:57–63

Dissanayake A, Piggott C, Baldwin C, Sloman KA (2010) Elucidating cellular and behavioural effects of contaminant impact (polycyclic aromatic hydrocarbons, PAHs) in both laboratory-exposed and field-collected shore crabs, *Carcinus maenas* (Crustacea: Decapoda) Mar. Environ Res 70:368–373

Doropoulos C, Ward S, Diaz-Pulido G, Hoegh-Guldberg O, Mumby PJ (2012) Ocean acidification reduces coral recruitment by disrupting intimate larval-algal settlement interactions. Ecol Lett 15:338–346

Duffy JE, Hay ME (2000) Strong impacts of grazing amphipods on the organization of a benthic community. Ecol Monogr 70:237–263

Dzieweczynski T (2011) Short-term exposure to an endocrine disruptor affects behavioural consistency in male threespine stickleback. Aquat Toxicol 105:681–687

Eby LA, Crowder LB, McClellan CM, Peterson CH, Powers MJ (2005) Habitat degradation from intermittent hypoxia: impacts on demersal fishes. Mar Ecol Prog Ser 291:249–261

Faucher K, Fichet D, Miramand P, Lagardère JP (2006) Impact of acute cadmium exposure on the trunk lateral line neuromasts and consequences on the "C-start" response behaviour of the sea bass (*Dicentrarchus labrax* L.; Teleostei, Moronidae). Aquat Toxicol 76:278–294

Faulk CK, Fuiman LA, Thomas P (1999) Parental exposure to ortho, paradichlorodiphenyltrichloroethane impairs survival skills of Atlantic croaker (*Micropogonias undulatus*) larvae. Environ Toxicol Chem 18:254–262

Ferrari MCMI, McCormick PL, Munday MG, Meekan DL, Dixson ÖL, Chivers DP (2011) Putting prey and predator into the CO_2 equation – qualitative and quantitative effects of ocean acidification on predator–prey interactions. Ecol Lett 14:1143–1148

Ferrari MCO, McCormick MI, Munday PL, Meekan MG, Dixson DL, Lönnstedt O, Chivers DP (2012) Effects of ocean acidification on visual risk assessment in coral reef fishes. Funct Ecol 26:553–558

Fingerman SW, Russell LC (1980) Effects of the polychlorinated biphenyl Aroclor 1242 on locomotor activity and the neurotransmitters dopamine and norepinephrine in the brain of Gulf killifish, *Fundulus grandis*. Bull Environ Contam Toxicol 25:682–687

Fleeger JW, Carman KR, Nisbet RM (2003) Indirect effects of contaminants in aquatic ecosystems. Sci Total Environ 317:207–233

Fong PP, Molnar N (2013) Antidepressants cause foot detachment from substrate in five species of marine snail. Mar Environ Res 84:24–30

Foote AD, Osborne RW, Hoelzel AR (2004) Environment – whale-call response to masking boat noise. Nature 428:910

Gaworecki KM, Klaine SJ (2008) Behavioral and biochemical responses of hybrid striped bass during and after fluoxetine exposure. Aquat Toxicol 88:207–213

Goncalves R, Scholze M, Ferreir AM, Martins M, Correia AD (2008) The joint effect of polycyclic aromatic hydrocarbons on fish behavior. Environ Res 108:205–213

Goto D, Wallace WG (2011) Altered feeding habits and strategies of a benthic forage fish (*Fundulus heteroclitus*) in chronically polluted tidal salt marshes. Mar Environ Res 72:75–88

Gravato C, Guilhermino L (2009) Effects of benzo(a)pyrene on seabass (*Dicentrarchus labrax* L): biomarkers, growth and behavior. Hum Ecol Risk Assess 15:121–137

Guler Y, Ford AT (2010) Anti-depressants make amphipods see the light. Aquat Toxicol 99:397–404

Gutierrez MF, Paggi JC, Gagneten AM (2012) Microcrustaceans escape behavior as an early bioindicator of copper, chromium and endosulfan toxicity. Ecotoxicology 21:428–438

Hagerman L, Szaniawska A (1986) Behaviour, tolerance and anaerobic metabolism under hypoxia in the brackish-water shrimp *Crangon crangon*. Mar Ecol Prog Ser 34:125–132

Hatch LT, Clark CW, Van Parijs SM, Frankel AS, Ponirakis DW (2012) Quantifying loss of acoustic communication space for right whales in and around a U.S. National Marine Sanctuary. Conserv Biol 26:983–994

Hernández-Moreno D, Pérez-López M, Soler F, Gravato C, Guilhermino L (2011) Effects of carbofuran on the sea bass (*Dicentrarchus labrax* L.): study of biomarkers and behaviour alterations. Ecotoxicol Environ Saf 74:1905–1912

Hughes JM, Chapman HF, Kitching RL (1987) Effects of sublethal concentrations of copper and freshwater on behaviour in an estuarine gastropod *Polinices sordidus*. Mar Pollut Bull 18:127–131

Hutcheson M, Miller DC, White AQ (1985) Respiratory and behavioral responses of the grass shrimp *Palaemonetes pugio* to cadmium and reduced dissolved oxygen. Mar Biol 88:59–66

Jones JC, Reynolds JD (1997) Effects of pollutants on reproductive behaviour in fishes. Rev Fish Biol Fish 7:463–491

Kalman J, Palais F, Amiard JC, Mouneyrac C, Muntz A, Blasco J, Riba I, Amiard-Triquet C (2009) Assessment of the health status of populations of the ragworm *Nereis diversicolor* using biomarkers at different levels of biological organization. Mar Ecol Prog Ser 393:55–67

Kavlock RJ et al (1996) Research needs for the risk assessment of health and environmental effects of endocrine disruptors – a report of the U.S. EPA-sponsored workshop. Environ Health Perspect 104:715–740

Kawaguchi M, Sugahara Y, Watanabe T, Irie K, Ishida M, Kurokawa D, Kitamura S-I, Takata H, Handoh IC, Nakayama K, Murakami Y (2012) Nervous system disruption and concomitant behavioral abnormality in early hatched pufferfish larvae exposed to heavy oil. Environ Sci Pollut Res Int 19:2488–2497

Kennedy CJ, Stecko P, Truelson B, Petkovich D (2012) Dissolved organic carbon modulates the effects of copper on olfactory-mediated behaviors of chinook salmon. Environ Toxicol Chem 31:2281–2288

Khoury JN, Powers E, Patnaik P, Wallace WG (2009) Relating disparity in competitive foraging behavior between two populations of fiddler crabs to the subcellular partitioning of metals. Arch Environ Contam Toxicol 56:489–499

Kienle C, Gerhardt A (2008) Behavior of *Corophium volutator* (Crustacea, Amphipoda) exposed to the water-accommodated fraction of oil in water and sediment. Environ Toxicol Chem 27:599–604

Koltes KH (1985) Effects of sublethal copper concentrations on the structure and activity of Atlantic silverside schools. Trans Am Fish Soc 114:413–422

Krang AS (2007) Naphthalene disrupts pheromone induced mate search in the amphipod *Corophium volutator* (Pallas). Aquat Toxicol 85:9–18

Krang AS, Dahlstrom M (2006) Effects of a candidate antifouling compound (medetomidine) on pheromone induced male search in the amphipod *Corophium volutator*. Mar Pollut Bull 52:1776–1783

Krang A-S, Ekerholm M (2006) Copper reduced mating behaviour in male shore crabs (*Carcinus maenas* L.). Aquat Toxicol 80:60–69

Kraus M, Kraus DB (1986) Differences in the effects of mercury on predator avoidance in two populations of the grass shrimp *Palaemonetes pugio*. Mar Environ Res 18:277–289

Krebs CT, Burns KA (1977) Long-term effects of an oil spill on populations of the salt-marsh crab *Uca pugnax*. Science 197:484–487

Lang WH, Miller DC, Ritacco PJ, Marcy M (1981) The effects of copper and cadmium on the behavior and development of barnacle larvae. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Biological monitoring of marine pollutants. Academic Press, New York, pp 165–203

Lesage V, Barrette C, Kingsley MCS, Sjare B (1999) The effect of vessel noise on the vocal behaviour of Belugas in the St. Lawrence River estuary, Canada. Mar Mamm Sci 15:65–84

Little EE, Finger SE (1990) Swimming behavior as an indicator of sublethal toxicity in fish. Environ Toxicol Chem 9:13–19

Liu CC, Chiu JMY, Li L, Shin PKS, Cheung SG (2011) Respiration rate and swimming activity of larvae of two sub-tidal nassariid gastropods under reduced oxygen levels: implications for their distributions in Hong Kong waters. Mar Pollut Bull 63:230–236

Long WC, Seitz R (2008) Trophic interactions under stress: hypoxia enhances foraging in an estuarine food web. Mar Ecol Prog Ser 362:59–68

Lower N, Moore A (2007) The impact of a brominated flame retardant on smoltification and olfactory function in Atlantic salmon (*Salmo salar* L) smolts. Mar Freshw Behav Physiol 40:267–284

Lynn SE, Egar JM, Walker BG, Sperry TS, Ramenofsky M (2007) Fish on Prozac: a simple, noninvasive physiology laboratory investigating the mechanisms of aggressive behavior in *Betta splendens*. Adv Physiol Educ 31:358–363

Matta MB, Linse L, Cairncross C, Francendese L, Kocan RM (2001) Reproductive and transgenerational effects of methylmercury or Aroclor 1268 on *Fundulus heteroclitus*. Environ Toxicol Chem 20:327–335

McCarthy ID, Fuiman LA, Alvarez MC (2003) Aroclor 1254 affects growth and survival skills of Atlantic croaker *Micropogonias undulatus*. Mar Ecol Prog Ser 252:295–301

McCauley RD, Fewtrell J, Popper AN (2003) High intensity anthropogenic sound damages fish ears. J Acoust Soc Am 113:638–642

McCormick SD, Hansen LP, Quinn TP, Saunders RL (1998) Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 55(supp. 1):77–92

McIntyre J, Baldwin DH, Meador JP, Scholz NL (2008) Chemosensory deprivation in juvenile coho salmon exposed to dissolved copper under varying water chemistry conditions. Environ Sci Technol 42:1352–1358

McIntyre J, Baldwin DH, Beauchamp DA, Scholz NL (2012) Low-level copper exposures increase visibility and vulnerability of juvenile coho salmon to cutthroat trout predators. Ecol Appl 22:1460–1471

Miksis-Olds JL, Miller JH (2006) Transmission loss in manatee habitats. J Acoust Soc Am 120:2320–2327

Miller PJO, Biassoni N, Samuels A, Tyack PL (2000) Whale songs lengthen in response to sonar. Nature 405:903

Mirkes DZ, Vernberg WB, DeCoursey PJ (1978) Effects of cadmium and mercury on the behavioral responses and development of *Eurypanopeus depressus* larvae. Mar Biol 47:143–147

Moore A, Lower N (2001) The impact of two pesticides on olfactory-mediated endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. Comp Biochem Physiol B 129:269–276

Moore A, Waring CP (2001) The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.). Aquat Toxicol 52:1–12

Moore A, Lower N, Mayer I, Greenwood L (2007) The impact of a pesticide on migratory activity and olfactory function in Atlantic salmon (*Salmo salar* L.) smolts. Aquaculture 273:350–359

Munari C, Mistri M (2011) Short-term hypoxia modulates *Rapana venosa* (Muricidae) prey preference in Adriatic lagoons. J Exp Mar Biol Ecol 407:166–170

Munari C, Mistri M (2012) Short-term sublethal hypoxia affects a predator–prey system in northern Adriatic transitional waters. Estuar Coast Shelf Sci 97:136–140

Munday PL, Dixson DL, Donelson JM, Jones GP, Pratchett MS, Devitsina GV, Doving KB (2009) Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. Proc Natl Acad Sci USA 106:1848–1852

Narahashi T (1996) Neuronal ion channels as the target sites of insecticides. Pharmacol Toxicol 78:1–14

Nilsson GE, Dixson DL, Domenici P, McCormick MI, Sørensen C, Watson S-A, Munday PL (2012) Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. Nat Clim Change 2:201–204

Nowacek DP, Thorne LH, Johnston DW, Tyack PL (2007) Responses of cetaceans to anthropogenic noise. Mamm Rev 37:81–115

Nowicki JP, Miller GM, Munday PL (2012) Interactive effects of elevated temperature and CO_2 on foraging behavior of juvenile coral reef fish. J Exp Mar Biol Ecol 412:46–51

Oakden JM, Oliver JS, Flegal AR (1984) Behavioral responses of a phoxocephalid amphipod to organic enrichment and trace metals in sediment. Mar Ecol Prog Ser 14:253–257

Olesiuk PF, Nichol LM, Sowden MJ, Ford JK (2002) Effect of the sound generated by an acoustic harassment device on the relative abundance and distribution of harbor porpoises (*Phocoena phocoena*) in Retreat Passage, British Columbia. Mar Mamm Sci 18:843–862

Oliveira C, Almeida J, Guilhermino L, Soares AM, Gravato C (2012) Acute effects of deltamethrin on swimming velocity and biomarkers of the common prawn *Palaemon serratus*. Aquat Toxicol 124–125:209–216

Oliveira C, Almeida JR, Guilhermino L, Soares AM, Gravato C (2013) Swimming velocity, avoidance behavior and biomarkers in *Palaemon serratus* exposed to fenitrothion. Chemosphere 90:936–944

Ososkov I, Weis JS (1996) Development of social behavior in larval mummichogs after embryonic exposure to methylmercury. Trans Am Fish Soc 125:983–987

Parks SE, Clark CW, Tyack PL (2007) Short- and long-term changes in right whale calling behaviour: the potential effects of noise on acoustic communication. J Acoust Soc Am 122:3725–3731

Parks SE, Johnson M, Nowacek D, Tyack PL (2011) Individual right whales call louder in increased environmental noise. Biol Lett 7:33–35

Pavlov DD, Chuiko GM, Gerassimov YV, Tonkopiy VD (1992) Feeding behavior and brain acetylcholinesterase activity in bream (*Abramis brama* L.) as affected by DDVP, an organophosphorus insecticide. Comp Biochem Physiol C 103:563–568

Peng S, Huang J, Xiong T, Huang M (2004) Effects of four heavy metals on toxicity and feeding behavior of soft coral (*Subergorgia suberosa*). J Oceanogr Taiwan Strait 23:293–301

Perez MH, Wallace WG (2004) Differences in prey capture in grass shrimp, *Palaemonetes pugio*, collected along an environmental impact gradient. Arch Environ Contam Toxicol 46:81–89

Phelps H, Pearson WH, Hardy JT (1985) Clam burrowing behaviour and mortality related to sediment copper. Mar Pollut Bull 16:309–313

Pichavant K, Person-Le-Ruyet J, Le Bayon N, Severe A, Le Roux A, Boeuf G (2001) Comparative effects of long-term hypoxia on growth, feeding and oxygen consumption in juvenile turbot and European sea bass. J Fish Biol 59:875–883

Pihl L, Baden SP, Diaz RJ, Schaffner LC (1992) Hypoxia-induced structural changes in the diet of bottom-feeding fish and Crustacea. Mar Biol 112:349–361

Pynnönen K (1996) Heavy metal-induced changes in the feeding and burrowing behaviour of a Baltic isopod, *Saduria* (*Mesidotea*) *entomon* L. Mar Environ Res 41:145–156

Reddy PS, Fingerman M (1995) Effect of cadmium chloride on physiological color changes of the fiddler crab *Uca pugilator*. Ecotoxicol Environ Saf 31:69–75

Reddy SLN, Venugopal NB (1993) Effect of cadmium on acetylcholinesterase activity and oxygen consumption in a freshwater field crab *Barytelphusa guerini*. J Environ Biol 14:203–210

Rehnberg BC, Schreck CB (1986) Acute metal toxicology of olfaction in coho salmon: behavior, receptors, and odor-metal complexation. Bull Environ Contam Toxicol 36:579–586

Reichelt-Brushett AJ, Harrison PL (2004) Development of a sublethal test to determine the effects of copper and lead on scleractinian coral larvae. Arch Environ Contam Toxicol 47:40–55

Reichmuth JM, Roudez R, Glover T, Weis JS (2009) Differences in prey capture behavior in populations of blue crab (*Callinectes sapidus* Rathbun) from contaminated and clean estuaries in New Jersey. Estuar Coast 32:298–308

Reichmuth JM, MacDonald J, Ramiriz J, Weis JS (2011) Fight or flight: an investigation of aggressive behavior and predator avoidance in two populations of blue crabs (*Callinectes sapidus* Rathbun) in New Jersey. Hydrobiologia 658:173–182

Reynolds JD, Jones JC (1999) Female preference for preferred males is reversed under low oxygen conditions in the common goby (*Pomatoschistus microps*). Behav Ecol 10:149–154

Ripley J, Foran C (2007) Influence of estuarine hypoxia on feeding and sound production by two sympatric pipefish species (Syngnathidae). Mar Environ Res 63:350–367

Risch D, Corkeron PJ, Ellison WT, Van Parijs SM (2012) Changes in humpback whale song occurrence in response to an acoustic source 200 km away. PLoS One 7(1):e29741. doi:10.1371/journal.pone.0029741

Roast SD, Widdows J, Jones MB (2001) Impairment of mysid (*Neomysis integer*) swimming ability: an environmentally realistic assessment of the impact of cadmium exposure. Aquat Toxicol 52:217–227

Rolland RM, Parks SE, Hunt KE, Castellote M, Corkeron PJ, Nowacek DP, Wasser SK, Kraus SD (2012) Evidence that ship noise increases stress in right whales. Proc R Soc Lond B 279:2363–2368

Romano TA, Keogh MJ, Kelly C, Feng P, Berk L, Schlundt C, Carder D, Finneran J (2004) Anthropogenic sound and marine mammal health: measures of the nervous and immune systems before and after intense sound exposure. Can J Fish Aquat Sci 61:1124–1134

Roper DS, Hickey CW (1994) Behavioural responses of the marine bivalve *Macomona liliana* exposed to copper- and chlordane-dosed sediments. Mar Biol 118:673–680

Roper DS, Nipper MG, Hickey CW, Martin ML, Weatherhead MA (1995) Burial, crawling and drifting behaviour of the bivalve *Macomona liliana* in response to common sediment contaminants. Mar Pollut Bull 31:471–478

Saaristo M, Craft JA, Lehtonen KK, Björk H, Lindström K (2009) Disruption of sexual selection in sand gobies (*Pomatoschistus minutus*) by 17α-ethinyl estradiol, an endocrine disruptor. Horm Behav 55:530–537

Sagasti A, Schaffner LC, Duffy JE (2001) Effects of periodic hypoxia on mortality, feeding and predation in an estuarine epifaunal community. J Exp Mar Biol Ecol 258:257–283

Sandahl J, Balswin D, Jenkins J, Scholz N (2004) Odor-evoked field potentials as indicators of sublethal neurotoxicity in juvenile coho salmon (*Oncorhynchus kisutch*) exposed to copper, chlorpyrifos, or esfenvalerate. Can J Fish Aquat Sci 61:404–413

Sandahl JF, Baldwin DH, Jenkins JJ, Scholz NL (2005) Comparative thresholds for acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos. Environ Toxicol Chem 24:136–145

Sárria MP, Santos MM, Reis-Henriques MA, Vieira NM, Monteiro NM (2011a) The unpredictable effects of mixtures of androgenic and estrogenic chemicals on fish early life. Environ Int 37:418–424

Sárria MP, Santos MM, Reis-Henriques MA, Vieira NM, Monteiro NM (2011b) Drifting towards the surface: a shift in newborn pipefish's vertical distribution when exposed to the synthetic steroid ethinylestradiol. Chemosphere 84:618–624

Saucier D, Astic L, Riouz P (1991) The effects of early chronic exposure to sublethal copper on the olfactory discrimination of rainbow trout, *Oncorhynchus mykiss*. Environ Biol Fish 30:345–351

Scarfe AD, Jones KA, Steele CE, Kleerekoper H, Corbett M (1982) Locomotor behavior of four marine teleosts in response to sublethal copper exposure. Aquat Toxicol 2:335–353

Scholz NL, Truelove NK, French BL, Berejikian BA, Quinn TP, Casillas E, Collier TK (2000) Diazinon disrupts antipredator and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*). Can J Fish Aquat Sci 57:1911–1918

Scott GR, Sloman KA (2004) The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquat Toxicol 68:369–392

Sebire M, Allen Y, Bersuder P, Katsiadaki I (2008) The model anti-androgen flutamide suppresses the expression of typical male stickleback reproductive behaviour. Aquat Toxicol 90:37–47

Sebire M, Scott AP, Tyler CR, Cresswell J, Hodgson DJ, Morris S, Sanders MB, Stebbing PD, Katsiadaki I (2009) The organophosphorous pesticide, fenitrothion, acts as an anti-androgen and alters reproductive behavior of the male three-spined stickleback, *Gasterosteus aculeatus*. Ecotoxicology 18:122–133

Sebire M, Katsiadaki I, Taylor NG, Maack G, Tyler CR (2011) Short-term exposure to a treated sewage effluent alters reproductive behaviour in the three-spined stickleback (*Gasterosteus aculeatus*). Aquat Toxicol 105:78–88

Shin P, Ng AWM, Cheung RYH (2002) Burrowing responses of the short-neck clam *Ruditapes philippinarum* to sediment contaminants. Mar Pollut Bull 45:133–139

Simpson SD, Munday PL, Wittenrich ML, Manassa R, Dixson DL, Gagliano M, Yan HY (2011) Ocean acidification erodes crucial auditory behavior in marine fish. Biol Lett 7(6):917–920, 10.1098

Skalski JR, Pearson WH, Malme CI (1992) Effects of sounds from a geophysical survey device on catch-per-unit-effort in a hook-and-line fishery for rockfish (*Sebastes* spp.). Can J Fish Aquat Sci 49:1357–1365

Sloman KA, McNeil PL (2012) Using physiology and behaviour to understand the responses of fish early life stages to toxicants. J Fish Biol 81:2175–2198

Smith GM, Weis JS (1997) Predator/prey interactions of the mummichog, *Fundulus heteroclitus*: effects of living in a polluted environment. J Exp Mar Biol Ecol 209:75–87

Smith GM, Khan AT, Weis JS, Weis P (1995) Behavior and brain correlates in mummichogs (*Fundulus heteroclitus*) from polluted and unpolluted environments. Mar Environ Res 39:329–333

Sobrino-Figueroa A, Cáceres-Martínez C (2009) Alterations of valve closing behavior in juvenile Catarina scallops (*Argopecten ventricosus* Sowerby, 1842) exposed to toxic metals. Ecotoxicology 18:983–987

Solé AM, Lenoir M, Durfort M, Quero C, Mas A, Lombarte A, van der Schaar M, López-Bejar M, Morell M, Zaugg S, Houégnigan L (2011) Low-frequency sounds induce acoustic trauma in cephalopods. Front Ecol Environ 9:489–493

Spachmo B, Arukwe A (2012) Endocrine and developmental effects in Atlantic salmon (*Salmo salar*) exposed to perfluorooctane sulfonic or perfluorooctane carboxylic acids. Aquat Toxicol 108:112–124

Staub GC, Fingerman M (1984) A mechanism of action for the inhibition of black pigment dispersion in the fiddler crab *Uca pugilator*, by naphthalene. Comp Biochem Physiol 79C:447–453

Staurnes M, Kroglund F, Rosseland BO (1996a) Water quality requirement of Atlantic salmon (*Salmo salar*) in water undergoing acidification or liming in Norway. Water Air Soil Pollut 85:347–352

Staurnes M, Hansen LP, Fugelli K, Haraldstad O (1996b) Short-term exposure to acid water impairs osmoregulation, seawater tolerance, and subsequent marine survival of smolt of Atlantic salmon (*Salmo salar* L.). Can J Fish Aquat Sci 53:1695–1704

Steirhoff KL, Targett T, Miller K (2006) Ecophysiological responses of juvenile summer and winter flounder to hypoxia: experimental and modeling analyses of effects on estuarine nursery quality. Mar Ecol Prog Ser 325:255–266

Stephens SM, Alkindi AYA, Waring CP, Brown JA (1997) Corticosteroid and thyroid responses of larval and juvenile turbot exposed to the water-soluble fraction of crude oil. J Fish Biol 50:953–964

Studholme AL, Bejda AJ, Olla BL (1987) Changes in burrowing, emergence, and feeding of the bloodworm, *Glycera dibranchiata* (Ehlers) induced by oil-contaminated sediment. In: Vernberg WB, Calabrese A, Thurberg FP, Vernberg FJ (eds) Pollution physiology of estuarine organisms. University of South Carolina Press, Columbia, pp 69–86

Sullivan BK, Buskey E, Miller DC, Ritacco PJ (1983) Effects of copper and cadmium on growth, swimming and predator avoidance in *Eurytemora affinis* (Copepoda). Mar Biol 77:299–306

Tanouka OF, Buffet PE, Amiard JC, Berthet B, Mouneyrac C, Amiard-Triquet C (2013) Integrated assessment of estuarine sediment quality based on a multi-biomarker approach in the bivalve *Scrobicularia plana*. Ecotoxicol Environ Saf 88:117–125

Taylor DL, Eggleston DB (2000) Effects of hypoxia on an estuarine predator–prey interaction: foraging behavior and mutual interference in the blue crab *Callinectes sapidus* and the infaunal clam prey *Mya arenaria*. Mar Ecol Prog Ser 196:221–237

Thetmeyer H, Waller U, Black KD, Inselmann S, Rosenthal H (2001) Growth of European sea bass (*Dicentrarchus labrax* L.) under hypoxic and oscillating oxygen conditions. Aquaculture 174:355–367

Thomas RE, Rice SD, Korn S (1987) Reduced swimming performance of juvenile coho salmon (*Oncorhynchus kisutch*) exposed to the water-soluble fraction of Cook Inlet crude oil. In: Vernberg WB, Calabrese A, Thurberg FP, Vernberg FJ (eds) Pollution physiology of estuarine organisms. University of South Carolina Press, Columbia, pp 127–137

Tierney KB, Ross PS, Jarrod HE, Delaney KR, Kennedy CJ (2006) Changes in juvenile coho salmon electro-olfactogram during and after short-term exposure to current-use pesticides. Environ Toxicol Chem 25:2809–2817

Timme-Laragy AR, Levin ED, Giulio RT (2006) Developmental and behavioral effects of embryonic exposure to the polybrominated diphenylether mixture DE-71 in the killifish (*Fundulus heteroclitus*). Chemosphere 62:1097–1104

Toppin SV, Heber M, Weis JS, Weis P (1987) Changes in reproductive biology and life history in *Fundulus heteroclitus* in a polluted environment. In: Vernberg W, Calabrese A, Thurberg F, Vernberg FJ (eds) Pollution physiology of estuarine organisms. University of South Carolina Press, Columbia, pp 171–184

Ugolini A, Pasquali V, Baroni D, Ungherese G (2012) Behavioural responses of the supralittoral amphipod *Talitrus saltator* (Montagu) to trace metals contamination. Ecotoxicology 21:139–147

Vasconcelos RO, Amorim MC, Ladich F (2007) Effects of ship noise on the detectability of communication signals in the Lusitanian toadfish. J Exp Biol 210:2104–2112

Viera LR, Gravato C, Soares AM, Morgado F, Guilhermina L (2009) Acute effects of copper and mercury on the estuarine fish *Pomatoschistus microps*: linking biomarkers to behavior. Chemosphere 76:1416–1427

Wahlberg M, Westerberg H (2005) Hearing in fish and their reactions to sounds from offshore wind farms. Mar Ecol Prog Ser 288:295–309

Wallace WG, Estephan A (2004) Differential susceptibility of horizontal and vertical swimming activity to cadmium exposure in a gammaridean amphipod (*Gammarus lawrencianus*). Aquat Toxicol 69:289–297

Wallace WG, Hoexum Brouwer TM, Brouwer M, Lopez GR (2000) Alterations in prey capture and induction of metallothioneins in grass shrimp fed cadmium-contaminated prey. Environ Toxicol Chem 19:962–971

Wang Y, Hu M, Cheung SG, Shin PK, Lu W, Li J (2012) Chronic hypoxia and low salinity impair anti-predatory responses of the green-lipped mussel *Perna viridis*. Mar Environ Res 77:84–89

Ward AJW, Duff AJ, Horsfall JS, Currie S (2008) Scents and scents-ability: pollution disrupts chemical social recognition and shoaling in fish. Proc R Soc B 274:101–105

Weis JS, Candelmo A (2012) Pollution and fish predator/prey behavior: a review of laboratory and field approaches. Curr Zool 58:9–20

Weis JS, Khan AA (1990) Effects of mercury on the feeding behavior of the mummichog, *Fundulus heteroclitus* from a polluted habitat. Mar Environ Res 30:243–249

Weis JS, Perlmutter J (1987a) Burrowing behavior by the fiddler crab, *Uca pugilator*: inhibition by the insecticide diflubenzuron. Mar Ecol Prog Ser 38:109–113

Weis JS, Perlmutter J (1987b) Effects of tributyltin on activity and burrowing behavior in the fiddler crab, *Uca pugilator*. Estuaries 10:342–346

Weis P, Weis JS (1974) Schooling behavior of *Menidia menidia* in the presence of the insecticide Sevin (carbaryl). Mar Biol 28:261–263

Weis JS, Weis P (1995a) Effects of embryonic exposure to methylmercury on larval prey capture ability in the mummichog, *Fundulus heteroclitus*. Environ Toxicol Chem 14:153–156

Weis JS, Weis P (1995b) Effects of embryonic and larval exposure to methylmercury on larval swimming performance and predator avoidance in the mummichog, *Fundulus heteroclitus*. Can J Fish Aquat Sci 52:2168–2173

Weis JS, Weis P (1998) Effects of lead on behaviors of larval mummichogs, *Fundulus heteroclitus*. J Exp Mar Biol Ecol 222:1–10

Weis JS, Samson J, Zhou T, Skurnick J, Weis P (2001) Prey capture ability by mummichogs (*Fundulus heteroclitus*) as a behavioral biomarker for contaminants in estuarine systems. Can J Fish Aquat Sci 58:1442–1452

Wibe AE, Nordtug T, Jenssen BM (2001) Effects of bis(tributyltin)oxide on antipredator behavior in threespine stickleback *Gasterosteus aculeatus* L. Chemosphere 44:475–481

Wibe A, Rosenqvist G, Jenssen BM (2002) Disruption of male reproductive behavior in threespine stickleback *Gasterosteus aculeatus* exposed to 17β-Estradiol. Environ Res 90:136–141

Williams MA, Coutant CC (2003) Modification of schooling behavior in larval Atherinid fish *Atherina mochon* by heat exposure of eggs and larvae. Trans Am Fish Soc 132:638–645

Wilson JE, Forward RB, Costlow JD (1985) Effects of embryonic exposure to sublethal concentrations of Dimilin® on the photobehavior of grass shrimp larvae. In: Vernberg FJ, Thurberg FP, Calabrese A, Vernberg WB (eds) Marine pollution and physiology: recent advances. University of South Carolina Press, Columbia, pp 377–396

Wilson JE, Forward RB, Costlow JD (1987) Delayed effects of diflubenzuron on the swimming and vertical distribution of *Palaemonetes pugio* larvae. In: Vernberg WB, Calabrese A, Thurberg FP, Vernberg FJ (eds) Pollution physiology of estuarine organisms. University of South Carolina Press, Columbia, pp 351–371

Xuereb B, Lefèvre E, Garric J, Geffard O (2009) Acetylcholinesterase activity in *Gammarus fossarum* (Crustacea Amphipoda): linking AChE inhibition and behavioural alteration. Aquat Toxicol 94:114–122

Yu A, Wang X, Zuo Z, Cai J, Wang C (2013) Tributyltin exposure influences predatory behavior, neurotransmitter content and receptor expression in *Sebastiscus marmoratus*. Aquat Toxicol 128–129:158–162

Zhou T, Weis JS (1998) Swimming behavior and predator avoidance in three populations of *Fundulus heteroclitus* larvae after embryonic and/or larval exposure to methylmercury. Aquat Toxicol 43:131–148

Zhou T, John-Alder HB, Weis P, Weis JS (1999a) Thyroidal status of mummichogs (*Fundulus heteroclitus*) from a polluted versus a reference habitat. Environ Toxicol Chem 18:2817–2823

Zhou T, Rademacher D, Steinpreis RE, Weis JS (1999b) Neurotransmitter levels in two populations of larval *Fundulus heteroclitus* after methylmercury exposure. Comp Biochem Physiol C 124:287–294

Zhou T, Scali R, Weis JS (2001) Effects of methylmercury on ontogeny of prey capture ability and growth in three populations of larval *Fundulus heteroclitus*. Arch Environ Contam Toxicol 41:47–54

Zizza M, Giusi G, Crudo M, Canonaco M, Facciolo RM (2013) Lead-induced neurodegenerative events and abnormal behaviors occur *via* ORXRergic/GABA$_A$Rergic mechanisms in a marine teleost. Aquat Toxicol 126:231–241

Part IV
Dealing with Pollutants

Chapter 10
Bioaccumulation/Storage/Detoxification

Abstract Bioavailability refers to the fraction of the total chemical in the environment that is available for absorption into biota. This depends on the chemical, the organism, and environmental conditions, such as temperature, DO, and pH. Uptake of contaminants generally is via the skin, respiratory system, or food, with food being a major route of uptake for species that are higher on the food web such as large carnivorous fishes or mammals. Hydrophobic compounds that have low water solubility (log octanol/water partition coefficient – log K_{ow} >6) are absorbed primarily from food, while compounds with low hydrophobicity (log K_{ow} <4) are more water-soluble, and are absorbed directly from the water, primarily by the gills. Hydrophobic chemicals including halogenated organics and metals that have low water solubility tend to be adsorbed to sediments, which are the main sink and source for uptake into biota (via pore water). They also partition onto particulates and small planktonic organisms. Once absorbed, only a portion of the chemical actually reaches the circulation for distribution around an animal's body. This amount is affected by processes including absorption, transport, biotransformation, and excretion via gills or kidneys. Within cells, metals can become associated with metallothioneins or metal rich granules, which make them unavailable, while metals associated with enzymes can cause toxicity. The subcellular distribution also affects the degree of trophic transfer. Persistent organic chemicals tend to accumulate in fatty tissue and to biomagnify up food webs. Organic chemicals are metabolized with the cytochrome P450 (CYP) enzyme system. Both metals and organic chemicals tend to accumulate in the liver or hepatopancreas; lipophilic chemicals accumulate in the blubber of large animals such as marine mammals.

While previous chapters have focused on what pollutants do to marine organisms, this chapter deals with what marine organisms do to pollutants – taking them up from the environment and doing something with them such as storage or metabolism. Uptake can be through the skin, gills, or gut and may involve adsorption, passive diffusion, active transport, or endocytosis. The specific kinetics of uptake relate to the concentration and nature of the contaminant. The term bioaccumulation is generally used to describe uptake, but there are specific terms

J.S. Weis, *Physiological, Developmental and Behavioral Effects of Marine Pollution*, DOI 10.1007/978-94-007-6949-6_10,

and acronyms that refer to variations in processes but are not always used correctly. *Bioaccumulation* refers to uptake from all sources in the environment. The bioaccumulation factor (BAF or BSAF) refers to the concentration in the organism relative to that of the sediment, when that is the major source of accumulation. If the concentration is normalized to the amount of lipid in the organism and the amount of organic carbon in sediments, the ratio may be referred to as the accumulation factor (AF). *Bioconcentration* is a more specific term that refers to accumulation from water. The bioconcentration factor (BCF) is the concentration of a chemical in the organism relative to that in the water. *Biomagnification* refers to increasing concentrations of a persistent contaminant from one trophic level to the next in a food chain, due to accumulation from food. The biomagnification factor (BMF) is the concentration at one trophic level divided by that at the trophic level below.

Keywords Absorption • Biomagnification • Depuration • Cytochrome • Detoxification • Granule • Hepatopancreas • Lysosome • Metallothionein • Mixed-function oxidase (MFO) • Stress protein • Trophically available metal (TAM) • Uptake

10.1 Metals

Life evolved in the presence of metals, and some metals are part of essential molecules (enzymes) and metabolic processes. Cu, Zn, and Fe are major essential metals, while trace amounts of Co Ni, Mn, Ni, Mb and Cr are also needed. Metals, particularly Zn, Cu, and Fe, may help with proper protein folding. Metal contamination of terrestrial and aquatic systems dates as far back as human civilization, but with the industrialization of the eighteenth century severe environmental impacts of metals released by mining and smelting began to pose threats to wildlife. The ionic form of most metals is the most bioavailable, and is taken up to the greatest degree. Of all the toxic metals, only mercury (as methylmercury) biomagnifies up food webs.

10.1.1 Bioaccumulation

Crustaceans

Since crustaceans are covered with an impervious chitinous exoskeleton, uptake in large animals is mostly via the gills or food, except perhaps directly after ecdysis, when the cuticle is thin and permeable. Smaller crustaceans can take up chemicals through the thin exoskeleton. The subsequent fate of the metal depends on the physiology of the animal and whether or not the metal is essential. The accumulation pattern of different metals varies considerably among taxa depending on the degree to which excretion plays a role and where the metal is stored. Essential metals tend to be regulated to optimum concentrations, above which excretion removes the excess. Potentially toxic metals must either be excreted or stored in a non-available form if they are not to cause damage. Toxicity occurs when the concentration exceeds the amount that can be stored in these forms or excreted. Metals tend to be stored in specific tissues such as the hepatopancreas (Rainbow 1988), which generally has the highest concentration of Cd, Zn, Cu, Pb, and Cr. However, significant levels may also be found in muscle, which has implications for human consumption of edible species. For example, in many crustaceans including blue crabs, Hg (which has caused serious effects in humans from consuming fish) accumulates largely in muscle (Reichmuth et al. 2010). Cd is taken up and accumulated from the water and food (Devi et al. 1996), and stored and detoxified mainly in the hepatopancreas (White and Rainbow 1986). Blue crabs accumulate Cd from food in the hepatopancreas and from water in the gills (Engel 1983). The order of Zn accumulation was found by Bagatto and Alikhan (1987) to be: hepatopancreas > exoskeleton > digestive tract > abdominal muscle. Engel et al. (1985) compared responses of blue crabs and lobsters to Cd, and found that both species accumulate it in the digestive gland (hepatopancreas) but only blue crabs accumulated significant amounts in the gills.

Cu in crustaceans is also essential as part of the hemocyanin molecule. It is regulated to a roughly constant level until bioavailability exceeds a threshold and net accumulation begins (White and Rainbow 1982; Rainbow and White 1989). Excess Cu and Zn can be rapidly depurated when the animals are put in clean water (Kouba et al. 2010). The shrimp *Palaemon elegans* can match excretion to uptake of certain metals such as Zn (Rainbow 1993). In other cases, such as the amphipod *Orchestia gammarellis*, excretion of Cu takes place from detoxified stores (Nassiri et al. 2000). Martins et al. (2011) showed that Cu flux into the gills of blue crabs was higher than into other tissues, and that both anterior and posterior gills were sites of accumulation at both high and low salinity. Experiments with isolated perfused gills showed a positive relationship between metal concentration in the incubation media and Cu accumulation in gills.

Lead is neither essential nor beneficial and accumulates mostly in the hepatopancreas. However, in the prawn *Penaeus monodon*, Pb granules were found in the antennal gland and to a lesser degree in the hepatopancreas. In the antennal gland, Pb granules were discharged into the lumen and excreted with the urine (Vogt and Quinitio 1994). Little Pb was transferred trophically from metal-rich worms to the prawn *Penaeus varians*. Prawns consuming metal-contaminated worms showed net accumulation only of Cd (Rainbow et al. 2006).

Since crustaceans periodically molt, depositing metals in the exoskeleton prior to molting provides a possible route for depurating contaminants. However, when grass shrimp (*P. pugio*) were exposed to Zn, or Cd and then placed in clean water to molt, relatively low percentages of their body burden were actually depurated via ecdysis, although about one-fourth of the Cd was removed. It appeared that much of the Cu in the exoskeleton was reabsorbed prior to molting (Keteles and Fleeger 2001). In contrast, fiddler crabs, *U. pugnax*, particularly those from a contaminated

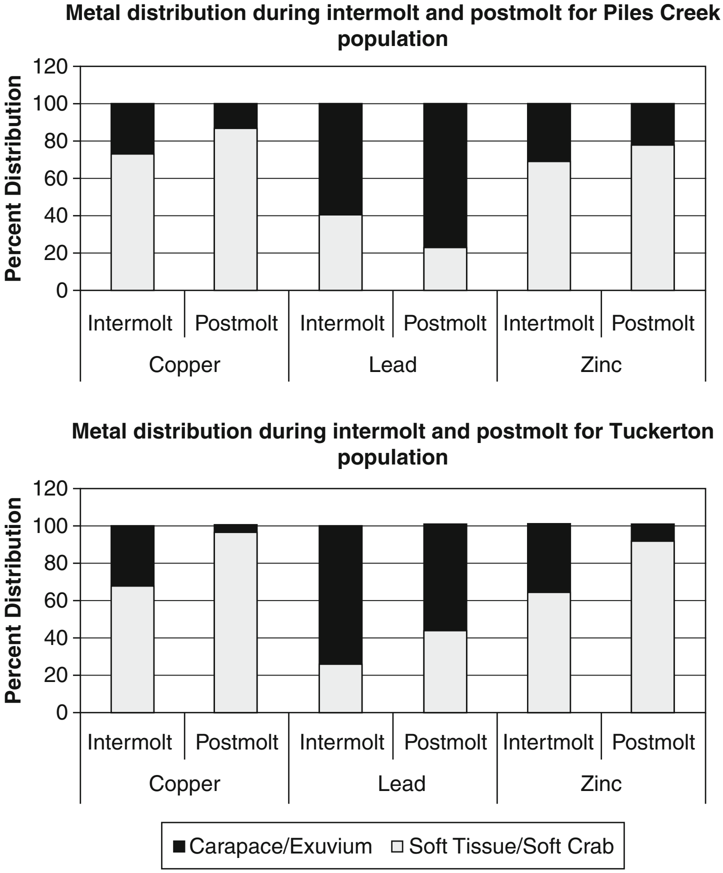


Fig. 10.1 Metal distribution between soft tissues and exoskeleton during intermolt and post-molt for the Piles Creek (polluted) population and Tuckerton (reference) population (Reprinted from Bergey and Weis 2007: 560, courtesy of Elsevier Publishing Co)

site, moved considerable amounts of toxic Pb and Hg from soft tissues into the exoskeleton prior to molting, while moving the essential Cu and Zn from the exoskeleton to the soft tissues (Bergey and Weis 2007) (Fig. 10.1). This was an effective way to depurate the more toxic metals.

Mollusks

Mollusks can accumulate metals from the water, sediments, and food. Assimilation efficiencies (AEs) and physiological turnover-rate constants (*k*) of Ag, Am, Cd, Co, Se, and Zn in the oyster C*rassostrea virginica*, the clams *Macoma balthica* and *Mercenaria mercenaria*, and the mussel *Mytilus edulis* were measured by Reinfelder et al. (1997) in radiotracer-depuration experiments. Egestion of unassimilated elements was highest during the first 24 h and then declined. The AEs of Ag, Cd, Se, and Zn were directly related to the proportion of each element in the cytoplasmic fraction of ingested phytoplankton; >80 % of metals in the algal cytoplasm were assimilated. *C. virginica*, *M. balthica*, and *M. mercenaria* assimilated about one-third of the Ag and Cd in the membrane/organelle fraction of ingested cells as well as the cytoplasmic fraction. The ratio of AE:*k*, which is proportional to the concentration in consumer: concentration in prey was greater for Cd, Se, and Zn than for Ag, Am, and Co. The ratio was lowest in *M. edulis*, indicating that this bivalve, widely used in biomonitoring, is inefficient at accumulating Ag, Cd, and Zn from ingested phytoplankton. This suggests that other bivalves might be better monitors of pollution.

Pan and Wang (2011) examined Hg accumulation in scallops *Chlamys nobilis*, clams *Ruditapes philippinarum*, oysters *Saccostrea cucullata*, green mussels *Perna viridis*, and black mussels *Septifer virgatus*, which showed different patterns of accumulation in terms of concentrations of meHg and total Hg (THg) (0.01–0.5 $\mu g\ l^{-1}$), and the ratio of meHg to THg. Scallops in 0.01 $\mu g\ l^{-1}$ meHg accumulated it at the fastest rate, about 5 $\mu g\ g^{-1}\ day^{-1}$. With the exception of black mussels, there was a significant relationship between efflux rates of Hg(II) and the THg concentrations (increasing from 0.02 to 0.06 day^{-1} with Hg concentration). Interspecific variations in the meHg to THg ratio were due to relative differences between the elimination rates of Hg(II) and meHg. Stable isotope analysis indicated that contrasting feeding niches of the species may affect Hg accumulation.

Mucus secretion is an indicator of stress but also a mechanism of depuration and protection for mollusks. Copper-exposed mussels *Perna viridis* secreted mucus with six times the concentration of Cu than the soft tissues (Sze and Lee 1995). Oysters (*C. virginica*) living on bulkheads of wood treated with CCA (chromated copper arsenate) accumulated extremely high levels of Cu (~200 $\mu g\ g^{-1}$ wet wt.) as well as some arsenic (~3 $\mu g\ g^{-1}$), enough Cu to acquire a green coloration. Highest concentrations were seen in small oysters compared to large ones, suggesting a growth dilution effect (Weis et al. 1993). Snails, *Thais* (*Stramonita*) *haemastoma floridana* that fed on these oysters gradually reduced their feeding, grew more slowly than snails fed clean oysters, and increased their body burden of copper about fourfold over 8 weeks (Weis and Weis 1993).

Gastropods are often predators in marine benthic environments, and trophic transfer is a major route of metal accumulation. The influences of prey composition on the trophic transfer, accumulation, subcellular distribution and metallothionein (MT) induction of Ag, As, Cd, Cu, Pb and Zn in *Nassarius siquijorensis* were investigated (Guo et al. 2013). The snails were fed clams *Ruditapes philippinarum*, mussels *Perna viridis*, oysters *Crassostrea angulata* or barnacles *Fistulobalanus albicostatus*, each differing in their metal accumulation and handling patterns. *N. siquijorensis* showed prey-specific bioaccumulation and trophic transfer of the metals. Body burdens in the viscera and muscles of the snail increased with increasing exposure period and metal concentration in the four prey. Calculated trophic transfer factors (TTFs) of the metals were the highest for clam and mussel prey, indicating that metal bioavailability from these prey was higher than that from barnacles and oysters. All the metals except Pb were enriched during transfer to the snails. The subcellular metal distribution in viscera was affected by prey species, but all induced MTs. Li et al. (2012) compared the accumulation of aqueous vs nanoparticle silver by the herbivorous gastropod, *Littorina littorea*, both in the presence and absence of contaminated algal food (sea lettuce, *Ulva lactuca*). Significant accumulation occurred in the gill, kidney, stomach and visceral mass after exposure to aqueous Ag in the absence of food. Despite the consumption of *U. lactuca* that was contaminated by Ag, no accumulation was observed from the dietary route. The levels of Ag in *U. lactuca* and in the snail feces clearly indicate throughput of Ag in the digestive system of the snail, but there was little mobilization of Ag from contaminated food. This is in contrast with previous studies, above. Trophic transfer of Ag from algae may be lower. When added as nanoparticles, accumulation of Ag was measured in the head and gill and only in the absence of contaminated food. That Ag was only detected in the head and gill suggests the association may be physical adsorption, with little Ag entering internal organs. These observations are also different from other studies, and suggest that Ag is most bioavailable to *L. littorina* when in solution, and that when in nanoparticle form, there is little transfer to internal organs.

Cd and Hg levels were measured in tissue samples of two loliginid (*Alloteuthis* sp. and *Loligo forbesi*) and two ommastrephid (*Todarodes sagittatus* and *Todaropsis eblanae*) squids by Pierce et al. (2008). Cd was generally higher in the ommastrephids in all tissues except muscle. Hg was higher in *T. sagittatus* than in the loliginids. In *L. forbesi*, Cd varied in relation to body size, geographic origin, and season. Cd levels decreased with increasing body size. This may be related to a shift in diet, since small *L. forbesi* feed on benthic invertebrates with relatively high Cd levels, while larger ones prey mainly on fish with lower Cd. In contrast, Hg levels increased with body size, and were highest at the end of the spawning season and in squid from the English Channel and the Scottish West Coast. It is likely that the Hg concentration in seawater plays an important part in its accumulation.

Metal uptake can be affected by ocean acidification. Lacoue-Labarthe et al. (2011) investigated effects of acidification (pH of 8.1, 7.85 and 7.60) on the accumulation of dissolved ^{110m}Ag, ^{109}Cd, ^{57}Co, ^{203}Hg, ^{54}Mn and ^{65}Zn radiotracers in the whole egg strand and in different compartments of the squid *Loligo vulgaris* during embryonic development and early hatchlings. In embryos, decreasing pH enhanced uptake of Ag and Zn, while Hg had the lowest uptake at the intermediate pH. Zn incorporation into statoliths increased with decreasing pH. Conversely, uptake of Cd and Mn decreased with decreasing pH, and the accumulation of Co was unaffected. In hatchlings, uptake of Ag increased with decreasing pH, that of Co was reduced at the lowest pH, and Hg had maximal uptake at the intermediate pH. Mn and Zn accumulation were not changed. These results suggest pH affects metabolism of embryos and hatchlings, and alters metal accumulation in various ways depending on the metal and pH.

Fishes

Fishes also take up metals from the water or food, and the type of food can influence the degree of trophic transfer. Dutton and Fisher (2011a) investigated the trophic transfer of As, Cd, Cr, Hg(II), and meHg from a benthic amphipod (*Leptocheirus plumulosus*) and an oligochaete (*Lumbriculus variegatus*) to killifish (*Fundulus heteroclitus*) using radioisotopes. Except for meHg, absorption efficiency (AE) varied among prey types. AEs were highest for meHg (92 %) and lowest for Cd (2.9–4.5 %) and Cr (0.2–4 %). Hg(II) showed the largest AE difference between prey type (14 % for amphipods vs 24 % for worms). Tissue distribution data showed that Cd and Hg(II) were mainly associated with the intestine, whereas As and meHg were transported throughout the body. Calculated trophic transfer factors (TTFs) suggest that meHg is likely to biomagnify at this trophic step at all ingestion rates, whereas As, Cd, Cr, and Hg(II) will not (Fig. 10.2). This is consistent with previous work indicating that of metals, only meHg biomagnifies.

The same authors (2011b) assessed the role of salinity on the uptake of As, Cd, Cr, inorganic Hg (Hg(II), and meHg into the euryhaline *F. heteroclitus* from the water. Fish were exposed to 4.98 nM ^{73}As, 0.73 nM ^{109}Cd, 0.51 nM ^{51}Cr, 0.51 nM ^{203}Hg(II), and 0.42 nM MeHg. Uptake rate constants were highest for meHg (0.79–2.29 $\mathrm{l\ g^{-1}\ day^{-1}}$), followed by Hg(II), Cd, Cr, and lowest for As. Cr showed no relationship with salinity; Cd, had an inverse relationship; and As, Hg(II), and meHg uptake increased as salinity increased. As and Cr were regulated by the fish, while Cd, Hg(II), and meHg were not. Concentration factors (CFs) were >1 at all salinities for Cd, Hg(II), and meHg, indicating that the fish were more enriched in the metal than the water, whereas As and Cr CFs were <1 at all salinities. Uptake rate was highest for meHg, followed by Hg(II), Cd, Cr, and lowest for As. As salinity increased, Cd increased in the viscera and decreased in the head and gills, suggesting that drinking to osmoregulate may account for some of the uptake. Salinity plays a major role in the toxicity of Zn to *F. heteroclitus*, with the greatest damage (oxidative stress) in freshwater, and the least in seawater (Loro et al. 2012). Shyn et al. (2012) examined uptake of two Zn concentrations: 15 and 75 $\mu\mathrm{g\ l^{-1}}$ in moderately hard freshwater, and 100 and 1,000 $\mu\mathrm{g\ l^{-1}}$ in 35 ppt salinity (saltwater) for 7 days. The ionic Zn concentrations were equivalent in the 75 $\mu\mathrm{g\ l^{-1}}$ freshwater treatment and the 100 $\mu\mathrm{g\ l^{-1}}$ saltwater treatment. Organ Zn distribution was quantified in different organs and different patterns of Zn accumulation were observed dependent on exposure medium. Despite lower exposure concentrations, fish accumulated more Zn in freshwater than in saltwater in all of the organs. Humic acid also plays a role in metal bioaccumulation (Dutton and Fisher 2012). While Cd uptake showed no relationship with humic acid concentration, Cr, Hg(II), and MeHg uptake showed an inverse relationship, and As uptake increased with increasing humic acid concentration. Concentration factors were >1 for Cd, Hg(II), and MeHg at all humic acid concentrations, indicating killifish were more enriched in the metal than the experimental media, whereas As and Cr generally had concentration

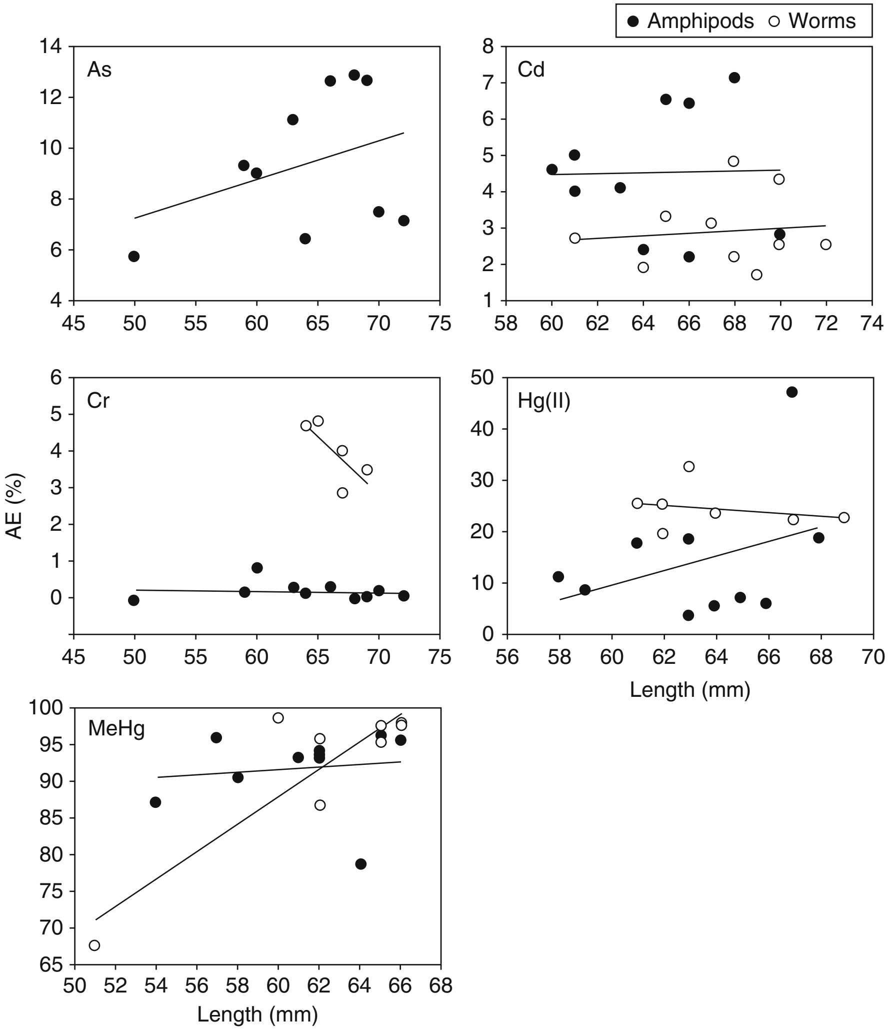


Fig. 10.2 Relationship between fish length and assimilation efficiency (AE) of metals examined for both prey types, amphipods and worms (R^2 values are 0.134 for As, 0.001 for Cd, 0.009 for Cr, 0.128 for Hg (II) and 0.015 for meHg after feeding on amphipods, and 0.012 for Cd, 0.576 for Cr, 0.068 for Hg(II) and 0.778 for meHg after feeding on worms) (Reprinted from Dutton and Fisher 2011a: 3441, courtesy of Elsevier Publishing Co)

factors <1. Gills generally had the highest concentration of all metals under all humic acid treatments. Thus, changes in humic acid concentration can influence the accumulation of aqueous metals in killifish.

Mercury accumulation throughout the lifespan of golden gray mullet *Liza aurata* was analyzed by Tavares et al. (2011) in muscle, gills, liver and brain in sites with differential Hg contamination. Hg was higher in all tissues in contaminated bays, as expected. The liver had the highest levels, ranging from 0.11 to 4.2 μg g^{-1}, followed by muscle, brain, and gills. Most sampling sites and tissues showed a dilution pattern as fish got bigger (Fig. 10.3). Organic Hg in muscle accounted for over 95 % of total Hg, and followed the same accumulation pattern of total Hg. While, in general, meHg biomagnifies and accumulates over time reaching highest levels in old large fish, if growth is particularly rapid somatic growth dilution can occur.

Similarly, in juvenile Atlantic salmon, large, fast-growing fish were found to have lower Hg concentrations than small, slow-growing ones (Ward et al. 2010). Hg concentration in the prey accounted for 59 % of the explained variation in the Hg concentration, and salmon growth rate accounted for 38 %. Juvenile European sea bass (*Dicentrarchus labrax*) and golden grey mullet (*L. aurata*) were surveyed in a Hg-contaminated estuary. Hg accumulated primarily in intestine, muscle, and liver, while gills and brain had the lowest Hg (Mieiro et al. 2012). *D. labrax* muscle levels were greater than *L. aurata*'s. Unlike the species above, muscle accounted for >87 % of the Hg tissue burden, whereas liver did not exceed 11 %. Organic Hg accumulation occurred mainly in liver and muscle, with *D. labrax* having higher loads. Organic Hg in prey items was also significantly greater in *D. labrax*; showing that feeding habits are important in determining metal accumulation. In Baltic Sea turbot (*Scophthalmus maximus*), muscle had the highest Hg concentration, with levels approaching 140 μg kg^{-1} in 30-cm fish, while livers had only 50–60 μg kg^{-1}. The BAF and BMF showed that muscle had maximum affinity for Hg, and thus best reflected environmental metals (Polak-Juszczak 2012). Finding the highest Hg concentration in muscle of edible species is a concern for human consumers.

The uptake of meHg has been found to be reduced by the presence of selenium in a number of organisms and Se can protect against some Hg toxicity. While this has been studied mostly in mammals, Huang et al. (2013) investigated interactive effects of Se and Hg on their absorption, disposition, and elimination in juvenile white sturgeon (*Acipenser transmontanus*). Selenium was provided as l-selenomethionine (SeMet). Sturgeon were orally intubated with a single dose of either 0 (control), SeMet (500 μg Se kg body weight^{-1}; BW), MeHg (850 μg Hg kg BW^{-1}), or their combination (500 μg Se kg BW^{-1} plus 850 μg Hg kg BW^{-1}). The combination of SeMet and MeHg significantly lowered blood concentrations of both Se and Hg and tissue Se. Even in the absence of exogenous SeMet, Se and Hg co-accumulated in tissue at a Se:Hg molar ratio greater than 1. Thus, similar to mammals, maintaining at least a 1:1 M ratio of Se and Hg is of great physiological importance. Interestingly, SeMet did not prevent Hg from accumulating in the brain.

Other Taxa

Corals accumulate metals in their skeletons, which can be used to record the chronology of metal pollution (Dodge and Gilbert 1984). Historic input of mine tailings in the Philippines was traced through variations in metals in *Porites* growth bands (David 2002). Peaks coincided with a documented release of mine tailings in

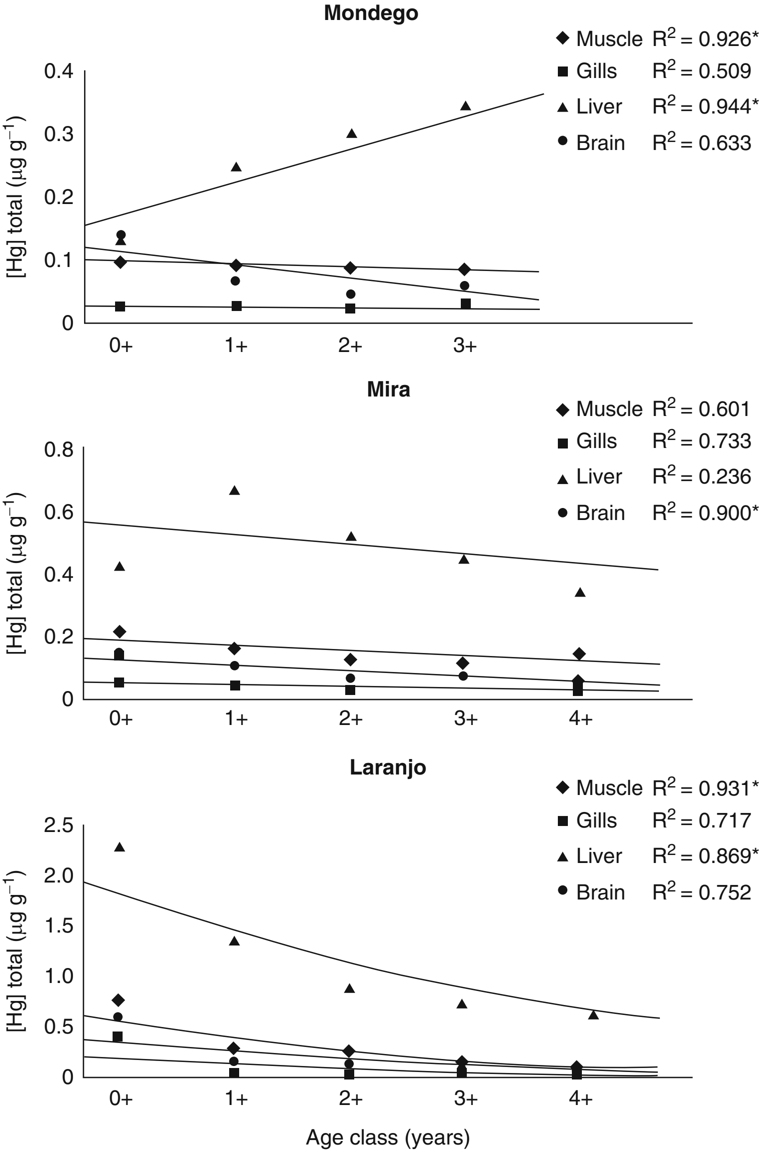


Fig. 10.3 Lifespan mercury accumulation in *Liza aurata* at different sites. Trendlines, calculated from average levels in each year class, represent the annual net accumulation of Hg by the fish. * = significant regression lines (Reprinted from Tavares et al. 2011: 319, courtesy Elsevier Publishing Co)

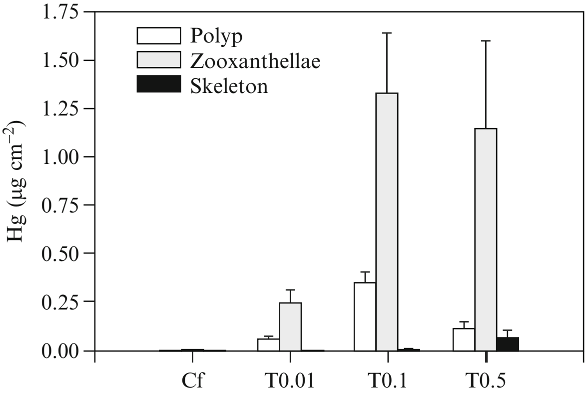


Fig. 10.4 *Porites astreoides*. Hg concentration per unit coral surface area for polyp tissue, zooxanthellae, and skeleton – and for each treatment (mean ± SE) (Reprinted from Bastidas and Garcia 2004: 137, courtesy Inter-Research)

the area during that year. Other metal peaks observed in coral samples correlated with years of high precipitation which may have resulted in increased sediment runoff in the region.

Despite the usefulness of coral skeletons for historical records, the symbiotic zooxanthellae accumulate greater metal concentrations than the skeleton or living coral tissue. Coral skeletons had consistently lower metal concentrations than the zooxanthellae, tissue and gametes. The loss of zooxanthellae during stress (bleaching) may help reduce metal loads (Reichelt-Brushett and McOrist 2003). Similarly, Bastidas and Garcia (2004) found most accumulation of Hg in zooxanthellae (Fig. 10.4). Corals exposed to 0.180 mg Hg l^{-1} accumulated 1.738 $\mu\mathrm{g\ cm}^{-2}$, 89 % of which was in the zooxanthellae, 7 % in polyps and 4 % in the skeleton. They thought that the capacity of zooxanthellae and the skeleton of *Porites astreoides* to concentrate Hg and the loss of zooxanthellae after Hg exposure suggest that the accumulation of Hg in these compartments functions as a detoxifying mechanism.

10.1.2 Subcellular Disposition/Detoxification

The cellular localization of metals is critical to their toxicity. Metals associated with metal-sensitive intracellular components (e.g., organelles and enzymes) can impair cell functioning. Metals tend to bind to proteins and may prevent the protein from functioning normally. For example, metals can bind to active sites of enzymes that play an essential role in the regulation of biochemical, metabolic, and physiological activities, changing their configurations and inhibiting their activities; Khoury et al. (2009) found that fiddler crabs (*U. pugnax*) from a contaminated site had elevated levels of metals in the heat-denatured proteins (enzymes). However, other proteins (e.g. metallothioneins, MT) can bind to metals and make them unavailable (to a degree). Thus, metals may be toxic and available or may be unavailable, depending on which protein they are bound to. Though not strictly a metal but a metalloid, arsenic accumulated by the deposit-feeding polychaete *Arenicola marina* is stored in the cytosol in heat stable proteins (~50 %) including metallothioneins (Casado-Martinez et al. 2012). The remaining As is accumulated mainly in the fraction containing cellular debris (~20 %), with the remainder in metal-rich granules (MRG), organelles and heat-sensitive proteins. A detoxified metal compartment including heat stable proteins and MRG can bind arsenic coming into the cells at a constant rate under sublethal conditions (Casado-Martinez et al. 2012). In environments with high levels of available metals, animals tend to have evolved mechanisms to enhance detoxification. The induction of detoxification systems such as MRG or MT may lead to tissue concentrations that are highly elevated. Wang et al. (2011) reported on a metal-contaminated estuary in Fujian Province, China, in which blue oysters (*Crassostrea hongkongensis*) and green oysters (*C. angulata*) were contaminated with very high levels of Cu (<14,000 $\mu g\ g^{-1}$ tissue dry wt, compared to about 700 in reference oysters) and other metals. Metal concentrations in blue oysters were as high as 1.4 and 2.4 % of whole-body tissue dry wt for Cu and Zn, respectively. Cellular debris was the main subcellular fraction binding the metals, but MRG were important for Cr, Ni, and Pb. With increasing Cu accumulation, its partitioning into cytosolic proteins decreased. In contrast, MT-like proteins bound more Zn as tissue Zn increased. In the most severely contaminated oysters, hardly any Cu or Zn was bound with the metal-sensitive fraction, which may explain survival in such contaminated environments.

Intracellular Localization and Trophic Transfer

Different subcellular compartments are associated with differing degrees of trophic transfer. Assimilation of trace metals by predators from prey is affected by the form of the metal in the prey, leading to the concept of a Trophically Available Metal (TAM) component in the prey depending on the subcellular fractioning of metals. Wallace and Lopez (1996) studied relationships between oligochaete subcellular Cd distribution and Cd absorption by a predator, the grass shrimp (*P. pugio*). Over time, there were increases in both the amount and proportion of Cd bound to the cytosolic fraction in the prey, which was considered to be induction of Cd-binding MTs. They found 1:1 relationships between the Cd in oligochaete cytosol and Cd adsorbed by shrimp, demonstrating that the metal bound to the MT fraction was available to higher trophic levels, and that factors influencing subcellular metal distribution in prey can alter trophic transfer to predators.

As originally defined, TAM consists of soluble metal and metal associated with organelles, the combination of which best explained results involving a crustacean predator feeding on polychaetes or mollusks. Rainbow et al. (2011) reviewed the literature and concluded that TAM as originally defined was frequently used in the literature as an absolute description of that component of metal that is trophically available in all prey to all consumers, but it is now clear that what is trophically available varies among prey species, consumers, and metals. Mechanisms involved in tolerance can be linked to trophic transfer of contaminants. Metals bound to MT would not be expected to interfere with cellular functions, but tend to be more available to predators than metals associated with insoluble cellular constituents. Seebaugh et al. (2005) fed *P. pugio* brine shrimp that had been exposed to Cd. An increase in metal associated with MT in the brine shrimp was associated with enhanced trophic transfer to the grass shrimp. Metals associated with the fractions containing MT, enzymes, and organelles were considered the TAM, which is available to predators (Seebaugh and Wallace 2004). A direct relationship was observed between the Cd and Zn in the TAM compartment in brine shrimp and absorption of these metals by *P. pugio*. Similarly, when Cd-exposed amphipods (*Gammarus lawrencianus*) were fed to grass shrimp, the amount of TAM was directly related to the shrimp's assimilation efficiency (Seebaugh et al. 2006).

Wallace and Luoma (2003) investigated subcellular partitioning of Cd and Zn in the bivalves *Macoma balthica* and *Potamocorbula amurensis* and found that metals associated with organelles, enzymes, and MT are trophically available to predators, and that this partitioning varies with species, animal size and metal. Clams were exposed to Cd and Zn and fed to shrimp *Palaemon macrodatylus*, or used to investigate subcellular partitioning. Shrimp fed Cd-contaminated *P. amurensis* absorbed ~60 % of ingested Cd, in accordance with the partitioning of Cd to the bivalve's TAM compartment (Fig. 10.5). About 34 % of the Cd and Zn in *M. balthica* was associated with TAM, while in *P. amurensis* it was metal-dependent (~60 % for Cd, ~73 % for Zn). TAM tended to decrease with increased clam size. *P. amurensis* poses a greater threat to predators because of higher tissue concentrations and greater partitioning of Cd as TAM.

Goto and Wallace (2009a) examined intracellular partitioning in prey as an indicator of metal trophic availability to mummichogs *Fundulus heteroclitus*, in metal-polluted salt marshes in New York, USA. Two common prey, *P. pugio* and *Nereis acuminata*, stored higher proportions of non-essential metals (particularly Pb) in insoluble (less trophically available) cellular components, as body burdens increased. In contrast, intracellular partitioning of essential metals (Cu and Zn) in prey varied relatively little among sites. Cd and Pb partitioning in *P. pugio* from different sites was associated with Cd and Pb body burdens in their mummichog predators, but bioaccumulation of Cu and Zn in mummichogs was similar among populations. This suggests that intracellular partitioning within prey may be only partially responsible for availability to a predator in polluted habitats, and that some predator-dependent processes may offset differential trophic availabilities from prey.

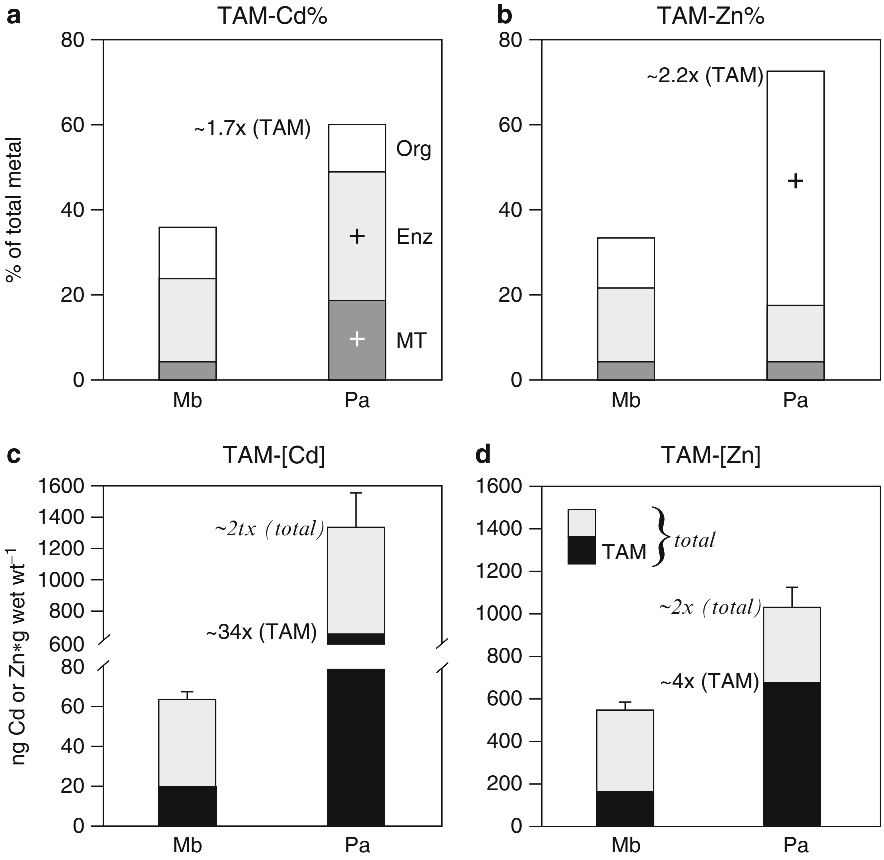


Fig. 10.5 *Macoma balthica* (Mb) and *Potamocorbula amurensis* (Pa) subcellular partitioning and accumulation of (**a**, **c**) Cd and (**b**, **d**) Zn following 14 days uptake. *Stacked bars* represent the proportional contribution of each subcellular fraction (organelles = open, enzymes = *light gray*, metallothioneins = *dark gray*) to the entire TAM compartment. Accumulated metal as ng metal *ug* wet wt^{-1} (Reprinted from Wallace and Luoma 2003: 130, courtesy of Inter-Research)

Furthermore, mummichogs consume other prey items besides grass shrimp. In contrast to the previous studies, marine fish *Terapon jurbua terepon* fed polychaetes, *Nereis diversicolor*, vs clams, *Scrobicularia plana*, from metal-polluted estuaries showed very different effects (Dang et al. 2012). Fish fed *S. plana* from Restronguet Creek which had body burdens of 9.55 Cr (μg g^{-1} dw), 1,321 Fe, 86 Cu, 3,184 Zn, 186 As, 45.8 Pb, and 0.93 Cd exhibited mortality, unlike those fed *N. diversicolor* (which had body burdens of 2.49 (μg g^{-1} dw) Cr, 529 Fe, 662 Cu, 214 Zn, 106 As, 1.19 Pb, and 0.27 Cd). Differences in metal content rather than subcellular metal distributions in the two food species appeared to be the cause of differential mortalities. Partial least squares regression revealed that Pb, Fe, Cd and Zn in the prey species co-varied most strongly with the observed mortality in fish consumers.

Metallothioneins

MTs are low molecular weight heat-stable proteins that are rich in cysteine and can bind high amounts of metals (Ag, Cu, Zn, Cd, Hg). They play a normal role in regulating the essential metals Cu and Zn but are also involved in detoxification of non-essential metals. Blue crabs that had not been exposed to elevated metals were examined for partitioning of Zn and Cu into different fractions at different phases of the molt cycle (Engel 1987). Cu and Zn in hemolymph decreased during molt, while these metals were highest in the digestive gland during premolt and lowest just after molt. Concentrations of MT were highest during intermolt and premolt and lowest just after ecdysis. MTs occur naturally and are actively involved in hemocyanin synthesis and Zn regulation. Their synthesis, primarily in hepatopancreas and gills, can be stimulated by metal exposure, and by binding metals MT limits their toxicity (Engel and Brouwer 1989). Most of the Cd taken up from solution by *Palaemon elegans* becomes bound to MT without being excreted (Rainbow and White 1989). Engel et al. (1985) compared responses of blue crabs and lobsters to Cd and Cu, and found that lobsters have a copper-MT in the digestive gland and a Cd-MT in the gills, while blue crabs have a Cd/Zn-MT and a Cu-MT in the digestive gland and a Cd-MT in gills. It has been assumed that if metals are sequestered by MT, an organism is protected from toxicity. However, Sanders and Jenkins (1984) found that when crab (*Rhithropanopeus harrisii*) larvae were exposed to elevated Cu, the accumulation of Cu in the MT pool was associated with growth inhibition. Different isoforms of MT appear to be involved in Cu metabolism versus detoxification in blue crabs (Schlenk and Brouwer 1991).

Mieiro et al. (2011) investigated exposure, Hg accumulation, and MT content of *Dicentrarchus labrax* and *Liza aurata*, in a Hg-polluted estuary. Total mercury (T-Hg) and MT content were determined in gills, blood, liver, kidney, muscle and brain. Tissues had different T-Hg accumulation patterns, and *D. labrax* accumulated generally higher levels. *D. labrax* had 0.6 and 0.4 mg kg^{-1} in muscle and brain, respectively, while *L. aurata* had 0.3 and 0.2 mg kg^{-1}. *D. labrax* had low MT in brain and Hg exposure did not induce its synthesis in other tissues, whereas *L. aurata* increased its MT in liver and muscle in response to Hg exposure. However, tissues did not show correlations between T-Hg and MT levels. The applicability of MT in fish tissues as biomarker of Hg exposure was uncertain. While Hg does bind to MT, most Hg in fishes is in organic form, meHg, which does not bind to MT and undergoes biomagnification, leading to increasing concentrations at higher trophic levels. MeHg is highest in muscle and liver.

Lysosomes

Lysosomes can sequester metals in many invertebrates, and thereby play an important role in detoxification. They accumulate metals and trap them in lipofuscin granules, making them unavailable to the cell. Lysosomal sequestration leads to elimination of the metals through the kidneys. Nassiri et al. (2000) investigated the amphipod *Orchestia*, which uses lysosomes in the ventral caeca for metal detoxification. When animals from contaminated and reference sites were compared, there was no difference in Zn uptake by the different populations when they were exposed to the same concentration of Zn. However, the contaminated population took up significantly less Cd. After laboratory exposure to Cu, Zn, and Cd, lysosomes contained Cu and Zn but not Cd. Lysosomes in cells of the ventral caeca appeared to be a major detoxification pathway for Cu and Zn in this species, while Cd bound to MTs. Ahearn et al. (2010) summarized investigations of lysosomal function in lobster hepatopancreas and described carrier-mediated transport processes on lysosomal membranes for accumulating metals from the cytoplasm. Metal transporters are linked with the uptake of anions that may then precipitate metal concretions within the lysosomes.

Insoluble Granules

Trace metals can be detoxified in the form of insoluble metal-rich granules (MRG) or deposits in tissues. Prawn juveniles, *Penaeus monodon*, were exposed to Cu and Pb to investigate the formation and accumulation of MRG in soft tissues and their excretion from the body (Vogt and Quinitio 1994). Cu-containing granules were accumulated primarily in the hepatopancreatic tubules. The amount and size of the granules increased along the tubules in accordance with the cells' age; the granules were released by discharge of senescent cells into the intestine and were added to the feces. In contrast, Pb-containing granules were found mostly in the thoracic extensions of the antennal gland, where they were individually discharged into the gland lumen by secretion and excreted with the urine. While metals associated with MT are available to predators, sequestering metals in granules reduces trophic transfer (Wallace and Lopez 1997), although the specific granule composition can influence the bioavailability of sequestered metals.

Stress Proteins

Heat shock proteins (HSPs), originally discovered after exposure to elevated temperature, play a role in protein folding and assembly and protect damaged cells from further damage. Various stresses can bring about changes in protein conformation; mechanisms to maintain proteins in their functional conformation are important. HSPs can repair proteins or eliminate them if they cannot be repaired. They act as molecular "chaperones" protecting other proteins. This family of proteins has been highly conserved in evolution and documented in many groups of organisms. HSPs are classified by their molecular weight. They are also induced by contaminant stress and have general protective functions, so are better termed "stress proteins" since they can confer increased tolerance to toxicants (Sanders 1993). Survival and stress protein response were investigated in the amphipod, *Gammarus fossarum*, (Schill et al. 2003). Low Cd^{2+} concentrations led to an induction of stress proteins

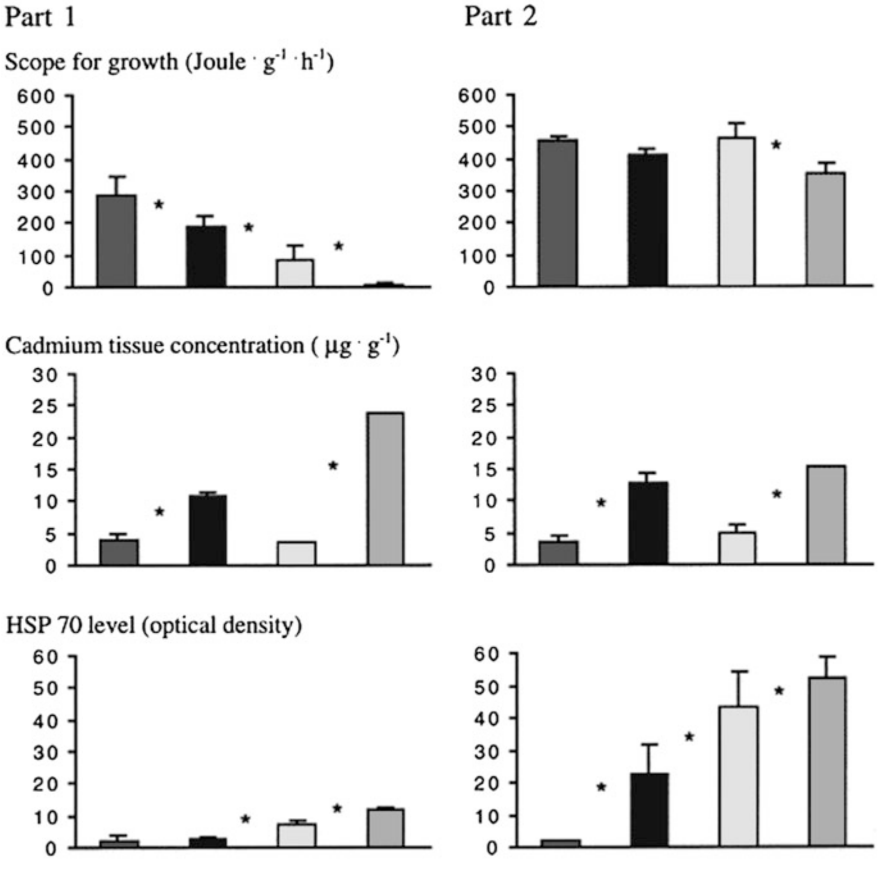


Fig. 10.6 Part 1 from *left to right*: control in 4° C, Cd in 4° C, heatshock 20° C, Cd with heatshock 20° C. Part 2 from *left to right*: control in 4° C, Cd in 4° C, pre-heated 20° C, Cd with pre-heat in 20° C. Mean values with standard error (bars) per g tissue dry wt. Central * = significant differences ($F < 0.05$) between temperature treatments (combined); other * = significant differences between Cd treatments within temperature treatments (Reprinted from Tedengren et al. 2000: 7, courtesy Elsevier Publishing Co)

while higher Cd^{2+} concentrations resulted in a reduced response, most likely due to pathological damage. Surviving individuals retained their capacity to produce stress proteins during recovery. Acquisition of tolerance by HSP induction is possible only up to a certain level (Eckwert et al. 1997). However, thermal acclimation confers resistance to other stresses such as toxic chemicals. For example, in the copepod, *Tigriopus japonicus* HSP70 is expressed in response to temperature and metals, and appears to be protective against both (Rhee et al. 2009). Stress proteins are utilized in mollusks to protect against metal as well as thermal stress. In *M. edulis*, exposure to elevated temperature led to increased tolerance to 20 µg l^{-1} Cd, correlated with increasing levels of HSP70 (Tedengren et al. 2000) (Fig. 10.6).

Increased HSP due to heat shock led to better fitness of Cd-exposed mussels in terms of scope for growth, even though the mussels had higher Cd body burdens (25 vs 15 $\mu g\ g^{-1}$).

Other Antioxidants

Ahmad et al. (2012) investigated the role of non-enzymatic antioxidants in the bivalve *Scrobicularia plana* exposed to Hg at moderately and highly contaminated sites in Ria de Aveiro (Portugal). Moderately contaminated sites had Hg levels 27× the Effects Range Low (ERL) of 0.15 mg kg^{-1}, while highly contaminated sites had Hg levels 132× the ERL. In moderate contamination, antioxidants were produced that allowed the animals to adjust. In higher contamination, *S. plana* failed to cope with Hg, and its defense abilities deteriorated. Gills in moderate levels increased non-protein thiols and glutathione, but in high contamination they increased levels of ascorbic acid.

10.2 Organic Contaminants

The uptake of foreign hydrocarbons presents organisms with problems of metabolism, storage and/or excretion. Chlorinated organics tend not to be metabolized, but bioaccumulated and stored in liver, hepatopancreas, or blubber of large marine organisms such as mammals. PAHs, which are also accumulated primarily in the liver or hepatopancreas, may be transformed into metabolites that can be excreted via the gills and kidneys. Sometimes, intermediate metabolites are more toxic than the original pollutant.

10.2.1 Accumulation of Halogenated Organics

As hydrophobic lipophilic compounds, chlorinated organics accumulate in fatty tissues, including yolk, liver/hepatopancreas, and blubber, and to biomagnify up food webs.

Crustaceans

Small crustaceans in the water column can take up organic contaminants from the water, while benthic species accumulate them primarily from sediments, which will have higher levels. Magnusson et al. (2007) studied accumulation of PCBs in pelagic copepods. Bioaccumulation factors (BAFs) were determined for ^{14}C-PCB 101 and ^{14}C-PBDE 99 in *Calanus finmarchicus* after exposure to either contaminated water or after being fed contaminated phytoplankton (the dinoflagellate *Prorocentrum minimum* or diatom *Thalassiosira weissflogii*). BAFs in algae were 7.6–8.0 for PCB 101 and 8.5–8.6 for PBDE 99. BAFs in copepods were significantly lower, 6.3–6.8 for PCB 101 and 7.6 for PBDE 99. For each chemical, the BAFs in copepods were independent of which algal species they had consumed, even though the bioaccumulation of both chemicals was higher in *P. minimum* than in *T. weissflogii*. Berrojalbiz et al. (2009) performed laboratory studies on bioconcentration (by passive uptake) of PAHs in phytoplankton (*Rhodomonas salina*) and subsequent accumulation in copepods (*Paracartia grani*) by both ingestion and diffusion. Both BCF and BAF showed significant correlation with the octanol–water partition coefficient (K_{ow}) for phytoplankton and zooplankton. BCF values for phytoplankton were two orders of magnitude higher than those for copepods. Fecal pellet analysis showed that for PAHs taken in by ingestion but not diffusion, elimination by defecation was important. Elimination was mainly by metabolism and depuration however. Uptake, depuration, egestion, and ingestion rates increased with hydrophobicity of the chemical, while metabolism was greater for the less hydrophobic compounds.

PAHs and PCBs were measured in the water column and in copepods, *Eurytemora affinis* from the Seine Estuary (Cailleaud et al. 2007). Total PCB and PAH levels in suspended particulate matter (SPM) and in copepods varied seasonally, with maximum levels during winter. PAH and PCB in the SPM ranged from 499 to 5,819 ng g^{-1} and from 58 to 463 ng g^{-1}, respectively. PCBs and PAHs bioaccumulated by *E. affinis* were 383–1,785 and 165–3,866 ng g^{-1}. Thus, copepods could have high accumulation factors (91,000 for PCBs and 17,000 for PAHs). The winter samples, with a high percentage of adults, had the highest PCB and PAH levels. Juveniles had the lowest concentrations.

Fiddler crabs, which dig burrows and process marsh sediments for food, take up PCBs from sediments (Marinucci and Bartha 1982; Clark et al. 1986). Another marsh burrower is the crab *Chasmagnathus granulate*. Sediment collected from inside and outside crab burrows as well as outside the crab bed, and cordgrass (*Spartina densiflora*) were collected from intertidal mudflat and marsh and analyzed for organochlorine (OC) compounds When lipid-normalized data for all OC pesticides were summed together, there were higher concentrations in crabs from the cordgrass habitat than crabs from the mudflat (Menone et al. 2000). There were no significant differences in percent organic matter of marsh and mudflat sediments, but the ΣOCs (normalized to organic carbon) were higher in marsh sediments than mudflat. Cordgrass rhizomes and roots contained high concentrations of OCs, indicating that it is important in determining the distribution of persistent contaminants and can be a source of these contaminants to marsh biota.

Biota-sediment accumulation factors (BSAFs) for some polychlorinated dibenzo-*p*-dioxin and dibenzofuran congeners (PCDD/Fs) in Dungeness crab (*Cancer magister*) hepatopancreas and associated sediments are concentration independent (as required by equilibrium partition models). In particular 2, 3, 7, 8-TCDD, 2, 3, 7, 8-TCDF, 2, 3, 7, 8-substituted pentachlorodibenzofurans, and the non-2, 3, 7, 8-hexachlorodibenzofurans (HxCDFs) fell into this group (Cretney and Yunker 2000). However, the BSAFs for other isomers exhibited significant, but nonlinear variations with sediment or crab PCDD/F concentrations. The nonlinear relationship between BSAFs and concentrations complicates predictions of the potential for dioxin and furan bioaccumulation in crabs. Blue crabs (*C. sapidus*) from Pensacola Florida (USA) were analyzed for 17 dioxins/furans, 12 dioxin-like PCB (DL-PCBs) congeners and levels compared to screening values (SV) calculated using U.S. EPA recommendations for establishing consumption advisories (Karouna-Renier et al. 2007). All samples exceeded the SV for dioxins/furans/DL-PCBs (0.098 pg g^{-1}), based on a Florida-specific consumption rate of 46 g seafood day^{-1}. Risks to human health were greatest from consumption of the hepatopancreas, suggesting that it should be avoided. Hepatopancreas and meat from blue crabs from contaminated Newark Bay NJ (USA) were subjected to congener specific analysis (Rappe et al. 1991). All samples were contaminated by 2,3,7,8-TCDD and other 2,3,7,8-substituted congeners as well as less hazardous PCDDs and PCDFs. A value exceeding 6,000 ppt wet tissue weight of 2,3,7,8-TCDD was found in one sample of hepatopancreas, which seemed to be the highest value so far reported in a food product. The meat, on the other hand, had only 100 ppt.

Pruell et al. (2000) exposed polychaetes, *Nereis virens*, to contaminated sediments from the Passaic River, NJ (USA) for 70 days, then fed them to lobsters, *Homarus americanus*, for up to 112 days. Sediments averaged 219.37 pg g^{-1} of 2,3,7,8-TCDD and 145.29 pg g^{-1} of 2,3,7,8-tetrachlorodibenzofuran (TCDF) dry weight. Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), 2,4,6,8-tetrachlorodibenzothiophene (TCDT), PCBs, and chlorinated pesticides were accumulated by polychaetes, some of which were also accumulated by lobsters exposed to contaminated sediment (CS) and/or fed contaminated polychaetes. Polychaetes exposed to CS for 70 days accumulated the tetrachlorinated congeners, including 2,3,7,8-TCDD (168.7 pg g^{-1} dw), 2,3,7,8-TCDF (72.0.9.7 pg g^{-1}) and 2,4,6,8-TCDT (474.131 pg g^{-1}). BSAF for 2,3,7,8, TCDD was 0.36 for polychaetes, 0.27 for lobster muscle and 0.8 for lobster hepatopancreas. BSAF for PCB 77 was 1.7 for polychaetes, 1.4 for lobster muscle and 4.8 for lobster hepatopancreas. Only the lesser chlorinated PCDDs and PCDFs (mostly tetra- and pentachlorinated congeners) and 2,4,6,8-TCDT were detected in the polychaetes and lobster. Alterations were noted in the PCB congeners in both species. The non-*ortho*-substituted PCBs became enriched in the polychaetes and especially the lobsters relative to the sediment, probably because these congeners were not metabolized. These congeners and the 2,3,7,8-TCDD toxicity equivalents of the mixtures were enriched by a factor of about six in the lobsters relative to the sediment. Shrimp can also bioaccumulate organochlorines, but generally to a lesser degree. While organochlorine compounds including PCBs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans were non-detectable in muscle of Northern shrimp, *Pandalus borealis*, from the Northwest Atlantic (Hellou et al. 1997), high levels were seen in the hepatopancreas, which has a high lipid content. Higher concentrations were observed in eggs in November (e.g. 10 ng g^{-1} wet wt PCBs) and in hepatopancreas of females in April (e.g. 40 ng g^{-1} wet wt. PCBs). A larger number of PCB and PCDF congeners were seen in the shrimp hepatopancreas than livers of finfish species previously sampled.

Mollusks

PCBs and DDT in mollusks and sediments at sites in the U.S. National Status and Trends Program were reported by O'Connor (1991). The dry weight-based chlorinated organic concentrations in mollusks generally exceeded those in nearby sediments by an order of magnitude. Highest concentrations of PCBs and DDT in mollusks were in the ranges of 1,000–4,000 and 400–1,000 ng g^{-1} (dry), respectively. While higher concentrations of contaminants can be found in localized hotspots, the NST data represent the distribution of concentrations over general areas of the coastal U.S.

Contamination levels and accumulation of PCBs and organochlorine pesticides (OCPs) were measured in bivalves from various sites along the coast of Korea (Kim et al. 2002). The dry weight based $\sum$PCBs and $\sum$OCPs ranged from 4.4 to 422 ng g^{-1} and from 9.95 to 131.37 ng g^{-1}, respectively. Organochlorine pesticides were mostly DDTs, HCHs, and chlordanes. At sites near urban and/or industrial areas levels were quite high, and the spatial distributions of DDTs were correlated with that of PCBs. Wang et al. (2008) investigated spatial patterns of OCPs, PCBs, and polybrominated biphenyl ethers (PBDEs) in mollusks of the coastal Chinese Bohai Sea. Seven species, including *Rapana venosa*, *Neverita didyma*, *Scapharca subcrenata*, *Mytilus edulis* (mussel), *Amusium*, *Meretrix meretrix*, and *Crassostrea talienwhanensis* (oyster) were investigated. Oysters and mussels, which have higher lipid contents than the other mollusks, had the highest concentrations of OCPs and PBDEs. Total OCP, PCBs and PBDE were 8.8 ng g^{-1} dw in oysters and 14.6 in mussels, while other mollusks generally had lower levels. Multivariate linear regression showed that the most influential factor for contaminant concentrations was the trophic level, followed by lipid content. However, concentrations correlated negatively with the total length, suggesting that these chemicals do not accumulate over time. However, different accumulation patterns were noted between oysters *Crassostrea angulata* and clams *Ruditapes decussatus* from the Sado estuary and Ria Formosa, respectively (Ferreira and Vale 1998). Oysters showed no significant differences in accumulation with size, but smaller clams had higher PCB levels than larger ones, indicating growth dilution. The PCB accumulation in same size classes of the two species, and the effect of PCB exposure on their lipids were investigated in the laboratory and again, no differences in oyster accumulation related to size were seen, but smaller clams accumulated PCB levels ten times higher than larger ones. PCB in the larger clams reached 360 ng g^{-1}, but small clams accumulated up to 2,400 ng g^{-1} and showed altered lipids. Thus, small and large individuals of the two species responded very differently in terms of PCB accumulation.

Ueno et al. (2003) measured PCBs, DDTs, CHLs (chlordane compounds), HCHs (hexachlorocyclohexane isomers) and HCB (hexachlorobenzene) in the liver of Japanese squid (*Todarodes pacificus*) from the Japan Sea and western North Pacific Ocean. PCBs (up to 5,600 ng g^{-1} lipid wt.) were the highest, the others were in the order of DDT > CHLs > HCHs > HCB. Studies on growth and seasonal variation suggest a rapid reflection of the pollution levels in seawater where and when they were collected, regardless of body-length and time of collection, indicating that Japanese common squid is a suitable bioindicator species for monitoring pollution.

Fishes

Sediment and food are the major pathways for organochlorine uptake. Accumulation may be affected by the proximity to contaminated sediments and prey, the magnitude of contamination, movement patterns, trophic status, growth rates, and duration of exposure (i.e., lifespan or fish age). Species-specific metabolism and detoxification of PCBs, reproductive (e.g. female depuration in eggs) and maturational patterns, and body fat (i.e., percent lipids) can affect the degree to which PCBs accumulate in tissues. Growth-related and species-specific accumulation of PCBs was studied for a variety of tidal flat organisms from the Ariake Sea, Japan (Nakata et al. 2002). Highest concentrations were found in omnivorous fishes, followed in decreasing order by crabs, herbivorous fishes, and mussels, showing that trophic level plays an important role in PCB accumulation. Age- and body length–dependent accumulations of PCBs were observed in herbivorous mudskippers. Among the three non-*ortho* coplanar PCBs, CB-77 was the predominant congener with an average of 1.5 ng g^{-1} (lipid weight) in herbivorous mudskippers. Concentrations were highly correlated with body length rather than age, which may indicate that the growth rate is important in determining PCB accumulation. The body burdens of PCBs averaged 870 ng in whole bodies and 97 ng in eggs, so the transfer rate to eggs was estimated to be about 10 % of female body burden. Comparison of PCB composition in eggs and whole body suggests the selective transfer of lower-chlorinated congeners to eggs. The relationship between BSAFs in organisms and log K_{ow} showed that omnivorous mudskippers accumulated higher concentrations of PCBs (averaging 2,900 ng g^{-1} lipid weight), which might be due to their greater feeding and/or higher trophic level.

Organochlorines were analyzed in cod (*Gadus morhua*), dab (*Limanda limanda*), plaice (*Pleuronectes platessa*) and lemon sole (*Microstomus kitt*) collected between 1991 and 2000 in Norway (Green and Knutzen 2003). PCB levels in liver of cod and dab showed a positive but weak correlation with length, but no correlation was found between PCBs and liver fat. Red mullet (*Mullus barbatus*), sea mullet (*Mugil cephalus*) and sea bass (*Dicentrarchus labrax*), from the Ebro Delta have different habitats and feeding behaviors. Higher levels of PCBs and DDTs were found in red mullet (20.3 and 16.8 ng g^{-1} wet wt. respectively) which has higher lipid content (Pastor et al. 1996). Red mullet and sea bass had a similar distribution pattern of PCBs, but DDTs and HCB (hexachlorobenzene, 10.0 ng g^{-1} wet wt.) were higher in sea mullet, probably due to direct uptake from the lagoons from which they were collected and where these pollutants are high. A decrease in concentrations with size (age) was generally observed in red mullet and sea bass, but less clearly in sea mullet. This decrease was more pronounced for DDTs, probably due to metabolic transformations. However, when data were normalized to lipid level, evidence for a positive uptake by sea mullet was seen, probably relating to its faster growth.

These results indicate that the accumulation of organochlorine compounds in coastal fishes from the same area depends on lipid content, habitat, diet, growth rate and the metabolism of each species.

Young-of-the year bluefish (*Pomatomus saltatrix*), a lipid-rich fish, were collected from the contaminated Hackensack River and from Tuckerton, a reference site. Hackensack fish were significantly smaller and had elevated levels of contaminants (Weis and Candelmo 2012). The mean PCB concentration in Hackensack bluefish (about 1,000 Aroclor ng g^{-1} wet wt) was 2–3 fold greater than that of the menhaden *Brevoortia tyrannus* and mummichog *Fundulus heteroclitus* prey found in their stomachs, showing biomagnification from prey to predator. The levels in the juvenile 4-month old bluefish exceeded the level considered safe for consumption.

Fish can accumulate PCBs from sediments or from food. Accumulation and trophic transfer were studied in a laboratory food chain consisting of sediments, polychaetes (*Nereis virens*) and fish (*Leiostomus xanthurus*). In phase I, fish and polychaetes were separately exposed to contaminated sediments (5.2 μg g^{-1} dw as Aroclor 1242 and 1254). In Phase II, the dietary fraction of PCB accumulation was determined by feeding exposed and control fish polychaetes with a known PCB body burden. Fish exposed to contaminated sediments and fed polychaetes from the same sediment accumulated more than twice as much PCB than fish in similar conditions but fed uncontaminated polychaetes. The dietary contribution of PCBs accounted for 53 % of the total body burden (Rubinstein et al. 1984). In a similar trophic transfer study, benthic copepods were exposed to sediments with Aroclor 1254 (83.3 μg PCBg^{-1} sediment) and then fed to juvenile *L. xanthurus* in uncontaminated sediments (DiPinto and Coull 1997). Experiments in which uncontaminated copepods were fed to fish in PCB-contaminated sediments were conducted to determine the relative roles of sediments vs prey in PCB uptake. Total PCBs measured in fish fed contaminated copepods averaged 380.4 ± 60 ng/fish, an average assimilation of 33 % of the PCB dose. Mean PCB body-burden concentrations in fish fed meals of PCB-contaminated copepods was 2,956 ng PCB/g fish dw. However, fish feeding on clean food in contaminated sediments accumulated five times as much PCB as fish feeding on contaminated prey in clean sediments, suggesting direct sediment exposure is more important than trophic transfer, unlike the Rubinstein study. This suggests an important role for the prey species, as was seen in studies of metals discussed previously. In terms of congeners, fish preferentially accumulated the tetrachlorinated congeners, which are less important in copepods and sediments.

Bioaccumulation of sediment-associated PCBs was examined in Atlantic cod (*Gadus morhua*) through diffusion from sediment and via dietary exposure (Ruus et al. 2012). To facilitate accumulation from sediment, it was continuously resuspended. To study dietary bioaccumulation, cod were fed polychaetes (*N. virens*) previously exposed to test sediments (ΣPCB 10 μg kg^{-1}) Polychaetes accumulated 17.4 ΣPCB μg kg^{-1}. Results suggest that the contaminated sediments of the inner Oslofjord are an important source of PCBs for cod, although additional contributions may also be important. Estimates of AE of ingested PCBs were found to be 30–50 %; the highest was for the lower chlorinated congeners (PCB-28 and -52).

In general, organochlorine compounds were lower in tropical Pacific fishes than in temperate fishes and showed less spatial variation (Kannan et al. 1995), despite continued use of these chemicals in tropical countries. This was attributed to the shorter residence time of semi-volatile chemicals in tropical waters. This study also found declining concentrations in walleye pollock (*Theragra chalcogramma*) from the Bering Sea.

The upper Hudson River in New York State was the site of a major PCB hot-spot discovered in the 1970s. Sloan and Armstrong (1988) studied migratory Hudson River fishes and found substantial declines in PCB levels since 1977, but concentrations in lipids were still an order of magnitude higher than background. Average annual percent declines for total PCB, Aroclor 1016, and Aroclor 1254 were 28, 42, and 5 %, respectively. PCB concentrations were slightly higher in upstream sites, and there was little variation among organs when PCB concentration was expressed on a lipid basis. The American eel (*Anguilla rostrata*) and blue crab (*C. sapidus*) hepatopancreas had concentrations >5 ppm, striped bass (*M. saxatilis*) had about 5 ppm, while Atlantic sturgeon (*Acipenser oxyrinchus*), shortnose sturgeon (*A. brevirostrum*), blueback herring (*Alosa aestivalis*), alewife (*A. pseudoharengus*), American shad (*A. sapidissima*), rainbow smelt (*Osmerus mordax*), Atlantic tomcod (*Microgadus tomcod*), and juvenile bluefish (*P. saltatrix*) all had <5 ppm.

Anadromous Pacific salmon may be exposed to PCBs in freshwater, estuaries, or the ocean. In freshwater, they eat insects and crustaceans, in the estuary they eat a wider variety of invertebrates and larval fish, and adults in the ocean eat more fish, increasing the likelihood of biomagnification. Most of their growth occurs in the sea before they return upstream. O’Neill et al. (1998) observed that chinook salmon (*Oncorhynchus tshawytscha*) had higher PCB concentrations than coho (*O. kisutch*) and that concentrations were higher in fish in marine than in freshwater areas. Marine chinook averaged 74.2 μg kg^{-1}, freshwater chinook averaged 49.1 μg kg^{-1}, while marine coho averaged 35.1 μg kg^{-1} and freshwater coho averaged 26.5 μg kg^{-1}, all statistically different from each other. For coho, lipid content and sampling site accounted for most of the variation in PCBs. Concentrations in chinook were highest in the older larger fish. The higher PCB levels in chinook may be the result of differences in migratory pattern and diet. While Puget Sound coho migrate to the sea in their second year and return to freshwater as 3-year-olds, chinook go to sea in their first or second year and return as 2-, 3-, 4-, and 5-year-olds. O’Neill et al. (1998) suggested that salmon accumulate most of their PCBs in Puget Sound and the ocean, and because chinook stay longer at sea they accumulate more. Also, adult chinook eat more fish, resulting in higher levels due to biomagnification. It has been found that contaminant levels in farmed salmon are higher than in wild fish (van Leeuwen et al. 2009). This may be related to the fish-based feed for farmed salmon.

Other Taxa

Marine birds and mammals, high in food webs, tend to accumulate high concentrations of PCBs and chlorinated pesticides. A number of studies have investigated accumulation of specific congeners or groups of congeners. Cacccamise et al. (2012) compared 1-month old and 4–5-month old black-footed albatross chicks and black-footed and Laysan albatross eggs on Midway atoll. In the 1-month old chicks, PCBs 99, 118, 138, 153, 170, 180 and 183 accounted for 36–78 % of the total PCBs, which were inversely related to the total body weights. In 4–5-month old chicks, those congeners accounted for much lower percentages; these old chicks had more of the less chlorinated congeners. The total toxic equivalents (TEQs) for the 1-month olds was 130–11,000 pg g^{-1} (lipid weight, lw), while total TEQs for the 4–5-month olds was 18,000–100,000 pg g^{-1}, reflecting bioaccumulation over time. The total concentration averaged 7.9 and 4.6 μg g^{-1} in Black footed and Laysan albatross eggs, respectively. The high concentrations could be due to the age and PCB level of the female producing the egg. Tanaka et al. (2013) analyzed PBDEs in adipose tissue of short-tailed shearwaters, *Puffinus tenuirostris* from the North Pacific Ocean. In 3 of 12 birds, they detected higher-brominated congeners, which are not present in the natural prey (pelagic fish) of the birds. The same compounds were present in plastic found in the stomachs of the three birds, suggesting the transfer of chemicals from ingested plastics to the tissues of marine organisms.

PCDDs, PCDFs and PCB congeners were investigated in beluga whale (*Delphinapterus leucas*), narwhal (*Monodon monoceros*) and other species at the same trophic level in two areas of Canada (Norstrom et al. 1992). There was a greater relative abundance of *meta-para*-unsubstituted PCBs in cetaceans compared with other species, suggesting that activity of cytochrome P450 monooxygenase enzymes causing CYP2B-type metabolism was low in beluga and narwhal. Lower relative levels of 2,3,7,8-TCDD were found in beluga from both areas, and selective reduction of non-*ortho* PCBs occurred in the highly PCB-contaminated St. Lawrence beluga. These compounds are potent inducers of cytochrome P450 CYP 1A enzymes (see next section), suggesting that enzymes for metabolizing TCDD-like substrates are present in the two cetaceans.

A wide range of contaminants was detected in the blubber of walruses, including PCBs (mean 2,000 ng g^{-1} lipid), DDE (mean 100 ng g^{-1} lipid), chlordanes (mean 2,500 ng g^{-1} lipid), toxaphenes (mean 80 ng g^{-1} lipid) and polybrominated diphenyl ethers (PBDEs) (mean 15 ng g^{-1} lipid). PCB and DDE levels were substantially lower than those of animals sampled 10 years earlier in the area, confirming a decrease of these compounds in the Arctic (Wolkers et al. 2006). However, compared to other marine mammals from Svalbard, walruses had higher PCB and chlordane levels but lower DDE, toxaphene, and PBDEs, possibly due to species- and location-specific differences in exposure and metabolism. The range in contaminant levels was wide, even though all were adult males from the same location. The PCB pattern in highly contaminated animals was different from that in animals with lower levels, with relatively more persistent PCBs in the highly contaminated group, suggesting that they were feeding at higher trophic levels; possibly eating seals in addition to mollusks (Wolkers et al. 2006).

CECs

Uptake of brominated vs chlorinated chemicals is not determined by the same physico-chemical properties of the environment. PBDEs and PCBs were measured by Dinn et al. (2012) in sediments and benthic invertebrates near outfalls in Victoria and Vancouver, B.C., Canada. PBDE levels in wastewater exceeded those of PCBs eightfold at Vancouver and 35-fold at Victoria. PBDE levels in benthic invertebrates were higher in Vancouver than Victoria, despite lower sediment levels, and correlated with organic carbon-normalized concentrations in sediment. Uptake of individual PBDE congeners was determined by sediment properties (organic carbon, grain size), while PCB congener uptake was governed by octanol-water partitioning coefficient. Results suggest that sediment quality guidelines for PBDEs should consider organic carbon-normalized concentrations. Where the PBDE: particulate organic carbon ratio is high, benthic invertebrates will have greater bioaccumulation.

Nakata et al. (2012) analyzed green and blue mussels collected from ten Asian countries to study the occurrence and distribution of emerging pollutants, synthetic musks and benzotriazole UV stabilizers (BUVSs) in Asia–Pacific coastal waters. Synthetic musks and BUVSs were detected in mussels from all countries, showing widespread distribution. High concentrations were seen in mussels from Japan and Korea, where levels were comparable or greater than those of PCBs, DDTs and PBDEs. Significant correlations were found between the concentrations of HHCB and AHTN, and between the concentrations of BUV-327 and BUV-328, which suggest similar sources of these compounds in commercial products.

10.2.2 Metabolism

Pathways of transformation of organic contaminants involve two phases. Phase I reactions hydrolyze or oxidize the molecule in order to make it more polar or water soluble. Phase II reactions involve the conjugation of the product of the Phase I reaction with another substance that makes it less bioactive and more readily excreted. The most common Phase I reaction is carbon oxidation. The enzymes responsible for oxidation of foreign compounds are mixed function oxidases (MFOs), which include the highly studied cytochrome P-450 (CYP) system. CYPs, found in many organisms and tissues, are involved in oxidative metabolism of a wide range of organic compounds including PAHs, PCBs, pesticides, and other chemicals. The mixed-function oxidase system requires NADH or NADPH and molecular oxygen to convert nonpolar PAHs into polar hydroxy derivatives and arene oxides (some of which may be more toxic than the parent compound). In Phase II, another molecule (such as acetate, glucuronic acid, sulfate, glycine, or glutathione) is conjugated to a susceptible group on the chemical, making it more readily excreted. For chlorine-containing molecules like DDT and PCBs, dechlorination is an important early step.

Crustaceans

Rates of detoxification in some crustaceans tend to be slow, and clearance time long. Petroleum hydrocarbons are metabolized by the CYP system. Their slow rate of detoxification partially accounts for their sensitivity to oil pollution (Burns 1976). More recent studies demonstrate the activity of CYP systems of crustaceans varies considerably among species. The shore crab *Carcinus maenas* has a high capacity for metabolizing PAHs with CYP enzymes. Expression of *CYP2* and *CYP3*-like genes fluctuated over the molt cycle with low expression during premolt and maximum expression during late post-molt, and was predominant in the hepatopancreas, while expression of *CYP4*-like genes was predominant in gills and epidermis (Dam et al. 2008). This supports previous biochemical studies showing that the hepatopancreas is the major site of CYP-mediated xenobiotic metabolism. In addition, the data showed that *CYP2* and *CYP3*-related genes responded to ecdysteroids and xenobiotics, while *CYP4*-related genes did not, and probably are involved in more general physiological functions such as fatty acid metabolism. The data also suggest that premolt crabs, with low gene expression, would be more susceptible to organic pollutants (Fig. 10.7).

Mothershead and Hale (1992) exposed molting and intermolt blue crabs (*C, sapidus*) to high levels of unsubstituted PAHs in a field site. Newly molted crabs had higher tissue burdens than intermolt crabs of cyclopentanophenanthrene, fluoranthene and pyrene, characteristic of the creosote-contaminated exposure site. The mean total concentration of these PAHs in hepatopancreas was 9,560 ng g^{-1} in newly molted crabs and 3,360 ng g^{-1} in intermolt crabs. Mean total PAH concentration in muscle was 1,380 ng g^{-1} in new molts and 498 ng g^{-1} in intermolts. The elevated tissue burdens at molt may be due to increased water uptake and shell permeability at ecdysis and/or decreased metabolism of PAH during molting. PAHs can be conjugated and then excreted in the urine. *C. maenas* were exposed to phenanthrene and pyrene (separately) at 0–200 μg l^{-1}. After 48 h, urine samples were taken and analyzed by immunoassay and UV-fluorescence spectrophotometry. Urinary levels were dose-dependent for both compounds (Fillman et al. 2004). *C. maenas* was exposed to waterborne pyrene for 48 h and depuration monitored over time. No unchanged pyrene was detected in urine from exposed crabs, which had transformed pyrene into three major conjugates that were excreted in the urine (Watson et al. 2004). Urinary levels reached a maximum 2–4 days after initial exposure and then decreased steadily.

While liver microsomes of fish could metabolize various CYP-mediated substrates with enzymes in the CYP1A and CYP3A subfamilies, hepatopancreatic microsomes of the shrimp *Alepocephalus antennatus* showed activity for only a few substrates, generally related to mammalian CYP2-like enzymes

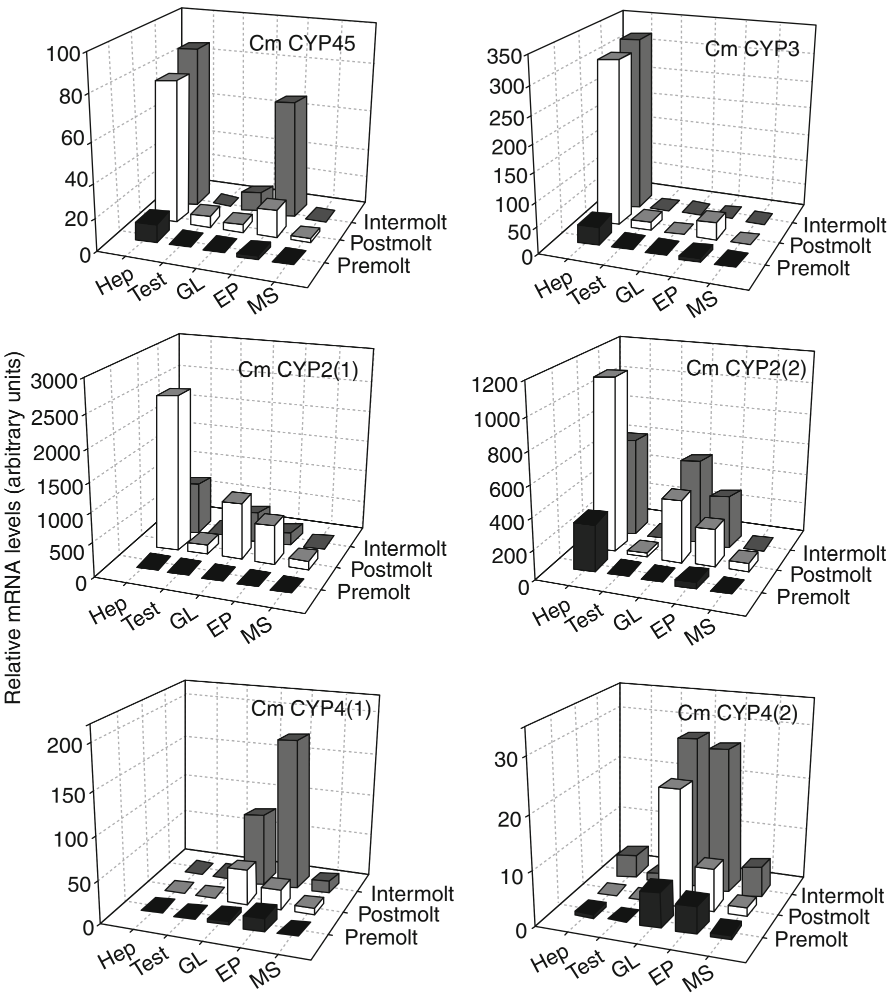


Fig. 10.7 Stage-specific expression of six CYP genes, coding for different CYP enzymes in different tissues. mRNA levels were analyzed with qPCR and normalized to ribosomal protein rpL 17. *Hep* hepatopancreas, *test* testes, *GL* gills, *EP* epidermis, *MS* muscle. Premoult D2-D3; Postmoult, B; Intermolt C3-C4 (Reprinted from Dam et al. 2008: 1136, courtesy Elsevier Publishing Co)

(Koenig et al. 2012). Furthermore, a direct relationship between metabolic activities and PCB accumulation profiles (which congeners were accumulated) was established. Ong et al. (2011) studied bioaccumulation and depuration of PAHs in *Penaeus monodon*, using anthracene as a model PAH. Food pellets spiked with anthracene were fed to *P. monodon*. At 20 mg kg^{-1}, the shrimp accumulated 0.1 % of the anthracene from the food, but depurated it twice as fast as its accumulation, showing that it is efficient in depurating hydrocarbons (Figs. 10.8 and 10.9).

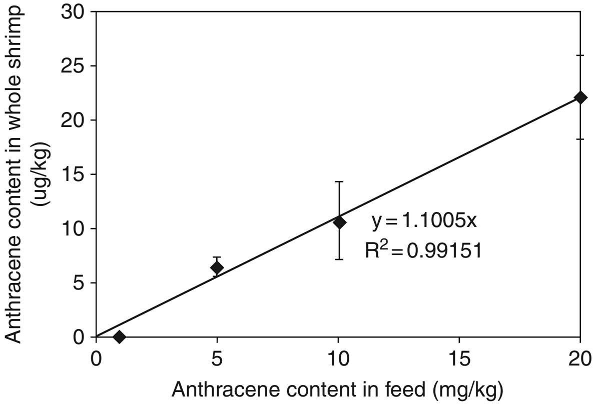


Fig. 10.8 Relationship between anthracene accumulated in shrimp and anthracene content in feed. Linear correlation (M ± SD) (Reprinted from Ong et al. 2011: 581, courtesy Elsevier Publishing Co)

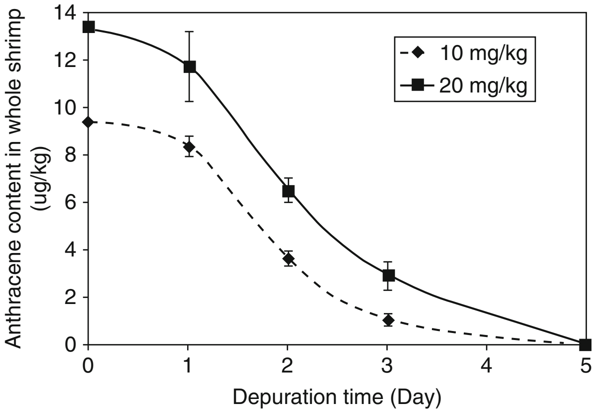


Fig. 10.9 Anthracene depuration in *P. monodon* after feeding with 10 or 20 mg kg^{-1} anthracene-contaminated feed for 30 days. Depuration was completed within 5 days (M ± SD) (Reprinted from Ong et al. 2011: 581, courtesy Elsevier Publishing Co)

Pesticides are stored and metabolized primarily in the hepatopancreas. Highly chlorinated compounds are metabolized very slowly, so they accumulate (Lee 1989). Lesser chlorinated compounds can be rapidly metabolized, conjugated, often to glutathione, and eliminated. Organophosphorus pesticides such as fenitrothion can be oxidized by the MFO system of crustaceans (Johnston and Corbett 1986). Hepatopancreases of *Eriocheir japonicus* from three Japanese rivers were analyzed for planar halogenated aromatic hydrocarbons (HAHs), including polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and coplanar PCBs. Crabs from the Tone river that runs through industrial, agricultural, and urban areas, had the highest concentrations of HAHs (4,100 pg g^{-1} fat weight). Identification of numerous PCDD and PCDF congeners in crabs from all three rivers provided evidence that one of the major sources of PCDDs and PCDFs was waste incineration. The higher TEQs in crabs from the Tone River (94.7 TEQ pg g^{-1} fat weight) were due to PCDDs and PCDFs, but the most important contributor was coplanar PCBs (49.95 %). The hepatopancreas contained glutathione S transferase (GST), and crabs with the highest TEQ levels had the highest GST activity (Ishizuka et al. 1998).

A variety of estuarine animals, including crabs (*C. sapidus*) and shrimp (*P. aztecus*) could metabolize tributyltin oxide (TBTO) which entered the animal via food or water. The hepatopancreas and stomach were important for metabolism of TBTO, while the gill, although important for uptake via the water, did not appear to be important in TBTO metabolism. *In vivo* studies showed dibutyltin (DBT) to be the major metabolite. The major metabolites in crab hepatopancreas were beta-hydroxybutyldibutltin and DBT. The CYP system appeared to be responsible for the oxidation of TBTO (Lee 1986).

Mollusks

Bioaccumulation and depuration experiments were performed by Yakan et al. (2011) with the mussel *M. galloprovincialis*. Benzo(a)anthracene (BaA) was chosen as the model PAH compound. Results showed that BaA can reach high levels in mussel tissues. When exposed for 15 days to water with 9 μg l^{-1}, mussels accumulated 17,180 ng g^{-1}, but rapidly depurated it when returned to clean water. A physiologically-based pharmacokinetic model (PBPK) was developed to describe the kinetics of 2,3,7,8-TCDD in the oyster *C. virginica* (Wintermyer et al. 2005). The estimated $t_{1/2}$ for elimination for a bolus dose of TCDD was 14–24 days based on experimental data and the model. The highest dioxin concentration was in the digestive gland followed by the mantle, gonad, hemolymph, gill, adductor muscle, and the kidney/heart. The PBPK model predicted the distribution and the elimination concentrations within each tissue compartment.

Stress proteins can be induced in mollusks in response to organic contaminants. Exposure to PAHs adsorbed onto clay particles and to suspended contaminated sediments induced HSP70 in *C. virginica*, which played a protective role (Cruz-Rodriguez and Chu 2002). Jonsson et al. (2006) found that exposure of mussels to brominated flame retardant, crude oil, bisphenol A and diallylphthalate all induced HSP70 expression. *C. virginica* showed only a limited ability to metabolize TBTO (Lee 1986).

Fishes

PCBs accumulate in fish primarily via food. Sex differences in PCB concentrations of adult fish have been attributed to females losing a substantial portion of their PCB body burden during spawning. There may also be sex differences in habitat utilization leading to sex differences in the PCB concentrations of prey and/or sex differences in growth efficiency, (growth divided by the amount of food consumption needed to achieve that growth). Based on analyses by Madenjian (2011), the first two mechanisms operate in relatively few fish, but can lead to males having PCB concentrations 2–3× higher than females. Growth efficiency operates in all fish, but results in only modest sex differences, with mature males having only 15–35 % higher PCB levels than mature females.

Fish metabolize PAHs using the CYP system, and CYP1A is a biomarker of environmental exposure to organic xenobiotic chemicals that act through the aryl hydrocarbon receptor. Many environments contaminated with organic chemicals, such as urban harbors, are also hypoxic. Rahman and Thomas (2012) found that hypoxia induces down-regulation of CYP1A expression in the Atlantic croaker, thereby interfering with metabolism of organic chemicals.

There are differences in PAH metabolism depending on life history stage. When mature eels are preparing to migrate downstream to reproduce in the ocean, the "silvering process" involves physiological and morphological changes including cessation of feeding. Reduced food intake results in a reduction of bile production and increased accumulation of PAH-metabolites in bile. Nagel et al. (2012) investigated the hydroxyl-metabolites of pyrene and phenanthrene in the bile of different maturation stages of eels, and found increasing PAH metabolite levels in bile during the silvering process. The highest rise was observed at the transition from pre migration stage III to the migrating stage IV, suggesting the beginning of cessation of feeding at this stage (Fig. 10.10).

Spot (*Leistomus xanthurus*) were able to metabolize tributyltin oxide (TBTO) which entered via food or water. The liver and intestine were important organs for TBTO metabolism, while the gill did not appear to be important. The major metabolites were beta-hydroxybutyldibutyltin and DBT. The CYP system appeared to be responsible for the oxidation of TBTO in liver microsomes. TBTO oxidation required NADPH and oxygen and was inhibited by carbon monoxide (Lee 1986).

Among contaminants of emerging concern are synthetic musks. Fernandes et al. (2013) investigated the metabolism and mode of action of the polycyclic musk galaxolide (HHCB) in the European sea bass, *Dicentrarchus labrax*, after an intraperitoneal injection of 50 mg HHCB kg body weight^{-1}. HHCB was actively metabolized and acted as a weak inhibitor of the synthesis of oxyandrogens in gonads of male fish. Both HHCB and a hydroxylated metabolite were detected in bile.

Other Taxa

The polychaetes *Nereis virens* and *Capitella capitata* have a CYP system that metabolizes PAHs. The system in *N. virens* is associated with microsomes in the lower intestine (Lee et al. 1979). In *C. capitata*, enzyme activity was seen only after exposure to petroleum hydrocarbons. Exposure of *Nereis succinea* to crude oil decreased growth rate and stimulated an increase in CYP activity. Elevated CYP activity was also seen in worms collected from a chronically polluted site (Lee et al. 1981).

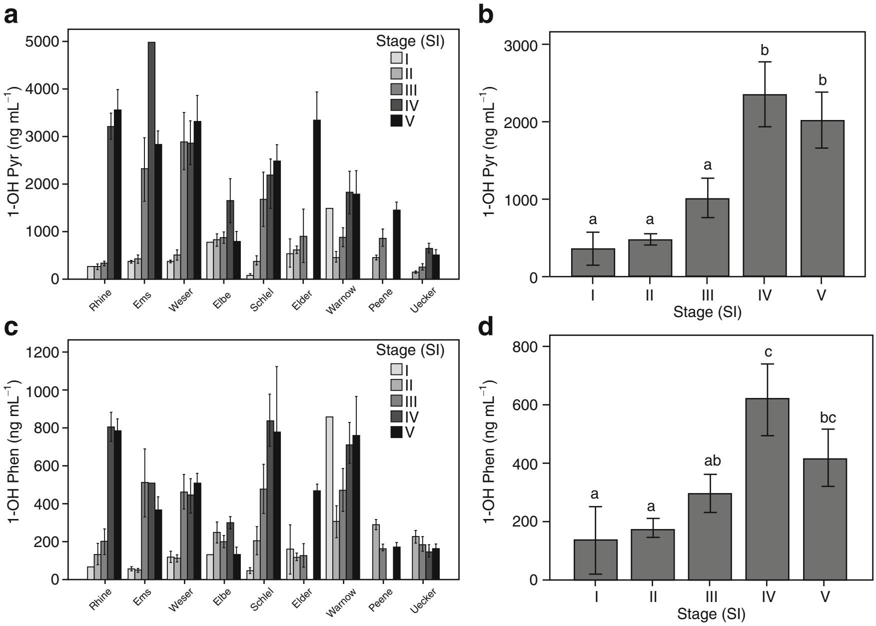


Fig. 10.10 Levels of PAH metabolites (ng ml^{-1}) in relation to silvering process in European eel. 1-hydroxypyrene (1-OH Pyr) (**a** and **b**); and hydrophenanthrene (1-OH Phen) (**c** and **d**) grouped from German rivers (**a** and **c**) or silvering index (SI) (**b** and **d**). SI 1 (*white*) to II (*slight gray*) for yellow eel, SI III (*gray*) for pre migrating eels and SI IV (*dark gray*) to V (*black*) for silver eel (M ± SE). *Letters* indicate significantly different groups, $p < 0.05$ (Reprinted from Nagel et al. 2012: 93, courtesy Elsevier Publishing Co)

10.3 Conclusions

Marine organisms incorporate chemicals in relation to their bioavailability in the environment, generally from water, food, or sediment. Intake can be via gills, digestive system, or the skin. Bioaccumulation is frequently (but not always) highest in the liver, or comparable organ in invertebrates (hepatopancreas), though levels in muscle tissue may also be high, which is of concern to human consumers of seafood. The subcellular distribution of the contaminant is of importance in determining its toxicity to the organism in question, as well as the potential for trophic transfer to animals higher in the food web. Chlorinated organic chemicals tend to be metabolized very slowly, so they tend to bioaccumulate and biomagnify. The ability to metabolize other organic contaminants such as PAHs via the CYP system of enzymes may reduce their toxicity and enable them to be excreted.

References

Ahearn GA, Sterling M, Mandal PK, Roggenbeck B (2010) Heavy metal transport and detoxification by crustacean epithelial lysosomes. In: Gerenczer GA (ed) Epithelial transport physiology. Humana Press, pp 49–71. doi: 10.1007/978-1-60327-229-2_3

Ahmad I, Mohmood I, Coelho JP, Pacheco M, Santos MA, Duarte AC, Pereira E (2012) Role of non-enzymatic antioxidants on the bivalves' adaptation to environmental mercury: Organ-specificities and age effect in *Scrobicularia plana* inhabiting a contaminated lagoon. Environ Pollut 163:218–225

Bagatto G, Alikhan MA (1987) Zinc, iron, manganese and magnesium accumulation in crayfish populations near copper-nickel smelters at Sudbury, Ontario. Can Bull Environ Contam Toxicol 38:1076–1081

Bastidas C, Garcia EM (2004) Sublethal effects of mercury and its distribution in the coral *Porites astreoides*. Mar Ecol Prog Ser 267:133–143

Bergey L, Weis JS (2007) Molting as a mechanism of depuration of metals in the fiddler crab, *Uca pugnax*. Mar Environ Res 64:556–562

Berrojalbiz N, Lacorte S, Calbet A, Saiz E, Barata C, Dachs J (2009) Accumulation and cycling of polycyclic aromatic hydrocarbons in Zooplankton. Environ Sci Technol 43:2295–2301

Burns KA (1976) Hydrocarbon metabolism in the intertidal fiddler crab *Uca pugnax*. Mar Biol 36:5–11

Caccamise SA, Wang J, Wu L, Woodward LA, Li QX (2012) Accumulation and toxicity assessment of polychlorinated biphenyls in black-footed albatross (*Diomedea nigripes*) from Midway Atoll, North Pacific Ocean. Ecol Ind 20:75–81

Cailleaud K, Forget-Leray J, Souissi S, Hilde D, LeMenach K, Budzinski H (2007) Seasonal variations of hydrophobic organic contaminant concentrations in the water-column of the Seine Estuary and their transfer to a planktonic species *Eurytemora affinis* (Calanoïda, copepoda). Part 1: PCBs and PAHs. Chemosphere 70:270–280

Casado-Martinez MC, Duncan E, Smith BD, Maher WA, Rainbow PS (2012) Arsenic toxicity in a sediment-dwelling polychaete: detoxification and arsenic metabolism. Ecotoxicology 21:576–590

Clark J, Patrick JM, Moore JC, Forester J (1986) Accumulation of sediment-bound PCBs by fiddler crabs. Bull Environ Contam Toxicol 36:571–578

Cretney WJ, Yunker MB (2000) Concentration dependency of biota-sediment accumulation factors for chlorinated dibenzo-*p* -dioxins and dibenzofurans in dungeness crab (*Cancer magister*) at marine pulp mill sites in British Columbia, Canada. Environ Toxicol Chem 19:3012–3023

Cruz-Rodriguez LA, Chu FL-E (2002) Heat shock response in the Eastern oyster *Crassostrea virginica* exposed to PAH sorbed to suspended artificial clay particles and to suspended field sediments. Aquat Toxicol 60:157–168

Dam E, Rewitz KF, Styrishave B, Andersen O (2008) Cytochrome P450 expression is moult stage specific and regulated by ecdysteroids and xenobiotics in the crab *Carcinus maenas*. Biochem Biophys Res Commun 377:1135–1140

Dang F, Rainbow PS, Wang WX (2012) Dietary toxicity of field-contaminated invertebrates to marine fish: effects of metal doses and subcellular metal distribution. Aquat Toxicol 120–121:1–10

David CP (2002) Heavy metal concentrations in growth bands of corals: a record of mine tailings input through time (Marinduque Island, Philippines). Mar Pollut Bull 46:187–196

Devi M, Thomas DA, Barber JT, Fingerman M (1996) Accumulation and physiological and biochemical effects of cadmium in a simple aquatic food chain. Ecotoxicol Environ Saf 33:38–43

Dinn PM, Johannessen SC, Ross PS, Macdonald RW, Whiticar MJ, Lowe CJ, van Roodselaar A (2012) PBDE and PCB accumulation in benthos near marine wastewater outfalls: the role of sediment organic carbon. Environ Poll 171:241–248

DiPinto L, Coull B (1997) Trophic transfer of sediment-associated polychlorinated biphenyls from meiobenthos to bottom-feeding fish. Environ Toxicol Chem 16:2568–2575

Dodge RE, Gilbert TR (1984) Chronology of lead pollution contained in banded coral skeletons. Mar Biol 82:9–13

Dutton J, Fisher N (2011a) Bioaccumulation of As, Cd, Cr, Hg(II), and MeHg in killifish (*Fundulus heteroclitus*) from amphipod and worm prey. Sci Total Environ 409:3438–3447

Dutton J, Fisher N (2011b) Salinity effects on the bioavailability of aqueous metals for the estuarine killifish *Fundulus heteroclitus*. Environ Toxicol Chem 30:2107–2114

Dutton J, Fisher N (2012) Influence of humic acid on the uptake of aqueous metals by the killifish *Fundulus heteroclitus*. Environ Toxicol Chem 31:2225–2232

Eckwert H, Alberti G, Kohler HR (1997) The induction of stress proteins (HSP) in *Oniscus asellus* (Isopoda) as a molecular marker of multiple heavy metal exposure. I. Principles and toxicological assessment. Ecotoxicology 6:249–262

Engel DW (1983) The intracellular partitioning of trace metals in marine shellfish. Sci Total Environ 28:129–140

Engel DW (1987) Metal regulation and molting in the blue crab *Callinectes sapidus*: copper, zinc, and metallothionein. Biol Bull 172:69–82

Engel DW, Brouwer M (1989) Metallothionein and metallothionein-like proteins: physiological importance. Adv Comp Environ Physiol 5:53–75

Engel D, Brouwer M, Thurberg FP (1985) Comparison of metal metabolism and metal-binding proteins in the blue crab and the American lobster. In: Vernberg FJ, Thurberg FP, Calabrese A, Vernberg WB (eds) Marine pollution and physiology: recent advances. University of South Carolina Press, Columbia, pp 229–245

Fernandes D, Dimastrogiovanni G, Blázquez M, Porte C (2013) Metabolism of the polycyclic musk galaxolide and its interference with endogenous and xenobiotic metabolizing enzymes in the European sea bass (*Dicentrarchus labrax*). Environ Pollut 174:214–221

Ferreira AM, Vale C (1998) PCB accumulation and alterations of lipids in two length classes of the oyster *Crassostrea angulata* and of the clam *Ruditapes decussates*. Mar Environ Res 45:259–268

Fillmann J, Watson GM, Howsam M, Francioni E, Depledge MH, Readman JW (2004) Urinary PAH metabolites as biomarkers of exposure in aquatic environments. Environ Sci Technol 38:2649–2656

Goto D, Wallace WG (2009a) Relevance of intracellular partitioning of metals in prey to differential metal bioaccumulation among populations of mummichogs (*Fundulus heteroclitus*). Mar Environ Res 68:257–267

Goto D, Wallace WG (2009b) Influences of prey- and predator- dependent processes on cadmium and methylmercury trophic transfer to mummichogs (*Fundulus heteroclitus*). Can J Fish Aquat Sci 66:836–846

Green N, Knutzen J (2003) Organohalogens and metals in marine fish and mussels and some relationships to biological variables at reference localities in Norway. Mar Pollut Bull 46:362–374

Guo F, Yang Y, Wang W-X (2013) Metal bioavailability from different natural prey to a marine predator *Nassarius siquijorensis*. Aquat Toxicol 126:266–273

Hellou J, Parsons D, Mercer G (1997) Organochlorine contaminants in the Northern shrimp, *Pandalus borealis*, collected from the Northwest Atlantic. Mar Environ Res 44:99–113

Huang S, Strathe AB, Fadel JG, Johnson ML, Lin P, Liu T-Y, Hung SS (2013) The interactive effects of selenomethionine and methylmercury on their absorption, disposition, and elimination in juvenile white sturgeon. Aquat Toxicol 126:274–282

Ishizuka M, Sakiyama T, Iwata H, Fukushima M, Kazusaka A, Fujita S (1998) Accumulation of halogenated aromatic hydrocarbons and activities of cytochrome P450 and glutathione *s*-transferase in crabs (*Eriocheir japonicus*) from Japanese Rivers. Environ Toxicol Chem 17:1490–1498

Johnston JJ, Corbett MD (1986) The uptake and in vivo metabolism of the organophosphate insecticide fenitrothion by the blue crab, *Callinectes sapidus*. Toxicol Appl Pharmacol 85:181–188

Jonsson H et al (2006) Expression of cytoskeletal proteins, cross-reacting with anti-CYP1A, in *Mytilus* sp exposed to organic contaminants. Aquat Toxicol 78(suppl 1):S42–S48

Kannan K, Tanabe S, Tatsukawa R (1995) Geographical distribution and accumulation features of organochlorine residues in fish in tropical Asia and Oceania. Environ Sci Technol 29:2673–2683

Karouna-Reiner N, Snyder RA, Allison JG, Wagner MG, Rao KR (2007) Accumulation of organic and inorganic contaminants in shellfish collected in estuarine waters near Pensacola, Florida: contamination profiles and risks to human consumers. Environ Pollut 145:474–488

Keteles K, Fleeger J (2001) The contribution of ecdysis to the fate of copper, zinc and cadmium in grass shrimp, *Palaemonetes pugio* Holthius. Mar Pollut Bull 42:1397–1402

Khoury J, Powers E, Patnaik P, Wallace WG (2009) Relating disparity in competitive foraging behavior between two populations of fiddler crabs to the subcellular partitioning of metals. Arch Environ Contam Toxicol 56:489–499

Kim SK, Oh JR, Shim WJ, Lee DH, Yim UH, Hong SH, Shin YB, Lee DS (2002) Geographical distribution and accumulation features of organochlorine residues in bivalves from coastal areas of South Korea. Mar Pollut Bull 45:268–279

Koenig S, Fernándes P, Solé M (2012) Differences in cytochrome P450 enzyme activities between fish and crustacea: relationship with the bioaccumulation patterns of polychlorobiphenyls (PCBs). Aquat Toxicol 108:11–17

Kouba A, Buřič M, Kosák P (2010) Bioaccumulation and effects of heavy metals in crayfish: a review. Water Air Soil Pollut 211:5–16

Lacoue-Labarthe T, Réveillac E, Oberhänsli F, Teyssié JL, Jeffree R, Gattuso JP (2011) Effects of ocean acidification on trace element accumulation in the early-life stages of squid *Loligo vulgaris*. Aquat Toxicol 105:166–176

Lee RF (1986) Metabolism of bis(tributyltin)oxide by estuarine animals. Presented at Oceans '86, Washington DC, pp 1182–1188. 10.1109/OCEANS.1986.1160361

Lee RF (1989) Metabolism and accumulation of xenobiotics within hepatopancreas cells of the blue crab, *Callinectes sapidus*. Mar Environ Res 28:93–97

Lee RF, Singer SC, Tenore K, Gardner WS (1979) Detoxification system in polychaete worms: importance in the degradation of petroleum hydrocarbons. In: Vernberg WB, Thurberg FP, Calabrese A, Vernberg FJ (eds) Marine pollution: functional responses. Academic Press, New York, pp 23–37

Lee RF, Stolzenbach J, Singer S, Tenore KR (1981) Effects of crude oil on growth and mixed function oxygenase activity in polychaetes, Nereis sp. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Biological monitoring of marine pollutants. Academic Press, New York, pp 323–334

Li H, Turner A, Brown MT (2012) Accumulation of aqueous and nanoparticulate silver by the marine gastropod *Littorina littorea*. Water Air Soil Pollut 224:1354–1357

Loro VL, Jorge MB, da Silva KR, Wood CM (2012) Oxidative stress parameters and antioxidant response to sublethal waterborne zinc in a euryhaline teleost *Fundulus heteroclitus*: protective effects of salinity. Aquat Toxicol 110–111:187–193

Madenjian CP (2011) Sex effect on polychlorinated biphenyl concentrations in fish: a synthesis. Fish Fish 12:451–460

Magnusson K, Magnusson M, Östberg P, Granberg M, Tiselius P (2007) Bioaccumulation of ^{14}C-PCB 101 and ^{14}C-PBDE 99 in the marine planktonic copepod *Calanus finmarchicus* under different food regimes. Mar Environ Res 63:67–81

Marinucci A, Bartha R (1982) Accumulation of the polychlorinated biphenyl aroclor 1242 from contaminated detritus and water by the saltmarsh detritivore, *Uca pugnax*. Bull Environ Contam Toxicol 29:326–333

Martins CG, Almeida DV, Marins LF, Bianchini A (2011) mRNA expression and activity of ion-transporting proteins in gills of the blue crab *Callinectes sapidus*: effects of waterborne copper. Environ Toxicol Chem 30:206–211

Meiro CL et al (2011) Metallothioneins failed to reflect mercury external levels of exposure and bioaccumulation in marine fish – considerations on tissue and species specific responses. Chemosphere 85:114–121

Menone ML, Bortolus A, Botto F, Aizpún de Moreno JE, Moreno VJ, Iribarne O, Metcalfe TL, Metcalfe CD (2000) Organochlorine contaminants in a coastal lagoon in Argentina: analysis of sediment, crabs and cordgrass from two different habitats. Estuaries Coasts 23:583–592

Mieiro CL, Coelho JP, Pacheco M, Duarte AC, Pereira ME (2012) Evaluation of species-specific dissimilarities in two marine fish species: mercury accumulation as a function of metal levels in consumed prey. Arch Environ Contam Toxicol 63:125–136

Mothershead R, Hale R (1992) Influence of ecdysis on the accumulation of polycyclic aromatic hydrocarbons in field exposed blue crabs (*Callinectes sapidus*). Mar Environ Res 33:145–156

Nagel F, Wagner C, Hanel R, Kammann U (2012) The silvering process of European eel (*Anguilla anguilla*) influences PAH metabolite concentrations in bile fluid: consequences for monitoring. Chemosphere 87:91–96

Nakata H, Sakai Y, Miyawaki T (2002) Growth-dependent and species-specific accumulation of polychlorinated biphenyls (PCBs) in tidal flat organisms collected from the Ariake Sea, Japan. Arch Environ Contam Toxicol 42:222–228

Nakata H et al (2012) Asia–Pacific mussel watch for emerging pollutants: distribution of synthetic musks and benzotriazole UV stabilizers in Asian and US coastal waters. Mar Pollut Bull 64:2211–2218

Nassiri Y, Rainbow PS, Amiard-Triquet C, Rainglet F, Smith BD (2000) Trace metal detoxification in the ventral caeca of *Orchestia gammarellis*. Mar Biol 136:477–484

Norstrom RJ, Muir DCG, Ford CA, Simon M, Macdonald CR, Béland P (1992) Indications of P450 monooxygenase activities in beluga (*Delphinapterus leucas*) and narwhal (*Monodon monoceros*) from patterns of PCB, PCDD and PCDF accumulation. Mar Environ Res 34:267–272

O'Connor T (1991) Concentrations of organic contaminants in mollusks and sediments at NOAA National Status and Trend sites in the coastal and estuarine United States. Environ Health Perspect 90:69–73

O'Neill S, West JE, Hoeman JC (1998) Spatial trends in the concentration of polychlorinated biphenyls (PCBs) in chinook (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) in Puget Sound and factors affecting PCB accumulation: results from the Puget Sound Ambient Monitoring Program. In: Proceedings of the Puget sound research '98. Washington Dept. of Fish and Wildlife, pp 312–328

Ong PT, Yong JC, Chin KY, Hii YS (2011) Bioaccumulation and depuration of anthracene in *Penaeus monodon* (Fibricius) through food ingestion. Chemosphere 84:578–584

Pan K, Wang WX (2011) Mercury accumulation in marine bivalves: influences of biodynamics and feeding niche. Environ Pollut 159:2500–2506

Pastor D, Boix J, Fernández V, Albaigés J (1996) Bioaccumulation of organochlorinated contaminants in three estuarine fish species (*Mullus barbatus*, *Mugil cephalus* and *Dicentrarcus labrax*). Mar Pollut Bull 32:257–262

Pierce GJ, Stowasser G, Hastie LC, Bustamante P (2008) Geographic, seasonal and ontogenetic variation in cadmium and mercury concentrations in squid (Cephalopoda: Teuthoidea) from UK waters. Ecotoxicol Environ Saf 70:422–432

Polak-Juszczak L (2012) Bioaccumulation of mercury in the trophic chain of flatfish from the Baltic Sea. Chemosphere 89:585–591

Pruell R, Taplin BK, McGovern DG, McKinney R, Norton SB (2000) Organic contaminant distributions in sediments, polychaetes (*Nereis virens*) and American lobster (*Homarus americanus*) from a laboratory food chain experiment. Mar Environ Res 49:19–36

Rahman MS, Thomas P (2012) Effects of hypoxia exposure on hepatic cytochrome P450 1A (CYP1A) expression in Atlantic croaker: molecular mechanisms of CYP1A down-regulation. PLoS One 7(7):e40825. doi:10.1371/journal.pone.0040825

Rainbow PS (1988) The significance of trace metal concentrations in decapods. Symp Zool Soc Lond 59:195–211

Rainbow PS (1993) The significance of trace metal concentrations in marine invertebrates. In: Dallinger R, Rainbow PS (eds) Ecotoxicology of metals in invertebrates. Lewis Publishers, Chelsea, pp 3–23

Rainbow PS, White SL (1989) Comparative strategies of heavy metal accumulation by crustaceans: zinc, copper and cadmium in decapod, an amphipod and a barnacle. Hydrobiologia 174:245–262

Rainbow PS, Poirier L, Smith BD, Brix KV, Luoma SN (2006) Trophic transfer of trace metals from the polychaete worm *Nereis diversicolor* to the polychaete *N. virens* and the decapod crustacean *Palaemonetes varians*. Mar Ecol Prog Ser 321:167–181

Rainbow PS, Luoma SN, Wang WX (2011) Trophically available metal – a variable feast. Environ Pollut 159:2347–2349

Rappe C et al (1991) Levels and patterns of PCDD and PCDF contamination in fish, crabs, and lobsters from Newark Bay and the New York Bight. Chemosphere 22:239–266

Reichelt-Brushett AJ, McOrist G (2003) Trace metals in the living and nonliving components of scleractinian corals. Mar Pollut Bull 46:1573–1582

Reichmuth JM, Weis P, Weis JS (2010) Bioaccumulation and depuration of metals in blue crabs (*Callinectes sapidus* Rathbun) from a contaminated and clean estuary. Environ Pollut 158:361–368

Reinfelder JM, Wang W-X, Luoma SN, Fisher NS (1997) Assimilation efficiencies and turnover rates of trace elements in marine bivalves: a comparison of oysters, clams and mussels. Mar Biol 129:443–452

Rhee J-S et al (2009) Heat shock protein (HSP) gene responses of the intertidal copepod *Tigriupus japonicus* to environmental toxicants. Comp Biochem Physiol 149C:104–112

Rubinstein N, Gilliam WT, Gregory NR (1984) Dietary accumulation of PCBs from a contaminated sediment source by a demersal fish (*Leiostomus xanthurus*). Aquat Toxicol 5:331–342

Ruus A, Daae IA, Hylland K (2012) Accumulation of polychlorinated biphenyls from contaminated sediment by Atlantic cod (*Gadus morhua*): direct accumulation from resuspended sediment and dietary accumulation via the polychaete *Nereis virens*. Environ Toxicol Chem 31:2472–2481

Sanders BM (1993) Stress proteins in aquatic organisms: an environmental perspective. Crit Rev Toxicol 23:49–75

Sanders BM, Jenkins KD (1984) Relationships between free cupric ion concentrations in sea water and copper metabolism and growth in crab larvae. Biol Bull 167:704–712

Schill R, Görlitz H, Köhler H-R (2003) Laboratory simulation of a mining accident: acute toxicity, hsc/hsp70 response, and recovery from stress in *Gammarus fossarum* (Crustacea, Amphipoda) exposed to a pulse of cadmium. Biometals 16:391–401

Schlenk D, Brouwer M (1991) Isolation of three copper metallothionein isoforms from the blue crab (*Callinectes sapidus*). Aquat Toxicol 20:25–33

Seebaugh DR, Wallace WG (2004) Importance of metal-binding proteins in the partitioning of Cd and Zn as trophically available metal (TAM) in the brine shrimp *Artemia franciscana*. Mar Ecol Prog Ser 272:215–230

Seebaugh DR, Goto D, Wallace WG (2005) Bioenhancement of cadmium transfer along a multilevel food chain. Mar Environ Res 59:473–491

Seebaugh DR, Estephan A, Wallace WG (2006) Relationship between cadmium assimilation by grass shrimp (*Palaemonetes pugio*) and trophically available cadmium in amphipod (*Gammarus lawrencianus*) prey. Bull Environ Contam Toxicol 76:16–23

Shyn A, Chalk SJ, Smith K, Charnock NL, Bielmyer GK (2012) Zinc distribution in the organs of adult *Fundulus heteroclitus* after waterborne zinc exposure in freshwater and saltwater. Arch Environ Contam Toxicol 63:544–553

Sloan RJ, Armstrong RW (1988) PCB patterns in Hudson River fish: II. Migrant and marine species. In: Fisheries research in the Hudson River. State University of New York Press, Albany, pp 325–350

Sze PW, Lee SY (1995) The potential role of mucus in the depuration of copper from the mussels *Perna viridis*. Mar Pollut Bull 31:390–393

Tanaka K, Takada H, Yamashita R, Mizukawa K, Fukuwaka M, Watanuki Y (2013) Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. Mar Pollut Bull 69(1–2):219–222

Tavares S, Oliveira H, Coelho JP, Pereira ME, Duarte AC, Pardal MA (2011) Lifespan mercury accumulation pattern in *Liza aurata*: evidence from two southern European estuaries. Estuar Coast Shelf Sci 94:315–321

Tedengren M et al (2000) Heat pretreatment increases cadmium resistance and HSP70 levels in Baltic Sea mussels. Aquat Toxicol 48:1–12

Ueno D, Inoue S, Ikeda K, Tanaka H, Yamada H, Tanabe S (2003) Specific accumulation of polychlorinated biphenyls and organochlorine pesticides in Japanese common squid as a bioindicator. Environ Pollut 125:227–235

Van Leeuwen SP, van Velzen MJM, Swart CP, van der Veen I, Traag WA, de Boer J (2009) Halogenated contaminants in farmed salmon, trout, tilapia, pangasius, and shrimp. Environ Sci Technol 43:4009–4015

Vogt G, Quinitio ET (1994) Accumulation and excretion of metal granules in the prawn, *Penaeus monodon*, exposed to water-borne copper, lead, iron and calcium. Aquat Toxicol 28:223–241

Wallace WG, Lopez GR (1996) Relationship between subcellular cadmium distribution in prey and cadmium trophic transfer to a predator. Estuar Coast 19:923–930

Wallace WG, Lopez GR (1997) Bioavailability of biologically sequestered cadmium and the implications of metal detoxification. Mar Ecol Prog Ser 147:149–157

Wallace WD, Luoma SN (2003) Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically available metal (TAM). Mar Ecol Prog Ser 257:125–137

Wang Y, Wang T, Li A, Fu J, Wang P, Zhang Q, Jiang G (2008) Selection of bioindicators of polybrominated diphenyl ethers, polychlorinated biphenyls, and organochlorine pesticides in mollusks in the Chinese Bohai Sea. Environ Sci Technol 42:7159–7165

Wang WX, Yang Y, Guo X, He M, Guo F, Ke C (2011) Copper and zinc contamination in oysters: subcellular distribution and detoxification. Environ Toxicol Chem 30:1767–1774

Ward DM, Nislow KH, Chen CY, Folt CL (2010) Rapid, efficient growth reduces mercury concentrations in stream-dwelling Atlantic salmon. Trans Am Fish Soc 139:1–10

Watson GM, Andersen O-K, Galloway TS, Depledge MH (2004) Rapid assessment of polycyclic aromatic hydrocarbon (PAH) exposure in decapod crustaceans by fluorimetric analysis of urine and haemolymph. Aquat Toxicol 67:127–142

Weis JS, Candelmo A (2012) Pollutants and fish predator/prey behavior: a review of laboratory and field approaches. Curr Zool 58:9–20

Weis JS, Weis P (1993) Trophic transfer of contaminants from organisms living by chromated-copper-arsenate (CCA)-treated wood to their predators. J Exp Mar Biol Ecol 168:25–34

Weis P, Weis JS, Couch J (1993) Histopathology and bioaccumulation in oysters (*Crassostrea virginica*) living on wood preserved with chromated copper arsenate. Dis Aquat Org 17:41–46

White SL, Rainbow PS (1982) Regulation and accumulation of copper, zinc and cadmium by the shrimp *Palaemon elegans*. Mar Ecol Prog Ser 8:95–101

White SL, Rainbow PS (1986) Accumulation of cadmium by *Palaemon elegans* (Crustacea: Decapoda). Mar Ecol Prog Ser 32:17–25

Wintermyer M, Skaidas A, Roy A, Yang Y-C, Georgapoulos P, Burger J, Cooper K (2005) The development of a physiologically-based pharmacokinetic model using the distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the tissues of the eastern oyster (*Crassostrea virginica*). Mar Environ Res 60:133–152

Wolkers H, van Bavel B, Ericson I, Skoglund E, Kovacs KM, Lydersen C (2006) Congener-specific accumulation and patterns of chlorinated and brominated contaminants in adult male walruses from Svalbard, Norway: indications for individual-specific prey selection. Sci Total Environ 370:70–79

Yakan SD, Henkelmann B, Schramm K-W, Okay OS (2011) Bioaccumulation depuration kinetics and effects of benzo(a)anthracene on *Mytilus galloprovincialis*. Mar Pollut Bull 63:471–476

Chapter 11
Tolerance

Abstract Tolerance is the ability of organisms to cope with stress, in this case to environmental pollutants. It appears to be a widespread phenomenon, and can be achieved by physiological acclimation or genetic adaptation. It can be assessed by comparing responses (lethal or sublethal) of individuals from different populations to the same degree of stress, e.g. the same concentration of a toxicant would produce less of an effect in a tolerant population. The phenomenon is well documented for metals and organic contaminants (for example, resistance of insect populations to insecticides is well-known). There are also cases in which tolerance has been looked for but not found in chronically exposed populations; probably more cases than have been reported in the literature, as this can be viewed as "negative data" and not reported. When enhanced tolerance does not occur in polluted populations, the reasons may be difficult to ascertain; it may be because detoxification mechanisms are adequate to cope with elevated exposures, or that dispersal and mixing between contaminated and reference populations obscures any observation of tolerance, or that the fitness costs counteract the selective advantage of the tolerance, or other reasons.

Compensatory responses to pollutants at the physiological level are referred to as "acclimation." Pre-exposure to chemicals can induce or enhance detoxification processes, discussed in the previous chapter, which reduces toxicity in pre-exposed organisms, either in the lab or at field sites. These responses (e.g. synthesis of MTs, CYPs) can mitigate effects on individuals experiencing moderate stress. Developing tolerance is energetically expensive, however, and may have deleterious effects on energy allocation. Thus exposure over extended periods may reduce fitness and increase selection pressure for genetically resistant individuals in populations that have variation and heritability for the response. The development of these populations that have adapted and become more resistant to pollution can be considered an evolutionary compensatory mechanism.

Keywords Costs • Cytochrome P450 • CYP • Energetics • Evolution • Food web • Metallothioneins • Sediment • Trade-offs

J.S. Weis, *Physiological, Developmental and Behavioral Effects of Marine Pollution*,
DOI 10.1007/978-94-007-6949-6_11,

11.1 Metals

Some metals (Zn, Cu) are essential for life, so organisms have developed ways to optimize their exposures and respond to higher levels. Metal tolerance is widespread, and is often associated with the storage of metals in a non-available form, rather than reduced uptake or increased elimination, although these mechanisms have also been observed. Metallothioneins (MTs) play important roles in the metabolism of essential metals and can be utilized in dealing with metal contaminants. For example, some contaminated populations have developed multiple copies of the gene for MT, enabling them to tolerate high metal concentrations (Soskine and Towkik 2010).

11.1.1 Fishes

While there are numerous papers about MTs and other protective mechanisms (see previous chapter) and many studies documenting enhanced metal tolerance in fish populations living in contaminated freshwater environments, reports on metal tolerance in marine species are less frequent. Early life stages of the killifish, *Fundulus heteroclitus*, inhabiting Piles Creek (PC), a contaminated industrialized estuary in New Jersey, U.S., have tolerance to Hg, one of the contaminants in the environment (Weis 2002). However, tolerance is manifested only in early life stages. Tolerance to meHg was seen in gametes (both sperm and eggs) and in developing embryos, which were less affected by meHg (Weis et al. 1981; Khan and Weis 1987a, b, c, d) (Fig. 11.1). A possible mechanism for tolerance of the embryos was reduced uptake through the chorion. While PC embryos were more tolerant to

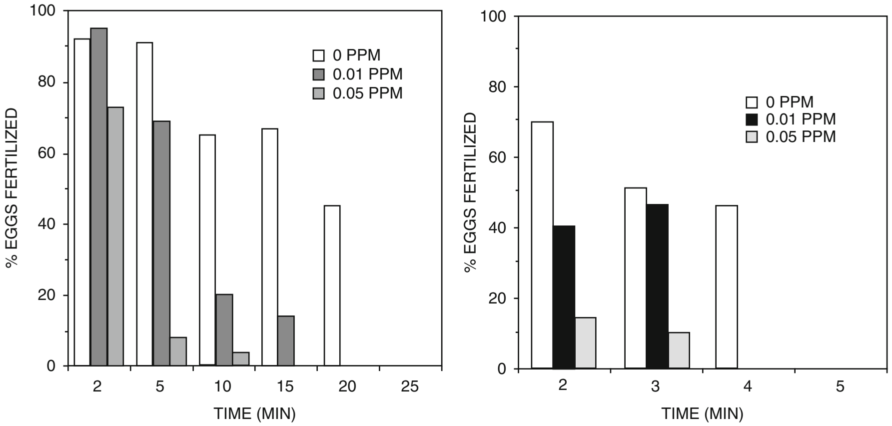


Fig. 11.1 (**a**) Effect of meHg on Piles Creek sperm mean fertilization success. (**b**) Effect of meHg on Long Island (reference) sperm mean fertilization success (Reprinted from Khan and Weis 1987a: 501, courtesy Springer Publishing Co)

meHg, they were less tolerant than embryos of reference populations to inorganic Hg. Tolerance was not exhibited in larvae or adults from the PC population, which showed signs of stress in terms of reduced growth and longevity (Weis and Weis 1989).

Mechanisms of copper tolerance in black-banded rainbowfish (*Melanotaenia nigrans*) were investigated by Gale et al. (2003). One population has been exposed to elevated Cu for over 40 years, due to mine leachate. The 96 h EC_{50} of exposed [E] fish was 8.3 times higher than that of reference [R] fish. Both E and R fish were exposed to low (L_{Cu}, 30 μg Cu l^{-1}) and elevated (E_{Cu}, 300 μg Cu l^{-1}) Cu for 24 and 48 h, respectively. Copper uptake in most tissues was less (up to 50 %) in E fish. Thus, the mechanism of tolerance was considered to be reduced uptake in the gills, rather than increased binding or elimination. Allozyme electrophoresis was performed and allozyme frequencies at the AAT-1 and GPI-1 loci were significantly different between E and R populations. Also, there was less heterozygosity in the E population. These results suggest that genetic selection may have occurred in the E population: selection for allozymes less sensitive to Cu may be another mechanism of tolerance.

Klerks and Lentz (1998) investigated resistance of mosquitofish *Gambusia affinis* to lead and zinc. Fish collected from highly contaminated Bayou Trepagnier (LA, USA) and exposed to Zn in a laboratory bioassay did not differ in their sensitivity from conspecifics from a control site. In contrast, Bayou Trepagnier fish did show increased resistance to lead. This difference, however, disappeared when both groups were kept for 34 days in clean water. This suggests that the elevated Pb resistance is due to physiological acclimation rather than adaptation at the population level. Annabi et al. (2009) reported resistance to Cd in a population of *G. affinis* from a polluted site in Tunisia. A genetic basis was inferred from assays performed on the F1 generation raised in the laboratory.

In the sheepshead minnow (*Cyprinodon variegatus*) heritability was estimated for tolerance to individual contaminants (phenanthrene, zinc) and to contaminant mixtures (phenanthrene plus zinc, and a complex mixture with three metals and three PAHs) (Klerks and Moreau 2001). Estimates were obtained from resemblances between relatives, parent–offspring pairs, and families of sibs and half-sibs. Heritabilities for resistance to these chemicals were low (with the high resemblances among sibs being due to common environmental and dominance genetic variation rather than additive genetic variation). Results were interpreted to mean that *C. variegatus* in contaminated environments is not likely to become resistant to these contaminants rapidly, and that resistance may develop even more slowly as more contaminants become involved. Adeyami and Klerks (2013) investigated Cu acclimation in the least killifish, *Heterandria formosa* using both lethal and sublethal endpoints, as well as potential mechanisms. Fish were exposed to either a background Cu level or to 15 μg l^{-1} Cu for 7 days and then exposed to a lethal level (150 μg l^{-1}) and monitored until all fish had died, and (during the first 8 h of this exposure) for whole-body Na levels and lipid peroxidation (LPO). Pre-exposed fish had a significantly longer time-to-death than controls. Neither whole-body Na nor LPO changed in the pre-exposed fish during the acute exposure, while both decreased in the control fish. Thus, acclimation was seen in both time-to-death and sublethal endpoints, and Cu toxicity may involve both Na loss and LPO. Acclimation may be brought about by prevention of these effects. A follow-up study on potential mechanisms used a similar pre-exposure/exposure design and quantified Cu accumulation, Na^+/K^+-ATPase activity, MT levels, and catalase activity. While Cu levels were higher in pre-exposed fish at T_0, net accumulation was faster in the controls during the high-level Cu exposure. Therefore, changes in accumulation dynamics may play a role in resistance. Cu-acclimated fish also had higher MT levels. There was no evidence of involvement of Na^+/K^+-ATPase.

Noel-Lambot (1981) observed in various species of unfed fishes (*Anguilla anguilla, Myoxocephalus scorpjus, Serranus cabrilla, Moena chryselis, Scorpaena* sp.), white mucus corpuscles in the intestinal lumen that contained high concentrations of Ca and Mg. In fish treated with Cd, Zn, or Cu, these corpuscles contained very high concentrations of these metals, potentially much of the body burden. It appeared that metals were accumulated in the corpuscles directly from ingested sea water and that the corpuscles limited their entry through the intestinal wall, thus protecting the fish against potentially hazardous concentrations of metals.

11.1.2 Crustaceans

In some classic studies, Bryan and colleagues (Bryan and Hummerstone 1971, 1973; Bryan and Gibbs 1983) studied invertebrates including the amphipod, *Corophium volutator* in Restronguet Creek (RC), Cornwall, England, an estuary with high metal concentrations, particularly Cu, from mining operations upstream. These amphipods were more tolerant to Cu than conspecifics from reference sites, as was the crab, *Carcinus maenas*, which was also more tolerant to Zn. Since crab larvae have a pelagic phase that distributes them widely, it is likely that the larvae settling in RC are derived from parents from far away with no history of metal exposure. Thus, selection would operate on individual crabs that settle in the contaminated area, with the more resistant ones able to survive due to physiological acclimation rather than genetic adaptation of the population. Investigators found that Zn resistance increased with crab size, supporting the idea of selection on individuals and physiological acclimation. The tolerant crabs accumulated less metal or excreted more, reducing their body burdens.

Fiddler crabs (*Uca pugnax*) from highly contaminated Piles Creek (PC) N.J., U.S.A. were more tolerant to meHg than crabs from a reference site, as indicated by the degree of growth inhibition in regenerating limbs and the degree of delay in time to molt (Callahan and Weis 1983). However, tolerance could not be induced by short-term pre-exposure to meHg in either *U. pugnax* or *U. pugilator*. An adaptive mechanism seen in PC fiddler crabs was the ability to move significant amounts of the toxic Hg and Pb from their soft tissues into their exoskeleton shortly before ecdysis, thus reducing their body burden (Bergey and Weis 2007).

Comparing grass shrimp (*P. pugio*) from PC and a reference site, Tuckerton (TK) N.J., U.S.A. Kraus et al. (1988) found no significant difference in larval tolerance to

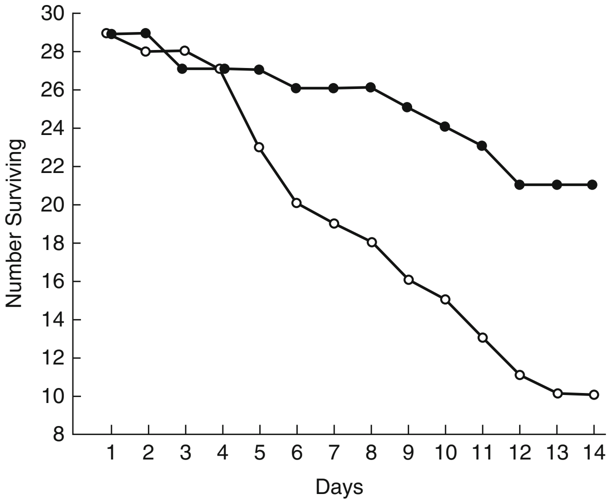


Fig. 11.2 Number adult shrimp from Piles Creek (PC) (black circles) and Big Sheepshead Creek (BSC, reference population) surviving in 0.025 mg l^{-1} meHg over 14 days (Reprinted from Kraus et al. 1988: 358, courtesy Springer Publishing Co)

$HgCl_2$. However, PC larvae were more tolerant to meHg than TK larvae. In contrast, PC adults were more tolerant than TK shrimp to both forms of Hg, suggesting that tolerance to meHg may be inherited, while tolerance to inorganic Hg had to be first induced by exposure earlier in life (Fig. 11.2).

Similarly, TK adults exposed to meHg or inorganic Hg (at 0.01 mg l^{-1}) had impaired regeneration of the telson, but PC shrimp were unaffected (Kraus and Weis 1988). Kraus and Kraus (1986) investigated effects of inorganic Hg and meHg (0.01 mg l^{-1}) on predator avoidance by PC and TK shrimp and found that while exposed TK shrimp were more vulnerable to predation, PC shrimp were unaffected by $HgCl_2$ and were less affected by meHg than TK shrimp. PC shrimp were also more tolerant to Cd, and pre-treatment of TK shrimp enhanced their tolerance to higher concentrations of Cd, showing acclimation (Khan et al. 1988).

A metal-tolerant population of the copepod *Tisbe holothuridae* collected from a polluted site was maintained for over 40 generations in the laboratory, after which time they remained more tolerant to Co^{2+} and Cr^{6+} than those from a clean area (Miliou et al. 2000). This suggests that under laboratory conditions there was little cost in maintaining tolerance, or that the tolerant phenotype had been genetically fixed.

Mangrove crabs *Ucides cordatus* and *Callinectes danae* were sampled from metal-polluted and clean mangroves in Brazil. Crabs from the polluted site showed greater ability to regulate blood osmotic concentrations at low salinity, but *U. cordatus* had reduced hypo-regulatory ability in seawater (Harris and Santos 2000). Differences in ion regulation were also seen. Differences in osmoregulation were considered possible adaptive changes after long-term exposure to contamination.

11.1.3 Mollusks

Bryan and Gibbs (1983) studied the bivalve, *Scrobicularia plana* from RC (see above), and found the clams to be resistant to Cu. Populations of the gastropod *Cerithidium rupestre* from Hg-polluted sites were much more tolerant to Hg than those from clean sites, presumably due to selection (Baker et al. 1985). Luoma et al. (1983) found tolerance to soluble Cu in the bivalve, *Macoma balthica*, varied substantially among populations within San Francisco Bay (US). Tolerance differed ten-fold or more over relatively small distances, suggesting geographical isolation of populations is not required for the development of differences in tolerance. Among five species of gastropods exposed to Cd in the laboratory, there was an association of allozyme genotypes with resistance; PGI genotypes of the dead animals had a higher proportion of heterozygotes than genotypes of the animals that survived the Cd treatment (Lavie and Nevo 1986).

Since larvae are generally more susceptible to contaminants than adults, it is of interest to see if they exhibit tolerance as well. Larvae were obtained from oysters (*C. gigas*) from a clean area (Arcachon Bay) and a polluted one (Bidassoa estuary) and exposed to Cu in the laboratory; their MT concentration was measured as well as biomarkers of oxidative stress. Biomarker responses and sensitivity to Cu for larvae from Arcachon Bay oysters were higher than for those from Bidassoa (Damiens et al. 2006).

Hoare et al. (1995) investigated effects of Cu on embryo development in different populations of *Mytilus edulis*. Concentrations of Cu that increased the rates of abnormality in populations from an unpolluted site (Menai Straits, Wales, UK) and to a lesser extent in an intermediately polluted site (Oosterschelde, The Netherlands) did not affect development of embryos from a polluted-site (Westerschelde, The Netherlands). Crosses between populations indicated that tolerance was mostly maternally determined, but there was also evidence of some paternal effect. An appreciable quantity of background embryo abnormality appeared to be sperm-mediated.

Metal exposure may induce specific metal-binding ligands. Metallothionein-like proteins appear to play an important role. Unger and Roesijadi (1996) investigated the effect of sublethal Cd on metallothionein (MT) mRNA accumulation in *Crassostrea virginica* preexposed to Cd. Initial treatments of control, 0.0044, 0.044, and 0.44 μM Cd for 21 days were followed by challenge with 0.44 μM Cd. MT mRNA accumulation during preexposure was concentration- and time-dependent. During the challenge, MT mRNA increased in all oysters and the concentration-dependence of MT mRNA in relation to preexposure concentration was maintained. MT mRNA during the challenge was significantly increased in oysters pre-exposed to the two highest Cd concentrations in comparison with the controls. These data demonstrate greater MT synthesis in individuals pre-exposed to low Cd concentrations and implicate new MT synthesis in acquired tolerance to Cd.

11.1.4 Other Taxa

Annelids, which tend to live in (and often eat) sediments, which are sinks for contaminants, can develop resistance. One of the earliest studies of tolerance was that of Bryan and Hummerstone (1971), who found that *Nereis diversicolor* in Restronguet Creek (RC, UK), had enhanced tolerance to Cu. Bryan and Hummerstone (1973) investigated potential mechanisms and found that tolerance to Zn was related to reduced uptake or greater excretion, so RC worms did not accumulate as much in their tissues. Uptake was studied further by Rainbow et al. (2009) who found that RC worms could balance increased uptake with detoxification, and had greater rates of storage detoxification of Zn in the form of crystals in the gut wall. Mouneyrac et al. (2003) tested tolerance of this species from metal-contaminated vs clean areas with a range of metals, and found increased tolerance to Cd, Cu, and Zn but reduced tolerance to Ag. Since tolerance to Cu and Zn in *N. diversicolor* was found to be inherited, it is possible to use the occurrence of tolerant individuals to map the ecological impact of contamination. In RC, the effect of Zn was restricted to an area within 1 km of the head of the estuary, and the impact of Cu was also greatest near the head of the estuary and became negligible by the estuary mouth. Sediment levels of $>$1,000 μg^{-1} Cu and 3,500 μg^{-1} Zn are not toxic to this tolerant population (Grant et al. 1989). To evaluate tolerance, Burlinson and Larwrence (2007) developed a behavioral bioassay for *Hediste (Nereis) diversicolor* subjected to Cu. Under metal stress worms showed attempts at burrowing, eversion of the proboscis and abnormal crawling. The bioassay consisted of exposing worms to increasing concentrations of Cu and recording the concentration at which the stress response was elicited. The behavioral end-points were shown to be a good predictor of time of death of Fal estuary worms under acutely toxic conditions. The bioassay would therefore allow the separation of tolerant phenotypes without mortality to the worm. Worms were not affected by consecutive bioassays and it was proposed that tolerance to more than one metal could be determined for individual worms.

However, some negative results have been seen also (Zhou et al. 2003). *N. diversicolor* from other differentially polluted sites in the UK had no difference in Zn or Cd tolerance. There were slight differences in Cu tolerance, but the most tolerant population was not the one from the most contaminated site.

It is also possible to increase tolerance in the laboratory by pre-exposing individual non-tolerant worms, *Neanthes arenaceodentata*, to Cu. Pre-exposed worms had lower uptake of Cu (Pesch and Hoffman 1982), an example of physiological acclimation. *Neanthes virens* was used to study effects of Ag on ion and water balance. Worms from a clean site showed an increase in K^+ and decrease in Ca^{2+} in coelomic fluid after accumulating 88 mg l^{-1} Ag, but worms from a Ag-contaminated site had effects only on Ca^{2+} (Pereira and Kanungo 1981). Exposed worms showed edema and a curled posture, effects which were more severe in the clean population, indicating that the exposed population had some Ag tolerance.

As tolerance comes with energetic and other costs, one would predict that when the contaminant stress decreases, the degree of tolerance might eventually decrease as well. A population of the oligochaete, *Limnodrilus hoffmeisteri*, in a highly Cd-polluted site in the Hudson River, Foundry Cove, (FC, where there had been a battery factory), was found to be tolerant to Cd. The elevated resistance in FC worms was genetically determined, as it was still present after two generations in clean sediment. Resistance had evolved rapidly (within 30 years). A laboratory selection experiment and estimates of the heritability of this resistance in *L. hoffmeisteri* from the control site, indicated that the resistance could have evolved in 1–4 generations. The laboratory selection resulted in a large increase in resistance after two generations of selection (Klerks and Levinton 1989). The resistant worms had significantly higher levels of a Cd-binding, MT-like protein than control worms. The elevated protein level was shown to be genetically determined and was considered partly responsible for the resistance. In addition, high levels of Cd were found in sulfur-rich granules, possibly in the form of cadmium sulfide (Klerks and Bartholomew 1991). As a result of U.S. Superfund legislation, the site was remediated and most of the Cd was removed, thereby removing the selection pressure for resistance. Following the cleanup, there was a rapid loss of resistance in ~9–18 generations, showing that the resistant worms were at a selective disadvantage relative to non-resistant ones after the pollution was no longer present, possibly reflecting a cost of resistance (Levinton et al. 2003). In contrast, *Nereis diversicolor* from Oued Souss, a highly contaminated site in Morocco, which had acquired tolerance to Cu and Zn due to a long-term exposure, maintained this tolerance after cessation of wastewater discharges in this site (Ait-Alla et al. 2006). This is different from what was observed by Levinton et al., (above). Higher catalase, GSTs and TBARs were observed in worms from Oued Souss than in those from reference sites.

At the community level, nematode communities from RC had increased Cu resistance due to a combination of three factors: (1) evolution of tolerance in some species, (2) an increase in abundance of tolerant species, and (3) the disappearance of more sensitive species. This combination of factors is called Pollution Induced Community Tolerance (PICT) (Millward and Grant 1995).

Non-indigenous species introduced via hull fouling may be able to adapt readily to metal-polluted environments, since they had previously adhered to a metallic boat hull that was probably coated with Cu-based antifouling paints. McKenzie et al. (2011) investigated Cu tolerance in the non-indigenous bryozoan *Watersipora subtorquata* from four populations from sites near Sydney, Australia. Colonies were collected, raised in the laboratory, and their offspring exposed to a range of Cu concentrations. Settlement and metamorphosis were measured, as were larval sizes for each colony. While there was no difference in tolerance among sites, there was a significant genotype × environment interaction, with a large variation in the response of colony offspring within sites. The variation in Cu tolerance suggests that there is potential within populations to adapt to elevated Cu, as tolerance is heritable. Larval size also differed significantly and was positively correlated with tolerance.

11.2 Organics

Tolerance to organic contaminants has also been noted frequently. Possible mechanisms of resistance include reduced uptake, increased sequestration, enhanced metabolism and excretion, or reduced sensitivity at the target sites. Mechanisms of transformation and elimination can be employed. CYPs are ubiquitous proteins that are used in normal metabolic transformations, and can be utilized to detoxify contaminants. Stress proteins are also highly conserved, and can protect organisms against both heat and chemical stressors. In many of the tolerance studies, CYP1A is involved. The mechanism by which TCDD, PCBs, and related chemicals cause induction of these enzymes is through the aryl hydrocarbon receptor (AHR), which is a transcription factor involved in regulation of genes for xenobiotic-metabolizing enzymes, including CYPs. The AHR is a receptor for chlorinated dioxins and other halogenated aromatic hydrocarbons. There is considerable evidence for its role in fishes, and some evidence that it plays a role in invertebrates as well. One might think AHR and CYPs would be enhanced in tolerant populations. However, in many of the populations that exhibit enhanced tolerance, the normal induction of CYP1A in response to exposure does not occur and/or the AHR has greatly reduced sensitivity to binding the chemicals – thus, if the receptors are impaired, the organism doesn't "see" the contaminants and toxic effects are not produced.

11.2.1 Fishes

Many studies have been performed by Nacci and colleagues on the evolution of tolerance in killifish (*F. heteroclitus*) to PCBs in New Bedford Harbor (NB), a highly contaminated site in Massachusetts, U.S. Nacci et al. (1999) compared embryos and larvae from reference sites and NB for sensitivity to dioxin-like compounds (DLCs); fish from NB were much more tolerant (Figs. 11.3 and 11.4).

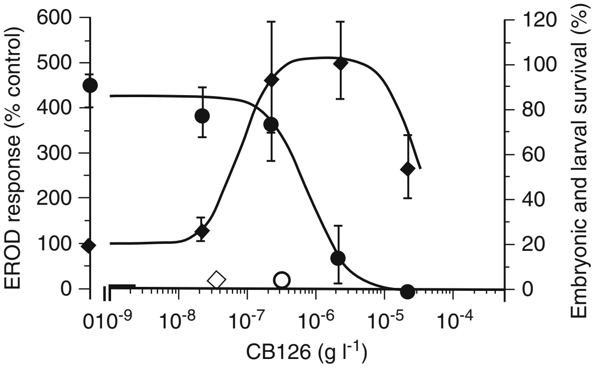
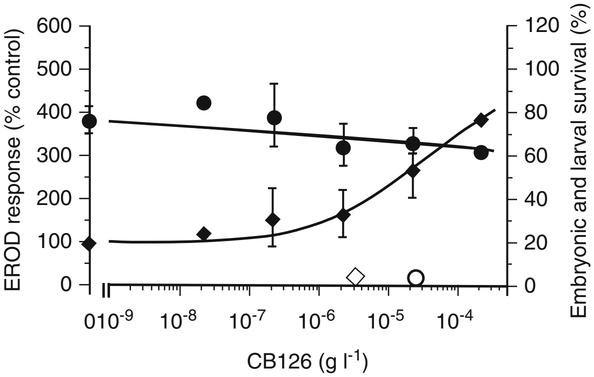


Fig. 11.3 Survival (*circles*), and EROD (*diamonds*) responses (±SD) to PCB 126 of West Island (reference population) killifish embryos (Reprinted from Nacci et al. 1999: 13, courtesy Springer Publishing Co)

Fig. 11.4 Survival (*circles*), and EROD (*diamonds*) responses (±SD) to PCB 126 of New Bedford Harbor killifish embryos (Reprinted from Nacci et al. 1999: 13, courtesy Springer Publishing Co)



Concentrations of DLCs similar to those measured in NB-collected fish eggs were lethal to reference embryos. Responsiveness was inherited and independent of maternal contaminant contributions. CYP1A was found to have very low inducibility in NB fish (Fig. 11.4). Thus, PCB contamination has selected for fish that are resistant. This adaptation may be a critical way that populations persist in this contaminated site. The NB population is also resistant to dioxin, which could not induce normal levels of CYP1A, showing that the Ah receptor signal pathway was altered in these fish (Bello et al. 2001) (Fig. 11.5).

Examining the possibility that the resistant NB population had reduced genetic diversity, McMillan et al. (2006) used AFLP markers, and found that genetic diversity did not differ among populations from contaminated vs reference estuaries. A possible reason that diversity may have been preserved could be because of large effective population sizes or because the mechanisms for adaptation to the contaminants affected only a small number of loci. Examining the issue of trade-offs, Nacci et al. (2009) investigated whether the NB population might be more susceptible to bacterial infection. They performed bacterial challenges of the marine pathogen *Vibrio harveyi* and found comparable survival by NB and reference fish, and improved survival by NB males. These results were inconsistent with hypothesized trade-offs of adaptation, and suggest that evolved tolerance in NB fish may include mechanisms that minimize the immunosuppressive effects of PCBs. Nacci et al. (2010) investigated many mummichog populations and found that they varied over four orders of magnitude in sensitivity to PCB126 and that this variation reflected the degree of contamination at the population's home site and was heritable between the F1 and F2 generations. The four most tolerant populations were from NB, Bridgeport, CT, Newark, NJ, and Norfolk, VA (all US), all highly contaminated sites (Fig. 11.6). The investigators found some similarities among the four highly tolerant populations, but they did not respond identically, and in at least one population, tolerance appeared to decrease in subsequent generations raised in the laboratory.

Roark et al. (2005) studied genetic adaptation in populations of the silversides, *Menidia menidia*, a migratory species residing seasonally in reference sites or

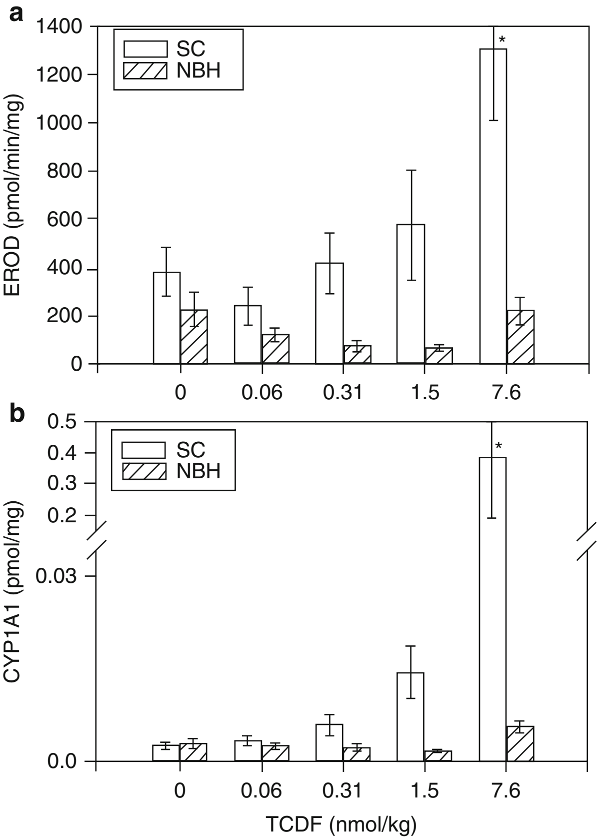


Fig. 11.5 Induction of hepatic CYP1A1 (EROD) activity (**a**) and protein (**b**) in SC (*open bars*, reference population) and NBH (resistant population, *hatched bars*) fish injected with corn oil or doses of TCDF. In (**a**) EROD activities were significantly different between the two populations. * = Significantly different from control from the same site (Reprinted from Bello et al. 2001: 81, courtesy Oxford University Press)

in NB. Offspring of *M. menidia* from NB were significantly less sensitive to embryonic exposure to the dioxin-like PCB 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) than offspring of reference fish. Analysis of ten polymorphic enzymatic loci indicated that juveniles from NB and an adjacent site had significant deviations from Hardy–Weinberg equilibrium at the phosphoglucomutase (*PGM**) locus. Survivors of embryonic laboratory exposure to PCB 126 indicated that *PGM** genotypes were associated with survival. Although a relationship was seen between tolerance and *PGM** genotype, mixing of populations during migration and the absence of multigeneration exposure at contaminated sites may limit adaptation in this species.

Prince and Cooper studied the *F. heteroclitus* population in Newark Bay NJ, highly contaminated with PCBs, metals, and dioxins. CYP1A was not inducible

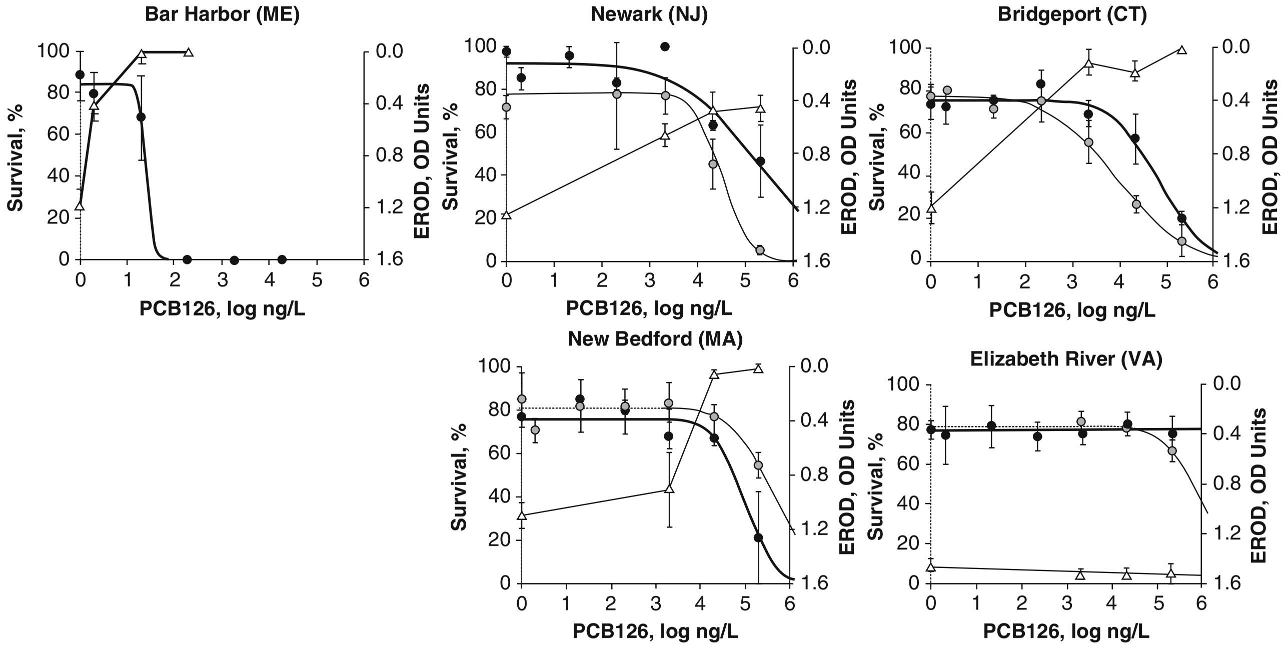


Fig. 11.6 Responses following lab exposure to PCB 126 for *F. heteroclitus* populations (mean + SE). (Lines = model predictions) for early life stage survival of progeny of field-collected parents (*black circles and lines*) or laboratory reared F2 progeny (*gray circles and lines*). Bar Harbor is the reference population, all others are contaminated. EROD activity, measured by optical density, is white triangles (Reprinted from Nacci et al. 2010: 858, courtesy Springer Publishing Co)

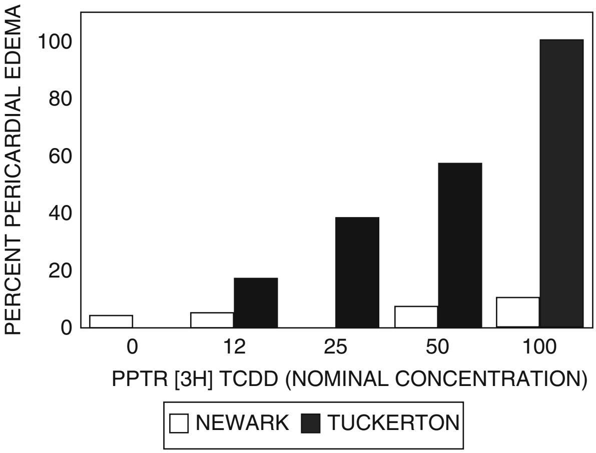


Fig. 11.7 Percentage of pericardial edema in Newark and Tuckerton *F. heteroclitus* embryos exposed to 0.05 % acetone (vehicle), 12, 25, 50, and 100 ppt [3H]TCDD (nominal), from fertilization to d 27 post-fertilization (Reprinted from Prince and Cooper 1995a, courtesy John Wiley & Sons)

in this population, which was highly tolerant to TCDD exposure. The Newark embryos, when exposed to 12–100 parts per trillion (ng l^{-1}) TCDD did not exhibit pericardial edema or death, seen in the reference population (Fig. 11.7). Resistance could not be attributed to reduced absorption of TCDD. Newark adults were also tolerant to TCDD (unlike the meHg tolerance of nearby PC fish discussed earlier, which was seen only in early life stages). When dermally treated, NB adults did not die or exhibit lesions seen in the reference population. Adults had elevated CYP450 activity, but induction of EROD (ethoxyresorufin-O-deethylase, a catalytic measurement of cytochrome P4501A induction) by TCDD exposure was much lower than in the reference population, suggesting an alteration in the AH receptor, similar to the NB fish.

Adults and laboratory-reared offspring of resistant NB fish showed decreased inducibility of CYP1A mRNA, CYP1A protein, and EROD enzyme activity, compared to fish from cleaner sites (Prince and Cooper 1995b; Elskus et al. 1999; Nacci et al. 1999). The laboratory-reared offspring had increased tolerance to PCBs as well as TCDD. Teratological effects of coplanar PCBs and TCDD in sensitive populations included pericardial edema, craniofacial malformations, yolk sac edema, and spine curvature, which probably were a result of altered vascular modeling, decreased blood flow and altered heart size and function (Antkiewicz et al. 2005), which were not seen in the tolerant populations. Blocking the expression of AHR2 protects against the cardiotoxicity of TCDD (Antkiewicz et al. 2006).

F. heteroclitus from a creosote-contaminated site (wood treatment facility) in the Elizabeth River, VA, US (ER) which has extremely high levels of PAHs, have liver neoplasms in moderate to high frequencies (see Chap. 8) (Vogelbein et al.

1990), but are more tolerant to PAHs than reference fish. Similar to the other tolerant populations, CYP1A was not inducible when exposed to PAHs or PCB126 (van Veld and Westbrook 1995; Meyer and DiGiulio 2002; Meyer et al. 2002).

The tolerant ER fish have elevated glutathione S-transferases, enzymes involved in biotransformation of xenobiotics, which could be involved in resistance (Armknecht et al. 1998). However, when reared in clean water or exposed to other contaminants, the F1 from ER had reduced fitness, indicating evolutionary costs of tolerance (Meyer and DiGiulio 2003). However, ER fish were tolerant to chlorpyrifos, permethrin, and carbaryl, suggesting that the adaptive phenotype was multi-faceted and that aspects other than CYP are likely to affect responses to contaminants (Clark and diGiulio 2012).

Wirgin and colleagues have studied Atlantic tomcod (*Microgadus tomcod*) from the Hudson River (HR), which is contaminated with PCBs and dioxins. HR tomcod have liver cancers (see Chap. 8), but have undergone evolutionary changes and become resistant to PCBs. They accumulate high levels of PCBs, PCDDs and PCDFs, but not PAH. The toxicity of these compounds and induction of CYP1A is mediated through the aryl hydrocarbon receptor (AHR), which has two forms, of which AHR2 is more active in fishes. HR fish have higher levels of CYP1A mRNA and bile metabolites of PAH than fish from four cleaner estuaries (Wirgin et al. 1994), but in the laboratory they had very reduced inducibility of CYP1A mRNA after treatment with PCBs and TCDD (Fig. 11.8) (Yuan et al. 2006a, b).

The reduced inducibility of CYP 1A mRNA and increased resistance to PCBs and TCDD in HR tomcod persisted in the F1 and F2 generations reared in the laboratory (Wirgin and Chambers 2006; Wirgin et al. 1992). Wirgin et al. (2011) investigated the mechanistic basis for the resistance and found that HR fish had variants in AHR not found elsewhere – there were deletions in the AHR2 gene. The fish are missing six base pairs of DNA of the AHR2 gene, and the two amino acids each triplet would code for. The mutated gene reduces the binding of molecules like TCDD to the receptor by fivefold, which lessens the toxicity. These changes were considered an evolutionary response to contaminant exposure. Authors felt AHR2 is a sensitive target for selection because of its regulatory role in the metabolism of contaminants. Evolutionary change probably resulted from selective pressure against sensitive phenotypes at susceptible early life stages.

Pollution resistance in populations of North American fishes, focusing on organic contaminants and genetic mechanisms, was reviewed by Wirgin and Waldman (2004).

Peña-Llopis et al. (2001) investigated the genetic basis of herbicide tolerance in eels in a laboratory study. *Anguilla anguilla* were exposed to 41.8 mg l^{-1} of the herbicide molinate in a time to death (TTD) test. Glutathione content (GSx, GSH, GSSG), glutathione reductase (GR) and γ-glutamyl transpeptidase (γ-GT) activities were determined in the liver and muscle of dead and surviving animals and compared with non-exposed eels. TTD was positively correlated to hepatic GSH, GSH:GSSG ratio, hepatic and muscular GR, but negatively correlated to muscular GSH. Eels that were able to induce GR activity, increase GSH and maintain the GSH:GSSG ratio in the liver showed greater survival than those that lost glutathione

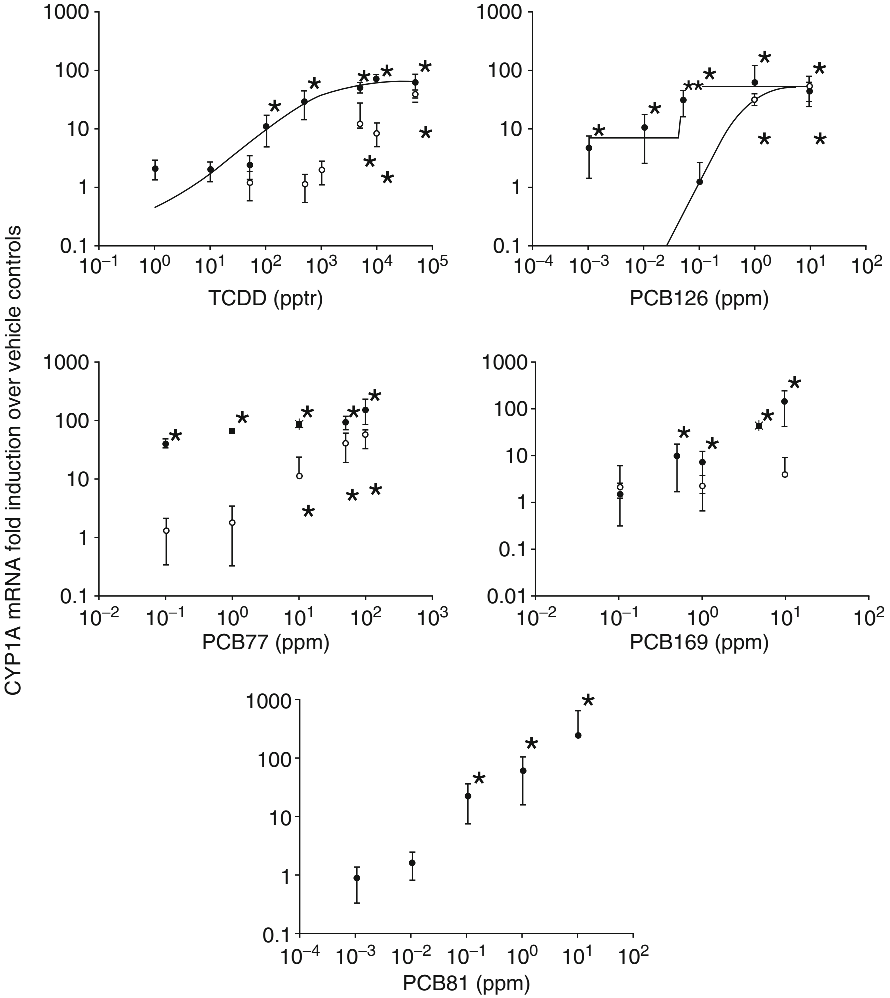


Fig. 11.8 Comparison of hepatic CYP1A m RNA expression in Miramichi River (*filled circles*, reference site) and Hudson River (*open circles*) tomcod injected with graded doses of TCDD or different PCBs.* = Significant difference from vehicle control for that population (Reprinted from Yuan et al. 2006a: 313, courtesy of Elsevier Publishing Co)

homeostasis. It would be interesting to follow up and see if these responses are greater in eels collected from herbicide-contaminated field sites than in those from clean sites.

In contrast to the above studies, resistance was not found in darter gobies (*Gobionellus boleosoma*) from a coastal marsh with a long history of PAH contamination (Klerks et al. 1997). A 2-week pre-exposure at the polluted site resulted in a decreased rather than an increased resistance in a subsequent laboratory exposure to polluted sediment; also fish from the contaminated sites did not show increased resistance to polluted sediment, confirming the lack of acclimation and of adaptation. No differences were detected in frequencies of allozyme genotypes between gobies from polluted vs a control site, and heterozygosity was similar in both populations. Authors suggested four explanations for the lack of resistance: (1) bioavailability of contaminants could have been low, although other evidence contradicts this. (2) The contaminated marsh has many different chemicals and development of resistance is less likely when more contaminants are involved. (This can also be ruled out since PC, NB, NBH, HR and other polluted sites discussed above also have multiple contaminants). (3) The hydrocarbon distribution at the contaminated marsh is very patchy, such that fish may avoid exposure to the highly-contaminated sediment. (4) Gene flow may be sufficiently high in this mobile species to prevent local adaptation. It would seem likely that explanation three or four (or some other) is correct. Another goby species, (*Gillichthys mirabilis*) from three differentially polluted southern California estuaries was studied by Forrester et al. (2003). Fish from each estuary were transplanted to cages in each estuary in reciprocal transplant experiments. The growth rates of caged fish, and the size-distribution of natural populations, showed the same pattern of difference among estuaries. The total pollutant burden at the site was related to the growth of caged fish. Fish in the field caging experiments, and other fish held in the laboratory under constant conditions, showed no difference in growth due to their estuary of origin. These results thus also suggest a lack of genetic adaptation or physiological acclimation. It is interesting that of a limited number of reports of lack of tolerance, gobies seem to be a group that does not exhibit tolerance. It is likely that many other taxa do not develop tolerance but publications reporting such are relatively rare.

11.2.2 Crustaceans

While there is considerable literature on development of tolerance to pesticides by freshwater crustaceans, reports of tolerance in marine species are limited. Grass shrimp (*P. pugio*) in the laboratory could be acclimated to specific metals and PAHs, but not to polluted sediments that contained a variety of contaminants. Similarly, shrimp from Pass Fourchon in Louisiana, a site with elevated PAHs and metals did not exhibit PAH tolerance (Klerks 1999). The explanation offered for the lack of tolerance in the shrimp was that acclimation is less likely when there are numerous contaminants; one contaminant may inhibit the detoxification of others, and energetic requirements of exposure to one contaminant may be offset by decreases in resistance to other types of contaminants. However, sites like PC, NB, HR and NB, discussed above, also have numerous contaminants, yet have tolerant animals (including grass shrimp at PC).

Harper-Arabie et al. (2004) investigated whether the allozymes of the enzymes glucose phosphate isomerase (GPI), phosphoglucomutase (GPM), or mannose phosphate isomerase (MPI) were related to survival of *P. pugio* during acute endosulfan, fluoranthene, and chromium(VI) exposures. *Palaemonetes pugio* were exposed in the laboratory to 6.3 μg l^{-1} endosulfan, 100 mg l^{-1} chromium(VI), or 0.6 mg l^{-1} fluoranthene. Dead shrimp were removed at approximately 15–30 m intervals and the individual's genotypes for the *Gpi*, *Mpi*, and *Pgm* enzymes were determined. Results indicated individuals that were heterozygous for the *Gpi* allozyme survived longer than the homozygous MM genotype when exposed to chromium(VI) or fluoranthene. No allozyme genotypes were related to tolerance to endosulfan. The results support the hypothesis that there is a genetic basis for tolerance in *P. pugio* during acute exposures to chromium(VI) and fluoranthene.

Carman et al. (2000) compared responses of meiofaunal communities from contaminated vs reference sediments to diesel oil. Responses at the two sites were generally similar, but several variables, including abundance of total nauplii, ostracods, and copepods were affected to a greater degree in the reference community. *Pseudostenhelia wellsi* (a benthic copepod) nauplii from the reference site showed greater adverse effects of diesel oil, suggesting increased tolerance of copepods from the contaminated site. However, no differences in tolerance were noted in meiobenthic copepods (*Microarthridion littorale*) populations exposed to a highly contaminated sediment mixture vs unexposed animals (Kovatch et al. 2000), despite the fact that significant genetic differences were found. The absence of enhanced tolerance may be because detoxification mechanisms are adequate to cope with elevated exposures, or that mixing between contaminated and reference populations obscures any observation of tolerance, or that the fitness costs counteract the selective advantage of tolerance.

11.2.3 Mollusks

Reduced uptake appears to be a common method by which mollusks acquire tolerance to organic pollutants. Mussels from the vicinity of the *Prestige* oil spill on the coast of Spain were investigated 4–8 years after the spill. The results showed that PAH pollution was still present, but bioaccumulation in *M. galloprovincialis* was low, compared to reference mussels. This suggests that the mussels in the area of the spill had been able to reduce their uptake of PAHs, as a method of tolerance (Fernández-Tajes et al. 2011). Reduced accumulation of PCBs by mollusks in contaminated sites has also been noted. Accumulation factors (AFs) for PCBs (the lipid normalized PCB concentration in organisms divided by the organic carbon normalized PCB concentration in sediments) were measured for PCBs in infaunal mollusks at field sites with a range of sediment Aroclor (A-1254) and total organic carbon (TOC) concentrations. The average AFs for A-1254 were higher at sites with lower contaminant concentrations (15.0–48.3 ng g^{-1}dry sediment) than at more contaminated sites (328–9,200 ng g^{-1}) (Lake et al. 1990).

Metabolic mechanisms of tolerance are also seen in mollusks. Yawetz et al. (2010) investigated defense mechanisms in mussels, *Donax trunculus,* from differentially polluted sites. Mussels from an oil-polluted site showed increased activity of the system of active transport of organic anions (SATOA) and the multixenobiotic resistance transporter (MXR) in the gills. In contrast, those collected near a PVC factory showed a decrease in SATOA activity and no increase in the activity of MXR in the gills. Those from the reference site demonstrated equilibrium between energy production and utilization, while in *Donax* from both the oil polluted and the PVC-polluted sites, the mitochondrial redox state reflected intensive consumption of energy. No significant changes were found in the activity of reduced glutathione s-transferase (GST) in the cytosolic fraction of the digestive gland of *Donax* from any of the three sites. These data demonstrate an increase in the anti-chemical defense systems and an intensification of energy metabolism in the mussels at polluted sites.

11.2.4 Other Taxa

Polychaete worms (*Neanthes arenaceodentata*) chronically exposed to petroleum hydrocarbons had developed enhanced tolerance (Rossi and Anderson 1978). However, tolerance could not be induced in the laboratory. In successive generations raised in WSF of fuel oil, F3 adults were no more tolerant than F1 animals. It appears that resistance was due to a selection process that selected over many generations for animals with genetic characteristics conferring resistance.

Physiological tolerance can arise from developing a way to metabolize the contaminant and excrete it. The opportunistic polychaete, *Capitella* sp. I accumulated fluoranthene from sediment in a concentration-dependent manner, but body burdens began to decrease after 2 days and were undetectable by 7 days, despite continued exposure to fluoranthene. Worms pre-exposed to PAH-contaminated sediment for 1 week excreted much more ingested fluoranthene than control worms and retained significantly less in their tissues than control worms (Forbes et al. 1996), indicating they had increased their metabolism and excretion of the hydrocarbon.

11.3 Hypoxia

As summarized by Hochachka et al. (1993) organisms use various defense strategies when confronted with low oxygen: (1) switching toward other metabolic pathways including anaerobic metabolism, (2) suppressing energy metabolism in order to balance ATP production and utilization, (3) minimizing the damage caused by physiological changes such as may occur by loss of ion homeostasis, and (4) using metabolic or other protective adaptations that are needed for recovery after hypoxia.

Marine animals initially respond to hypoxia by attempting to maintain oxygen delivery (e.g. increasing respiration rate, number of red blood cells, or oxygen binding capacity of hemoglobin or hemocyanin), then later by conserving energy (metabolic depression, reduced activity, down-regulation of protein synthesis and down-regulation/modification of certain regulatory enzymes). Upon exposure to prolonged hypoxia, animals must eventually resort to anaerobic respiration. Hypoxia reduces growth and feeding, which eventually affects fitness. Many marine organisms can detect and actively avoid hypoxia. Some benthic animals leave their burrows and move up to the sediment surface, making them more vulnerable to predation. Under chronic hypoxic conditions, there is a general tendency for benthic suspension feeders to be replaced by deposit feeders; demersal fish by pelagic fish; and macrobenthos by meiobenthos. Microflagellates and nanoplankton also tend to dominate in the phytoplankton community (Wu 2002).

11.3.1 Fishes

Some fish can engage in aquatic surface respiration (ASR), getting their oxygen from the air. Active swimmers can avoid hypoxic areas, but some are more likely to remain in hypoxic water than others due to differences in physiological tolerance and movement responses. Determining avoidance responses is important for identifying the species most susceptible to impacts of hypoxia. A trawl survey was used to examine avoidance responses of blue crabs (*C. sapidus*) and several fish: pinfish (*Lagodon rhomboides*), spot (*Leiostomus xanthurus*), Atlantic croaker (*Micropogonias undulatus*), bay anchovy (*Anchoa mitchilli*), and flounders (*Paralichthys dentatus* and *P. lethostigma*) to chronic hypoxia and episodic hypoxic upwelling events in the Neuse River Estuary, NC, USA (Bell and Eggleston 2005). Trawl collections quantified changes in distribution and abundance patterns during normoxia, chronic hypoxia, and hypoxic upwelling events. Pinfish, anchovies, blue crabs, and flounder abundance increased with increasing DO. The two taxa most closely associated with the bottom (blue crabs and flounder) showed the strongest avoidance response to hypoxia. All taxa showed a stronger avoidance response to chronic hypoxia than to episodic hypoxic upwelling, which was attributed to reduced ability to avoid the rapid intrusions of hypoxic water during episodic events, or to increased risks of predation in shallow habitats, which may force some individuals back into hypoxic water. Froeschke and Stunz (2012) used mesocosm choice experiments to examine how habitat selection in pinfish (*Lagodon rhomboides*) and Atlantic croaker (*M. undulatus*) was affected by substrate, DO, food availability, and predation risk. Both species strongly avoided low DO, but at moderate levels of DO, substrate and food influenced selection. Both species avoided predators even when the alternative habitat had low DO, suggesting that predation risk is more important than DO for habitat selection. Swimming speed and indicators of stress (blood cortisol and lactate) were measured in Atlantic cod to assess if a shift in physiological homeostasis preceded changes in behavior or vice versa (Herbert and Steffenson 2005). Swimming speed increased when DO was reduced rapidly, an avoidance response. However, swimming speed was reduced at moderate DO and continued to drop under progressively deep hypoxia. Elevations in plasma cortisol and blood lactate indicated physiological stress but only at levels near the critical oxygen tension. Reduced activity is adaptive for survival in low DO. Mandic et al. (2009) noted that sculpins with higher hypoxia tolerance inhabited the O_2 variable intertidal zone, while species with lower hypoxia tolerance inhabited the more stable subtidal zone. Hypoxia tolerance is associated with enhanced O_2 extraction capacity, which has three principal components: routine O_2 consumption rate; mass-specific gill surface area; and hemoglobin O_2-binding affinity. Differences in the concentration of ATP and GTP provide a system with plasticity for survival in a highly O_2 variable environment.

Inheritable tolerance and acclimation to sulfide (related to eutrophication and low DO in salt marshes) was found in California killifish *F. parvipinnis* exposed to dissolved sulfide (Bagarinao and Vetter 1993). The high sulfide tolerance, particularly of concentrations typical of salt marshes, was explained by mitochondrial sulfide oxidation. Sulfide tolerance and mitochondrial sulfide oxidation did not diminish in fish held in the laboratory in sulfide-free water for 1–2 months. Seasonal differences in capacity of gulf killifish, *F. grandis*, to tolerate hypoxia were studied by Love and Rees (2002), who measured the frequency of aquatic surface respiration (ASR) during gradual reduction in DO and survival time during severe hypoxia, and found both to be significantly affected by season. Fish collected in summer did not engage in ASR until the DO concentration dropped below that required to cause ASR during other seasons. Laboratory acclimation to low DO did not change the relationship of ASR and DO, suggesting that the seasonal effect on ASR was not due simply to previous exposure. Fish collected in the summer and winter had longer survival times in severe hypoxia than fish collected in the fall. Seasonal variation in ASR and survival suggest that tolerance may be subject to acclimatization. Increased tolerance in summer could increase survival in low DO, which prevails during the summer. In comparison to other marsh fishes, *F. heteroclitus* is highly tolerant of low oxygen (D'Avanzo and Kremer 1994; Smith and Able 2003), with little or no mortality occurring until DO drops below 1 mg l^{-1}. *F. heteroclitus* does not avoid water of 1 mg l^{-1} in laboratory choice experiments, whereas other marsh fish do (Wannamaker and Rice 2000). During chronic exposure to oxygen tensions near their P_{crit}, the oxygen-binding capacity of blood increases due to increased hematocrit and hemoglobin oxygen affinity (Greaney et al. 1980). The increased hematocrit is likely due to several factors, including splenic contraction and synthesis and possibly maturation of red blood cells stimulated by erythropoietin (Lai et al. 2006). As oxygen drops below P_{crit}, *F. heteroclitus* and *F. grandis* utilize anaerobic metabolism. Increased glycolysis is reflected by the accumulation of lactate in blood and tissues after both acute and chronic hypoxic exposure (Cochran and Burnett 1996).

ASR and avoidance are not viable options for sluggish bottom dwelling fishes such as flatfish. Maxime et al. (2000) examined turbot (*Scophthalmus maximus*) during progressive severe hypoxia followed by recovery. Fish initially increased their ventilation amplitude and frequency, which maintained standard O_2 consumption over a broad range of DO, until a critical level of 30 mmHg. The hyperventilation induced a moderate blood alkalosis, compensated by a lactic acidosis. Blood pH did not decrease below control values, which could be explained by the retention of most of the lactate produced in muscle, and by a high capacity for H^+ excretion. During the recovery period, a marked increase in O_2 uptake (oxygen debt repayment) was related to lactate elimination. When energy contributions of aerobic and anaerobic processes were assessed in terms of ATP, the anaerobic contribution during hypoxia was >20 % of the total energy budget and compensated for the reduced aerobic metabolism. The high value of O_2 tension in arterial blood in normoxia and during recovery from hypoxia showed high diffusing capacity of gills, also contributing to the high tolerance of turbot for low DO.

Nilsson and Östlund-Nilsson (2008) reviewed the literature on the relation of fish size to hypoxia tolerance and concluded that body size *per se* has little impact on the ability to take up oxygen during low DO conditions, primarily because the respiratory surface area matches the metabolic rate over a wide size range. In cases where size-related differences are seen in a species, these were considered likely to reflect adaptation to different life-styles or habitats. However, during severe hypoxia and anoxia where fish must rely on glycolysis for survival, large individuals have a clear advantage because small ones will run out of glycogen or reach lethal levels of anaerobic end-products (lactate and H^+) sooner, due to their higher mass-specific metabolic rate. Those species that have evolved extreme adaptations to hypoxia, such as hemoglobin with very high oxygen affinity or alternative anaerobic end-products, reveal that evolutionary adaptation is more important in developing hypoxia tolerance than physiological acclimation.

11.3.2 Crustaceans

Active swimmers like shrimp can actively avoid hypoxic areas. When confronted with a gradient of dissolved oxygen, *Metapenaeus ensis* were able to avoid hypoxic areas and move to oxygenated water. Their ability to detect and avoid hypoxia may enhance their survival (Wu et al. 2002). Behavior and mortality of juvenile Norway lobster (*Nephrops norvegicus*) changed with lowered DO; energetically costly activities were reduced, and general activity declined (Eriksson and Baden 1997). In normoxia, juveniles walked and burrowed, but when exposed to hypoxia they became inactive with occasional outbursts of escape swimming. To increase oxygen availability they raised their bodies. However, oxygen saturations of 25 % were lethal within 24 h. Juveniles were more sensitive than adults. Copepods, *Acartia clausi* from the polluted Elefsis Bay (Greece) were much more tolerant of low DO conditions than ones from reference areas (Kerambrun et al. 1993). A common way of increasing tolerance is to synthesize more hemocyanin (Hc) or increase its oxygen affinity. Blue crabs (*Callinectes sapidus*) were held in hypoxic (50–55 mmHg) water for 7–25 days. Lactate, urate, and Ca^{+2} all raised the O_2 affinity of blue crab hemocyanin (Hc); by 25 days, blood lactate and urate had risen slightly, and Ca^{+2} had increased dramatically. Hc concentration had also increased by 25 days. At both 7–25 days there was an increase in hemocyanin-O_2 affinity and a change in subunit composition (DeFur et al. 1990).

The extent to which exposure to hypoxia (40, 30, and 20 % sat) produce an increase in the concentration of Hc in Norway lobster *Nephrops norvegicus,* depends on the initial Hc concentration and the intensity of the hypoxia. While Hc did increase with decreasing pO_2, individuals with relatively high initial Hc levels did not increase Hc further. The greatest hypoxia-related increase in Hc was in individuals with the lowest initial Hc concentrations. The changes in Hc concentration took place over a short time scale (hours rather than days) (Spicer and Baden 2001).

11.3.3 Mollusks

Metabolic depression and arrest are common responses among mollusks to low DO. Starved *Crassostrea virginica* and *Thais haemastoma* were anoxia tolerant; their metabolic rates were depressed under anoxia to 75 and 9 % of the normoxic rate (Stickle et al. 1989). Ueda et al. (2009) investigated whether tolerance to low oxygen could be selected for in oysters and examined the expression of heat shock protein 70 (HSP 70) in control and anoxia-challenged juvenile oysters. *C. virginica* were collected from an area (CP) considered to have normoxic conditions and an area (WH) with periodic anoxia. F1 oysters were produced from CP and WH parents that survived anoxia for 96 h and from both parental stocks not exposed to anoxia. The F1 oysters were subsequently exposed to anoxia or normoxia, and expression of HSP 70 was examined. Three HSP 70 isoforms were expressed in both anoxia- and normoxia-exposed oysters from all groups. In general, there were not significant differences in the expression of these proteins between the control and anoxia-treated oysters, suggesting no selection for tolerance. They did not report on the actual anoxia tolerance of the F1 oysters, however.

Two South African mussels show marked differences in their tolerance to low DO. *Choromytilus meridionalis* occurs low on the shore and on rocks associated with sand, while *Perna perna* occurs higher on the shore on rocks that are not usually influenced by sand. *C. meridionaus* withstands prolonged exposure to hypoxia (<1.00 ppm O_2: $LT_{50} > 30$ days) longer than *P. perna* with LT_{50} value of ≈ 6.5 days for hypoxia. *C. meridionalis* activates anaerobic pathways more readily than *P. perna* when exposed to hypoxia, and shows a much stronger tendency to close its valves and to reduce its heart rate. With declining oxygen tension it regulates oxygen uptake down to a lower level (2 ppm O_2) than *P. perna* (3 ppm O_2) (Marshall and McQuaid 1993).

Kurochkin et al. (2009) studied metabolic responses to prolonged anoxia and subsequent recovery in anoxia-tolerant *C. virginica* and effects of Cd on these processes. Anoxia led to anaerobic respiration, as indicated by accumulation of L-alanine, acetate, and succinate. Prolonged anoxia caused a decline in the maximum activity of electron transport chain and oxygen uptake by mitochondria. During reoxygenation, there was a significant overshoot of mitochondrial oxygen uptake (<70 % above normoxic values). Mild mitochondrial uncoupling in anoxic tissues and a subsequent strong stimulation during recovery may help to restore redox status and protect against formation of damaging reactive oxygen species (ROS). Exposure to Cd inhibited anaerobic metabolism, abolished the stimulation of mitochondrial oxygen uptake, and led to oxidative stress and a loss of mitochondrial capacity during the recovery period.

11.3.4 Other Taxa

Many polychaetes including the lugworm, *Arenicola marina,* live in eutrophic benthic habitats subject to frequent hypoxia. They are able to shift gradually from aerobic metabolism to anaerobic metabolism, accompanied by a drop in ATP demand and production. The activity of the enzyme glycogen phosphorylase plays a major role in switching to anaerobic metabolism (Kemp 1993). The intertidal polychaete *Scoloplos armiger* in anaerobic conditions produces energy in a similar manner, but is less tolerant. Energy is produced from phosphagen stores and from the conversion of glycogen to fatty acids, mainly propionate and acetate. *S. armiger* is able to maintain aerobic metabolism down to a Pw_{O2} of ~20 Torr and even at a Pw_{O2} of 10 Torr retained some aerobic metabolism. However, *S. armiger* cannot reduce its energy demands to the same degree as *A. marina* and has a relatively small pool of glycogen, which may account for why it is only moderately resistant. Its recovery from anoxia is slower than in *A. marina.* In the field during low tide *S. armiger* goes up into the oxic layer, where it can maintain aerobic metabolism (Schöttler and Grieshaber 1988).

The number of forms of enzymes involved in glycolysis can affect the degree of hypoxia tolerance of a species. Nine polychaete species (*Paraprionospio pinnata, Nephtys ferruginea, Glycera americana, Haploscoloplos* sp., *Lumbrineris composta, Sigambra bassi*, *Aricidea pigmentata*, *Cossura chilensis*, and *Pectinaria chilensis*) were assayed for lactic dehydrogenase, octopine dehydrogenase, strombine dehydrogenase and alanopine dehydrogenase. Each species had a characteristic number of the pyruvate oxidoreductases, ranging from 4 in *Paraprionospio pinnata* to 1 in *Pectinaria chilensis*. The pyruvate saturation curves suggest that NADH is oxidized at different rates depending on the amino acid used in the reaction with pyruvate. The data indicate that organisms with more than one pyruvate oxidoreductase have greater metabolic capacity to cope with low DO because these enzymes would better regulate the pyruvate consumption rate during the transition period. Thus, the dominance of *Paraprionospio pinnata* in hypoxic areas, and its worldwide distribution is consistent with its high number of pyruvate oxidoreductases with different pyruvate consumption rates (González and Quiñones 2000).

Low oxygen is also found in deep sea hydrothermal vent habitats (though not due to pollution). The polychaete *Methanoaricia dendrobranchiata* lives at hydrocarbon seeps in a microhabitat that is always very hypoxic and sulfidic. A suite of adaptations to low DO were described by Hourdez et al. (2002). The worms can regulate their oxygen consumption down to about 870 Pa oxygen, have a large gill surface area, a small diffusion distance from sea water to blood, a very high hemoglobin oxygen affinity and a Bohr effect that is pronounced at high oxygen saturations. They can withstand extended periods of anoxia even though when fully saturated, the hemoglobin binds sufficient oxygen for only 31 min of aerobic metabolism.

11.4 Climate Change/Ocean Acidification

A special issue of Proceedings of the Royal Society has been devoted to the idea of "evolutionary rescue" in changing environments (ER) as a way to maintain biodiversity in the face of climate change. In the introductory article, Gonzalez et al. (2013) explain that ER is the idea that evolution might occur sufficiently fast to arrest population decline and allow recovery before extinction results. ER provides a perspective on evolutionary dynamics that focuses on short time-scales, genetic variants of large effects, and absolute rather than relative fitness. Contributions in the issue cover conceptual developments and modeling, as well as theoretical and experimental results. Populations under severe stress may be rescued by natural selection, but its operation has ecological and genetic constraints. Whether evolution will be rapid enough to rescue declining populations will depend upon the population size, the degree of genetic variation, the degree of maladaptation and the evolutionary history. A factor that may limit their ability to adapt is the rate at which beneficial mutations can become established. Population persistence can also be influenced by phenotypic plasticity (acclimation), and by the evolution of plasticity itself. These articles are general and none focus on the oceans or pH.

Kelly and Hofmann (2012) reviewed current literature on the potential for adaptation to elevated $p\mathrm{CO}_2$ in marine organisms. Although the number of papers is currently quite small, they argued that data on physiological effects, natural variation in pH and lessons learned from previous work on adaptation can all inform predictions and priorities for future research. They argued that selection is one of the most important forces maintaining intraspecific genetic variation. Unlike temperature, pH lacks a strong global gradient, and so selection may maintain less adaptive variation for pH than for temperature. However, long-term data sets for natural pH variation are scarce, so pH gradients may be more common than previously observed. Two important effects of elevated $p\mathrm{CO}_2$ are reduced calcification and changes in metabolism. Detailed understanding of physiological mechanisms underlying these effects is important for predicting the ability to acclimatize or adapt. They recommended that future research should assess adaptation to local pH conditions and measure the capacity for adaptation to acidified conditions in natural populations.

11.4.1 Fishes

Juvenile anemonefish, *Amphiprion melanopus*, which show an increase in metabolic rate and decreases in length, weight, condition and survival in response to acidification, can adjust to the pH expected by 2100 (1,000 μatm CO_2 and a temperature rise of 1.5–3.0 °C) if their parents were also raised in more acidic water (Miller et al. 2012). Under those circumstances, juveniles were able to compensate for the change, although it is not known if the tolerance persists throughout their lives. How parent fish pass on the ability to deal with acidity to their offspring is not known. The time interval is too short for it to be genetic adaptation in the normal sense. Thus some fish species have greater capacity to cope than was previously thought. Anemonefish are particularly hardy, however, and may not be representative of all marine fish.

Munday et al. (2012) used a field-based experiment to test for differential survival associated with variation in CO_2 tolerance in a wild population of coral-reef fishes. Juvenile damselfish, *Pomacentrus wardi* exhibited variation in their response to elevated (700 μatm) pCO_2 when tested in the laboratory and this influenced their behavior in the field. Individuals that were sensitive to elevated pCO_2 (changing normal response to predator odor) were more active and moved farther from shelter in natural coral reef habitat and consequently had higher mortality from predation than fish that were more tolerant to elevated pCO_2. Authors concluded that if individual variation in CO_2 tolerance is heritable, this selection of phenotypes tolerant to elevated pCO_2 could potentially help mitigate the effects of ocean acidification.

Adaptation to climate change also involves temperature tolerance. Klerks and Blaha (2009) compared heat tolerance of fish collected from pairs of sites with different thermal regimes, using least killifish (*Heterandria formosa*) and eastern mosquitofish (*Gambusia holbrooki*). They measured heat tolerance as temperature-at-death and time-to-death when field-collected fish were exposed in the laboratory to increasing water temperatures. For the four pairs of populations that were compared, two differed in heat tolerance. Fish from one site with above-normal temperatures had reduced heat tolerance indicative of stress, rather than tolerance. Fish from another site with above-normal temperatures had elevated heat tolerance, which appeared to be due to acclimation rather than adaptation, since it was not maintained in their offspring.

For coastal wetland species, sea level rise results in salt water intrusion, which can be another stress. Populations of the eastern mosquitofish, *Gambusia affinis,* with exposure to saline environments develop adaptations for increased survival in high salinity (Purcell et al. 2008). Fish from brackish and intermediate marshes had increased salinity tolerance compared to fish from freshwater marshes. They tested the descendents of fish from the fresh and brackish marshes that were reared for two generations in fresh water, and found that descendents of fish from brackish marshes had higher survival in saline water than descendents of fish from freshwater, implying genetic adaptation. Purcell et al. (2012) evaluated the genetic structure of *G. affinis* populations previously shown to have adaptations for increased salinity tolerance. They found that gene flow was higher between populations experiencing different salinity regimes within an estuary than between similar marsh types in different estuaries, suggesting the development of saline-tolerant phenotypes is due to local adaptation.

11.4.2 Crustaceans

Crustaceans appear to be very variable in vulnerability to ocean acidification due to differences in lifestyle and in the ability to compensate for environmental change. It is predicted that strong iono- and osmo-regulating species are likely to be the most tolerant to acidification, because they have the compensatory mechanisms to respond to acid–base disruptions. These species tend to inhabit shallow coastal environments with freshwater inputs, where they experience natural variations in seawater pCO2, pO2, salinity and temperature. The ability to compensate for the effects of ocean acidification can also vary with lifestyle. Decapods with high rates of activity, for example, have a greater capacity for passive compensation of hemolymph acid–base disturbances (i.e. buffering by non-bicarbonate buffers) than slow-moving, relatively inactive species due to differences in respiratory variables (Whitely 2011).

11.4.3 Mollusks

Amerala et al. (2011) sampled oyster, gastropod and crab populations at sites close to and far from drains discharging acid sulfate soil (ASS) runoff, hypothesizing that reduced populations of smaller-sized individuals would be found at the more acidified sites. Oysters *Saccostrea glomerata* and gastropods (*Bembicium auratum*) were less abundant at ASS-affected than reference sites in New South Wales, Australia, but impacts were smaller than predicted. Although gastropod populations were dominated by smaller individuals, oyster populations were skewed towards larger individuals at affected sites, and abundances of both species were comparable to reference estuaries. Authors felt that behavior, physiological acclimation and/or genetic selection may be responsible, or that populations may recover between the rain events that bring in acidic runoff. Thus, at the population level, these calcifying organisms showed some resistance to acidic runoff.

Parker et al. (2012) exposed adult Sydney rock oysters, *Saccostrea glomerata* to ambient and elevated $p\mathrm{CO}_2$ during reproductive conditioning and measured development, growth and survival of their larvae. Elevated $p\mathrm{CO}_2$ caused a reduction in growth, development rate and survival. However, exposing adults to elevated $p\mathrm{CO}_2$ during reproductive conditioning had positive effects on larvae. Larvae spawned from adults exposed to elevated $p\mathrm{CO}_2$ were larger, developed faster and had similar survival as larvae spawned from adults exposed to ambient $p\mathrm{CO}_2$. This suggests that these oysters have the capacity to acclimate or adapt to elevated $p\mathrm{CO}_2$.

11.4.4 Other Taxa

Corals

Some corals can adapt to high temperatures and/or low pH. Populations that were able to survive a massive bleaching event in 2010 in Southeast Asia were ones that had previously experienced severe bleaching in 1998. In Indonesia, corals responded to higher temperatures in a typical way, with branching species such as staghorn corals suffering severe die-offs. But at sites in Singapore and Malaysia, normally susceptible *Acropora* corals appeared healthy and unbleached. The locations that had less severe bleaching in 2010 had bleached in 1998. In contrast, the site that had severe bleaching in 2010 had not bleached in 1998. It appears that the corals that had bleached in 1998 had subsequently adapted and/or acclimatized to thermal stress (Guest et al. 2012). Genetic changes in the corals may play a role in increasing tolerance to temperature. Some of the *Acropora hyacinthus* corals in the back reef of Ofu Island, American Samoa, thrive in pools with daily heat fluctuations of up to 6 °C. To find the molecular basis of this resilience, Barshis et al. (2013) compared gene activity in heat-resistant and heat-sensitive corals by measuring their ‘transcriptome’ – the RNA molecules transcribed from the genes – under different temperatures. They found a suite of genes that are present in both types of corals but are more highly expressed in the heat-resistant corals, which had 60 stress-related genes turned on even before the experiment began. These genes, which code for a number of antioxidants and heat shock proteins, are “frontloaded” in the resistant corals – already turned on and ready to work even before the stress began.

Corals may also adapt by hosting varieties of algal symbionts that are more temperature tolerant. Increasing numbers of coral species have been found to be able to host multiple algal symbionts. Being capable of hosting symbionts that survive in warmer temperatures suggests they have potential to adapt to warmer temperatures (Silverstein et al. 2012). However, some confounding evidence was found by Putnam et al. (2012) who collected 132 samples of coral from reefs around Moorea, analyzed DNA to determine the types of dinoflagellates present, and reviewed the scientific literature for information on how well each type of coral dealt with environmental changes. Surprisingly, the corals that hosted many diverse dinoflagellates were less resilient. For example, flexible corals such as *Acropora* and *Pocillopora* that hosted a variety of symbionts fared the worst after a mass bleaching in 1994. Corals that were more selective about their symbionts coped more successfully. It would appear that the symbionts, though less diverse, were more temperature tolerant. Complicating the picture further, Hume et al. (2013) studied heat-tolerant corals in the Persian Gulf, and found that although both the coral host and the associated algae need to withstand the high temperatures, the algae belong to a group not known for its thermal tolerance. The algae found in most of the corals in Abu Dhabi reefs were previously described as a 'generalist strain' that is usually not found in corals exposed to high levels of heat stress.

Bleaching was studied in two species of octocorals, *Phenganax parrini* and *Sarcothelia* sp. both of which had similar responses (Parrin et al. 2012). While symbionts detach or die leaving the polyps bleached, large numbers of the symbionts accumulate in the stolons. The presence of symbionts in the stolons may be a mechanism to help the colonies recover from bleaching if the symbionts subsequently migrate back into the polyps.

Climate change includes both ocean acidification and temperature increase. Edmunds (2011) exposed *Porites* corals to different temperatures and pH for 1 month in the laboratory, and provided them with brine shrimp as food. Increasing the amount of food reduced the effects of acidification. Both calcification and biomass were sustained at high $p\mathrm{CO}_2$ by increased food, showing that this species can resist effects of 1 month in high $p\mathrm{CO}_2$ through heterotrophy. This mechanism may play a role in determining the extent to which corals can resist the long-term effects, and it depends on adequate amounts of suitable plankton food being available, which is also necessary to survive bleaching.

Organisms that form calcium carbonate skeletons produce it in one of two forms, aragonite and calcite. McCulloch et al. (2012) suggest that those with aragonite skeletons, such as *Porites* and *Acropora*, have molecular pumps that enable them to regulate their internal acid balance, which buffers them from the changes in seawater pH. This up-regulation of pH at the site of calcification provides them with enhanced resilience to the effects of acidification, while those that use the calcite pathway lack this resilience. The cold-water coral *Lophelia pertusa* can keep growing even in low pH if given time to adjust to the new conditions (Form and Riebesell 2012). Coral branches were kept at a range of $p\mathrm{CO}_2$ levels while temperature was constant and corals were fed well. In the short term, a pH decrease of only 0.1 unit caused a decline of growth by about one third, but after 6 months the corals seemed to adapt; those in high $p\mathrm{CO}_2$ grew even faster than controls. However, since acidification is only one aspect of climate change, more studies are needed to investigate interactions of carbon dioxide, temperature, and food availability. Further evidence for adaptation to long-term exposure to very low carbonate concentrations was seen by Thresher et al. (2011) who examined the distribution and skeletal characteristics of corals along a natural deep-sea concentration gradient on seamounts. Carbonate under-saturation had little effect on depth distribution, growth, or skeletal composition of live scleractinians or gorgonians, with corals growing, often abundantly, in waters as much as 20–30 % under-saturated.

Coccolithophores/Algae

Coccolithophores, phytoplankton with calcite shells, have been shown to be vulnerable to acidification. However, when *Emeliana huxleyi* were cultured under increased CO_2 conditions, after 1 year (500 generations) they grew and calcified better than non- adapted control populations when tested under acidified conditions (Lohbeck et al. 2012).

Collins (2011) used an experimental evolution approach to investigate adaptation in changing environments, using fitness and the total number of reproductive events to quantify adaptation of phytoplankton communities to global change, where environmental variables change continuously. She examined the possible scenario that climate change will not acidify the ocean because microbes will absorb the extra carbon in photosynthesis, store it and release oxygen, a logical scenario because many microorganisms are photosynthesizers. To test this idea, algae were grown in high CO_2, and 1,000 generations later some surprising responses were seen. Some of the algae in high CO_2 did not store it any more. Others photosynthesized much faster, but were no longer able to use the extra carbon for growth. Thus, the notion that phytoplankton will efficiently absorb all the extra CO_2 would appear to be unlikely.

11.5 Costs of Tolerance

11.5.1 Energy Costs

Processes involved in developing tolerance are energetically expensive and may have deleterious effects on energy allocation. To adjust to the metabolic costs of a chemical stressor, a shift in allocation of energy occurs at the expense of other energy-demanding processes such as growth. For example, mucus secretion in the gastropod *Patella vulgata* uses almost 25 % of the animal's energy (Davies et al. 1990). In dogwhelks, *Nucella lapillus,* exposed to Cd, reduced oxygen consumption and glycogen level could be linked to the production of mucus and metallothioneins (Leung et al. 2000). The production of enzymes to detoxify organic contaminants also has energy costs (Calow 1991). Many of the studies described in Chap. 3 report decreased metabolic rate, which reflects energy costs. Thus contaminant exposure over extended periods may reduce fitness, which increases selection pressure for more resistant individuals in populations that exhibit variation and heritability for their response. Populations subject to strong selection pressure for a particular stress may show a reduction in genetic variation, since the more susceptible genotypes would have disappeared and only the resistant remain. This may in turn increase their vulnerability to other types of stresses, which is a cost of tolerance. For example, in Cd-polluted environments, the upregulation of heat shock proteins is partially suppressed in the oyster *Crassostrea virginica*, resulting in reduced thermotolerance (Ivanova et al. 2009). Thus, populations with tolerance to some pollutants may have lower tolerance to others and lower survival ability in the longer term. Grant et al. (1989) demonstrated that the genetically based tolerance of *Nereis* worms in Restronguet Creek to Cu was a disadvantage at sites with low levels of metals. The metabolic costs of the tolerance produced a much lower scope for growth than seen in non-resistant populations. The loss of Cd-tolerance in the Foundry Cove (Hudson River) *Limnodrilus* worms after clean-up of the Superfund site (Levinton et al. 2003) demonstrates that the tolerance had a cost and when the selection pressure for Cd-tolerance was removed, non-tolerant worms were at an advantage.

Meyer and DiGiulio (2003) reported that F1 and F2 offspring of PAH-resistant killifish from the creosote-contaminated Elizabeth River were less tolerant of low DO than the F1 and F2 from fish from reference sites. They also found that the F1 but not F2 offspring from ER had reduced growth and survival in clean conditions. The F1 offspring were more susceptible to phototoxicity and oxidative stress. Frederick et al. (2007) similarly found that adult ER fish had lower antibody responses and increased susceptibility to bacterial pathogens. However, since these tests were on fish taken from ER, rather than their offspring raised in the laboratory, it cannot be established if this is a “cost” of tolerance or a direct effect of PAHs and other contaminants on the immune system.

It is often difficult to separate out costs of tolerance from direct or indirect sublethal effects of the contaminants. For example, metal-tolerant *Nereis diversicolor* from the Seine River exhibited lower physiological and population status than those from a reference site (Mouneyrac et al. 2010). Reduced feeding (as described in many studies) is a common response to contaminants, and will also have negative effects on the energy budget. This could be responsible for the observations of Mouneyrac et al. (2010). Also, killifish from Piles Creek that are resistant to meHg in early life stages show reduced feeding, predator avoidance, growth, and longevity as adults (Weis and Weis 1989). These may be costs of tolerance, but are more likely to be direct effects of the contaminants. It is necessary to study laboratory-raised F2 and F3 generations to distinguish true costs of tolerance from direct effects of the contaminated environment.

11.5.2 Food web Costs

Metals

Mechanisms that allow organisms to tolerate contaminants could be a risk to consumers if tolerant prey accumulate high concentrations of contaminants and transfer them to less well-adapted predators. In addition, for organisms that produce mucus in response to contaminants (e.g. mussels), the secreted mucus that has high contaminant levels (Sze and Lee 1995) may then be consumed by other species. Trophic transfer can have major, even lethal impacts. For example, shrimp that

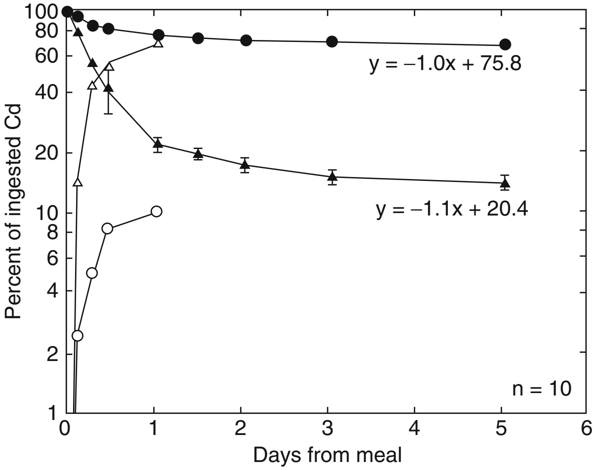


Fig. 11.9 Percentage retention (*filled symbols*, mean + SE) and egestion (*open symbols*) of ^{109}Cd by grass shrimp fed ^{109}Cd-labelled South Cove (reference site, *circles*) or Foundry Cove (*triangles*) *Limnodrilus hoffmeisteri* (Reprinted from Wallace et al. 1998: 232, courtesy Inter-Research)

consumed metal-rich *N. diversicolor* from Restronguet Creek had elevated mortality (Rainbow et al. 2006), suggesting that the shrimp were not as tolerant as their polychaete prey. In many invertebrates, metals complexed with MT in the cytosol are transferred to predators, while metals stored in metal-rich granules (MRG) tend to be unavailable to predators (Fig. 11.9) (Wallace et al. 1998). However, some predators may be able to acquire metals from MRG in their food (Rainbow et al. 2007) (Fig. 11.10) so differences in trophic transfer of metals are also affected by the digestive processes of the predator, which will affect the predator's body burden.

Organics

Despite the ability of many organisms to degrade organic compounds, many xenobiotics accumulate in biota to varying degrees, especially persistent organic pollutants (POPs), such as DDT, PCBs, PBDEs, and dioxins. These are metabolized poorly, and therefore are readily passed up food chains, biomagnifying in the process. Therefore predators (including humans) acquire higher concentrations than their prey. Fish consumption advisories remain in many places, due to unacceptably high levels of chemicals such as PCBs in fish or crab muscle tissue. Tolerant prey can accumulate greater concentrations to pass onto their predators. For example, crustaceans fed PCB-tolerant phytoplankton *Nannochloris oculata* accumulated more PCBs than those fed sensitive *Isochrysis galbana* (Wang et al. 1998).

In contrast, PAHs tend to be better metabolized and do not biomagnify because they are rapidly transformed at higher trophic levels (especially vertebrates), so

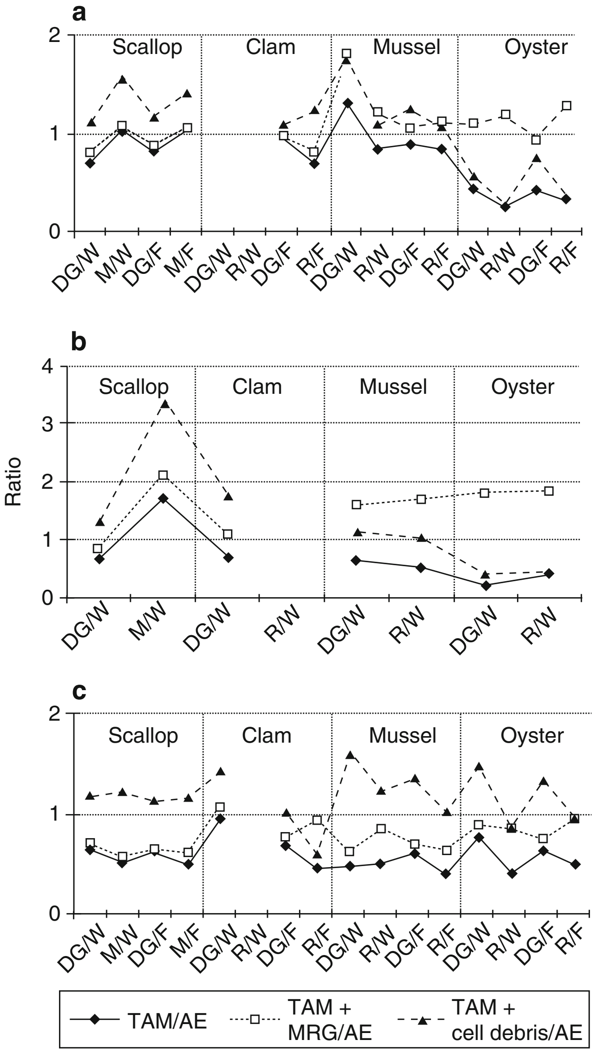


Fig. 11.10 *Nassarius festivus*. Ratio of percentage distributions of accumulated radiolabelled (**a**) Cd (**b**) Ag, and (**c**) Zn in the combined subcellular fractions of trophically available metal (TAM), TAM + metal-rich granules (MRG), and TAM + cell debris in tissues (*DG* digestive gland, *M* adductor muscle, *R* remaining soft tissues) of four bivalves, after uptake from water (W) or food (F), to percentage assimilation efficiency (AE) (Reprinted from Rainbow et al. 2007: 133, courtesy Inter-Research)

their levels may be "trophically diluted" along the food web. But this is not always the case. The oligochaete worm *Monophlephorus rubroniveus* has a high tolerance of and a low ability to metabolize and eliminate PAHs. It bioaccumulates high concentrations of fluoranthene from sediments, which it passes on to consumers such as grass shrimp (Filipowicz et al. 2007). Prey that do transform PAHs may produce carcinogenic metabolites, which are then passed on to consumers. English sole fed B[a]P-contaminated polychaetes showed slower growth, increased CYP1A, and hepatic DNA adducts (Rice et al. 2000). Bottom-dwelling fish in areas contaminated with PAHs have higher incidence of tumors (see Chap. 8), probably partly due to PAHs acquired from their prey.

11.6 Discussion and Conclusions

It is clear that pollutants can cause physiological changes that reduce their toxicity and exert selection pressure on populations in contaminated environments. Reduced accumulation and increased depuration are effective ways to become more resistant to a chemical. Other mechanisms include synthesis of MTs and other metabolic detoxification methods. In addition to physiological acclimation, many species are able to evolve to become more tolerant to the contaminants they are exposed to. Life evolved in the presence of metals, and MTs and other protective mechanisms are found in a diversity of species including microorganisms, invertebrates, plants, and vertebrates, enabling them to resist toxic effects. The most common defense against toxic organic chemicals are the cytochrome P450s (CYPs), highly diverse enzyme systems that may have been used initially to defend animals against natural plant toxins, and subsequently became utilized to defend against the growing numbers of toxic organic chemicals produced by industry.

However, organisms do not have the luxury of coping with one chemical at a time, but must deal with numerous chemicals in some coordinated fashion.

Tolerance is an issue relevant to toxicity testing for choosing organisms for “standard toxicity tests,” since the origin of the animals used in bioassays will affect their responses to chemical stress. It is also relevant to the field of biomarkers. Biochemical biomarkers in organisms from polluted field sites will be different (reduced) if the organisms have developed tolerance.

The acquisition of tolerance to pollutants in the environment allows populations of organisms to sustain themselves in highly contaminated sites, even though they may show negative effects such as reduced abundance, reduced genetic diversity, altered behavior, liver tumors, etc. Thus, while some organisms can develop tolerance to pollutants in their environment, this comes at a cost, often energetic. Also, in cases in which high concentrations of contaminants are accumulated, this can pose risks to non-tolerant consumers, including humans who eat seafood containing elevated levels of methylmercury, PCBs, or other contaminants that biomagnify through food webs. As Klerks et al. (2011) noted in a review, approaches used over the past few decades have limitations, including difficulty in separating the various genetic and environmental variance components, since responses depend on specific populations and test conditions. To improve insights into the long-term consequences of adaptation, it is important to look not only at resistance itself, but also at the fitness consequences and responses in other characteristics.

Developments in molecular genetics have yielded additional insights. Quantitative genetics is benefiting from the use of molecular tools and is becoming an important field for studying evolutionary toxicology.

Other reviews on the overall topic of tolerance have been published recently including Amiard-Triquet (2011, 2013), Johnson (2011), Berthet et al. (2011), Rainbow and Luoma (2011), Romeo and Wirgin (2011) and others in the same volume edited by Amiard-Triquet, Rainbow and Romeo.

As contaminant levels are reduced as a result of environmental clean-ups and reduction of inputs, negative effects on marine species are expected to decline, along with tolerance and bioaccumulation, resulting in a healthier environment for both marine species and humans. Such "good news" has been seen in many areas and is expected to continue for conventional pollutants like metals, oil, and persistent organic compounds. However, with respect to CECs, warming temperatures, and ocean acidification, the future health of the marine environment is likely to worsen, since there has been no progress in curbing these inputs. Whether or not organisms will be able to evolve tolerance to the inevitable increased levels of stressors associated with climate change remains in question. Research to date suggests that coral reefs and some shellfish may face very serious consequences although some species will undoubtedly be able to thrive, while others may disappear. Those who care about the environment can only hope and advocate for international agreements that will result in major reductions in greenhouse gases in the near future.

References

Adeyami JA, Klerks PL (2013) Occurrence of copper acclimation in the least killifish *Heterandria formosa*; and associated biochemical and physiological mechanisms. Aquat Toxicol 130–131: 51–57

Ait-Alla A, Mouneyrac C, Durou C, Moukrim A, Pellerin J (2006) Tolerance and biomarkers as useful tools for assessing environmental quality in the Oued Souss estuary (Bay of Agadir, Morocco). Comp Biochem Physiol 143C:23–29

Amerala V, Cabrala HN, Bishop MJ (2011) Resistance among wild invertebrate populations to recurrent estuarine acidification. Estuar Coast Shelf Sci 93:460–467

Amiard-Triquet C (2011) Pollution tolerance from fundamental biological mechanisms to ecological consequences. In: Amiard-Triquet C, Rainbow PS, Romeo M (eds) Tolerance to environmental contaminants. CRC Press, Boca Raton, pp 1–23

Amiard-Triquet C (2013) Biomarkers of defense, tolerance and ecological consequences. In: Amiard-Triquet C, Amiard J-C, Rainbow PS (eds) Ecological biomarkers: indicators of ecotoxicological effects. CRC Press, Boca Rato, pp 45–74

Annabi A, Messaoudi I, Kerkeni A, Said K (2009) Comparative study of the sensitivity to cadmium of two populations of *Gambusia affinis* from two different sites. Environ Monit Assess 155:459–465

Antkiewicz DS, Burns CG, Carney SA, Peterson RE, Heideman W (2005) Heart malformation is an early response to TCDD in embryonic zebrafish. Toxicol Sci 94:368–377

Antkiewicz DS, Peterson RE, Heideman W (2006) Blocking expression of AHR2 and ARNT1 in zebra larvae protects against cardiac toxicity of 2,3,7,8-tetrochlorodibenzo-p-dioxin. Toxicol Sci 94:175–182

Armknecht SL, Kaatari SL, van Veld PA (1998) An elevated glutathione S-transferase in creosote-resistant mummichog (*Fundulus heteroclitus*). Aquat Toxicol 41:1–16

Bagarinao T, Vetter RD (1993) Sulphide tolerance and adaptation in the California killifish *Fundulus parvipinnis*, a salt marsh resident. J Fish Biol 42:729–748

Baker R, Lavie B, Nevo E (1985) Natural selection for resistance to mercury pollution. Experientia 41:697–699

Barshis D, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR (2013) Genomic basis for coral resilience to climate change. Proc Natl Acad Sci USA. 110:1387–1392

Bell GW, Eggleston DB (2005) Species-specific avoidance responses by blue crabs and fish to chronic and episodic hypoxia. Mar Biol 146:761–770

Bello SM, Franks DG, Stegeman JJ, Hahn ME (2001) Acquired resistance to Ah receptor agonists in a population of Atlantic killifish (*Fundulus heteroclitus*) inhabiting a marine superfund site: in vivo and in vitro studies on the inducibility of xenobiotic metabolizing enzymes. Toxicol Sci 60:77–91

Bergey L, Weis JS (2007) Molting as a mechanism of depuration of metals in the fiddler crab, *Uca pugnax*. Mar Environ Res 64:556–562

Berthet B, Leung KM, Amiart-Triquet C (2011) Inter- and intraspecific variability in tolerance: implications for bioassays and biomonitoring. In: Amiard-Triquet C, Rainbow PS, Romeo M (eds) Tolerance to environmental contaminants. CRC Press, Boca Raton, pp 49–84

Bryan GW, Gibbs PE (1983) Heavy metals in the Fal estuary, Cornwall: a study of long-term contamination by mining waste and its effects on estuarine organisms. Occ Publ Mar Biol Assoc UK 2:1–112

Bryan GW, Hummerstone LM (1971) Adaptation of the polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of heavy metals. I. General observations and adaptation to copper. J Mar Biol Assoc UK 51:845–863

Bryan GW, Hummerstone LM (1973) Adaptation of the polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of zinc and cadmium. J Mar Biol Assoc UK 53:839–857

Burlinson FC, Larwrence AJ (2007) Development and validation of a behavioural assay to measure the tolerance of *Hediste diversicolor* to copper. Environ Pollut 145:274–278

Callahan P, Weis JS (1983) Methylmercury effects on regeneration and ecdysis in fiddler crabs (*Uca pugilator, U. pugnax*) after short-term and chronic pre-exposure. Arch Environ Contam Toxicol 12:707–714

Calow P (1991) Physiological costs of combating chemical toxicants: ecological implications. Comp Biochem Physiol C 100:3–6

Carman KR, Fleeger JW, Pomarico SM (2000) Does historical exposure to hydrocarbon contamination alter the response of benthic communities to diesel contamination? Mar Environ Res 49:255–278

Clark BW, DiGiulio RT (2012) *Fundulus heteroclitus* adapted to PAHs are cross-resistant to multiple insecticides. Ecotoxicology 21:465–474

Cochran RE, Burnett LE (1996) Respiratory responses of the salt marsh animals, *Fundulus heteroclitus*, *Leiostomus xanthurus*, and *Palaemonetes pugio* to environmental hypoxia and hypercapnia and to the organophosphate pesticide, azinphosmethyl. J Exp Mar Biol Ecol 195:125–144

Collins S (2011) Many possible worlds: expanding the ecological scenarios in experimental evolution. Evol Biol 38:3–14

D'Avanzo C, Kremer JN (1994) Diel oxygen dynamics and anoxic events in an eutrophic estuary of Waquoit Bay, Massachusetts. Estuaries 17:31–139

Damiens G, Mouneyrac C, Quiniou F, His E, Gnassia-Barelli M, Roméo M (2006) Metal bioaccumulation and metallothionein concentrations in larvae of *Crassostrea gigas*. Environ Pollut 140:492–499

Davies MS, Hawkins SJ, Jones HD (1990) Mucus production and physiological energetics in *Patella vulgata* L. J Mollus Stud 56:499–503

DeFur P, Mangum C, Reese JE (1990) Respiratory responses of the blue crab *Callinectes sapidus* to long-term hypoxia. Biol Bull 178:46–54

Edmunds P (2011) Zooplanktivory ameliorates the effects of ocean acidification on the reef coral *Porites* spp. Limnol Oceanogr 56:2402–2410

Elskus AA, Monosson E, McElroy AE, Stegeman JJ, Woltering DS (1999) Altered CYP1A expression in *Fundulus heteroclitus* adults and larvae: a sign of pollution resistance? Aquat Toxicol 45:99–113

Eriksson SP, Baden SP (1997) Behaviour and tolerance to hypoxia in juvenile Norway lobster (*Nephrops norvegicus*) of different ages. Mar Biol 128:49–54

Fernández-Tajes J, Rábade T, Laffon B, Méndez J (2011) Monitoring follow up of two areas affected by the *Prestige* oil four years after the spillage. J Toxicol Environ Health 74: 1067–1075

Filipowicz AB, Weinstein JE, Sanger DM (2007) Dietary transfer of fluoranthene from an estuarine oligochaete (*Monopylephorus rubroniveus*) to grass shrimp (*Palaemonetes pugio*): influence of piperonyl butoxide. Mar Environ Res 63:132–145

Forbes V, Forbes TL, Holmer M (1996) Inducible metabolism of fluoranthene by the opportunistic polychaete *Capitella* sp. I. Mar Ecol Prog Ser 132:63–70

Form AU, Riebesell U (2012) Acclimation to ocean acidification during long-term CO2 exposure in the cold-water coral *Lophelia pertusa*. Glob Change Biol 18:843–853

Forrester GE et al (2003) Growth of estuarine fish is associated with the combined concentration of sediment contaminants and shows no adaptation or acclimation to past conditions. Mar Environ Res 56:423–442

Frederick LA, Van Veld PA, Rice CD (2007) Bioindicators of immune function in creosote-adapted estuarine killifish, *Fundulus heteroclitus*. J Toxicol Environ Health 70A:1433–1442

Froeschke JT, Stunz GW (2012) Hierarchical and interactive habitat selection in response to abiotic and biotic factors: the effect of hypoxia on habitat selection of juvenile estuarine fishes. Environ Biol Fishes 93:31–41

Gale SA, Smith SV, Lim RP, Jeffree RA, Petocz P (2003) Insights into the mechanisms of copper tolerance of a population of black-banded rainbowfish (*Melanotaenia nigrans*) (Richardson) exposed to mine leachate, using $^{64/67}$Cu. Aquat Toxicol 62:135–153

González RR, Quiñones RA (2000) Pyruvate oxidoreductases involved in glycolytic anaerobic metabolism of polychaetes from the Continental Shelf off Central-south Chile. Estuar Coast Shelf Sci 51:507–519

Gonzalez A, Ronce O, Ferriere R, Hochberg ME (2013) Evolutionary rescue: an emerging focus at the intersection between ecology and evolution. Philos Trans R Soc B 368. doi:10.1098/rstb.2012.0404

Grant A, Hateley JG, Jones NV (1989) Mapping the ecological impact of heavy metals on the estuarine polychaete *Nereis diversicolor* using inherited metal tolerance. Mar Poll Bull 20:235–238

Greaney GS, Place AR, Cashon RE, Smith G, Powers DA (1980) Time course of changes in enzyme activities and blood respiratory properties of killifish during long-term acclimation to hypoxia. Physiol Zool 53:136–144

Guest JR, Baird AH, Maynard JA, Muttaqin E, Edwards AJ et al (2012) Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. PLoS One 7(3):e33353. doi:10.1371/journal.pone.0033353

Harper-Arabie RM, Wirth EF, Fulton MH, Scott GI, Ross PE (2004) Protective effects of allozyme genotype during chemical exposure in the grass shrimp, *Palaemonetes pugio*. Aquat Toxicol 70:41–54

Harris RR, Santos MC (2000) Heavy metal contamination and physiological variability in the Brazilian mangrove crabs *Ucides cordatus* and *Callinectes danae* (Crustacea: Decapoda) Mar. Biol 137:691–703

Herbert NA, Steffenson JF (2005) The response of Atlantic cod, *Gadus morhua*, to progressive hypoxia: fish swimming speed and physiological stress. Mar Biol 147:1403–1412

Hoare K, Beaumont AR, Davenport J (1995) Variation among populations in the resistance of *Mytilus edulis* embryos to copper: adaptation to pollution? Mar Ecol Prog Ser 120:155–161

Hochachka P, Lutz PL, Sick T, Rosenthal M, van den Thillart G (1993) Volume overview: hypoxia defenses and adaptational strategies. In: Hochachka PW (ed) Surviving hypoxia: mechanisms of control and adaptation. CRC Press, Boca Raton, p 571

Hourdez S, Weber RE, Green BN, Kenney JM, Fisher CR (2002) Respiratory adaptations in a deep-sea orbiniid polychaete from Gulf of Mexico brine pool NR-1: metabolic rates and hemoglobin structure/function relationships. J Exp Biol 205:1669–1681

Hume B, D'Angelo C, Burt J, Baker AC, Riegl B, Wiedenmann J (2013) Corals from the Persian/Arabian Gulf as models for thermotolerant reef-builders: prevalence of clade C3 Symbiodinium, host fluorescence and ex situ temperature tolerance. Mar Pollut Bull. doi:10.1016/j.marpolbul.2012.11.032

Ivanova AV, Taylor C, Sokolova IM (2009) Effects of elevated temperature and cadmium-exposure on stress protein response in eastern oyster *Crassostrea virginica* (Gmelin). Aquat Toxicol 91:245–254

Johnson EL (2011) Tolerance to contaminants. Evidence from chronically exposed populations of aquatic organisms. In: Amiard-Triquet C, Rainbow PS, Romeo M (eds) Tolerance to environmental contaminants. CRC Press, Boca Raton, pp 25–48

Kamp G (1993) Intracellular reactions controlling environmental anaerobiosis in the marine annelid *Arenicola marina*, a fresh look at old pathways. In: Hochachka P, Lutz PL, Sick T, Rosenthal M, van den Thillart G (eds) Surviving hypoxia: mechanisms of control and adaptation. CRC Press, Boca Raton, p 571

Kelly M, Hofmann GE (2012) Adaptation and the physiology of ocean acidification. Funct Ecol. doi:10.1111/j.1365-2435.2012.02061.x

Kerambrun P, Thessalou-Legaki M, Verriopoulos G (1993) Comparative effects of environmental conditions in eutrophic polluted and oligotrophic nonpolluted areas of the Saronikos Gulf (Greece) on the physiology of the copepod *Acarcia clausi*. Comp Biochem Physiol C 105:415–420

Khan AT, Weis JS (1987a) Effects of methylmercury on sperm and egg viability of two populations of killifish, *Fundulus heteroclitus*. Arch Environ Contam Toxicol 16:499–505

Khan AT, Weis JS (1987b) Effects of mercuric chloride on eggs and juvenile viability in two populations of killifish. Mar Pollut Bull 18:504–505

Khan AT, Weis JS (1987c) Effects of methylmercury on egg and juvenile viability in two populations of *Fundulus heteroclitus*. Environ Res 44:272–278

Khan AT, Weis JS (1987d) Effects of mercuric chloride on sperm and egg viability in two populations of the mummichog *Fundulus heteroclitus*. Environ Pollut 48:263–273

Khan AT, Weis JS, D'Andrea L (1988) Studies of cadmium tolerance in two populations of grass shrimp *Palaemonetes pugio*. Bull Environ Contam Toxicol 40:30–34

Klerks PL (1999) Acclimation to contaminants by the grass shrimp *Palaemonetes pugio*: individual contaminants vs mixtures. Ecotoxicol 8:277–286

Klerks PL, Bartholomew PR (1991) Cadmium accumulation and detoxification in a Cd-resistant population of the oligochaete *Limnodrilus hoffmeisteri*. Aquat Toxicol 19:97–112

Klerks PL, Blaha MA (2009) Heat tolerance as an indicator of climate change impacts: general considerations and a case study in Poeciliid fish. Environ Bioindic 4:46–66

Klerks PL, Leberg PL, Lance RF, McMillan DJ, Means J (1997) Lack of development of pollutant-resistance or genetic differentiation in darter gobies (*Gobionellus boleosoma*) inhabiting a produced-water discharge site. Mar Environ Res 44:377–395

Klerks PL, Lentz SA (1998) Resistance to lead and zinc in the western mosquitofish *Gambusia affinis* inhabiting contaminated Bayou Trepagnier. Ecotoxicology 7:11–17

Klerks PL, Levinton JS (1989) Rapid evolution of metal resistance in a benthic oligochaete inhabiting a metal-polluted site. Biol Bull 176:135–141

Klerks PL, Moreau CJ (2001) Heritability of resistance to individual contaminants and to contaminant mixtures in the sheepshead minnow (*Cyprinodon variegatus*). Environ Toxicol Chem 20:1746–1751

Klerks PL, Xie L, Levinton JS (2011) Quantitative genetics approaches to study evolutionary processes in ecotoxicology; a perspective from research on the evolution of resistance. Ecotoxicology 20:513–523

Kovatch CE, Schizas NV, Chandler GT, Coull BC, Quattro JM (2000) Tolerance and genetic relatedness of three meiobenthic copepod populations exposed to sediment-associated contaminant mixtures: role of environmental history. Environ Toxicol Chem 19:912–919

Kraus M, Kraus DB (1986) Differences in the effects of mercury on predator avoidance in two populations of the grass shrimp *Palaemonetes pugio*. Mar Environ Res 18:277–289

Kraus ML, Weis JS (1988) Differences in effects of mercury on telson regeneration in two populations of the grass shrimp, *Palaemonetes pugio*. Arch Environ Contam Toxicol 17:115–120

Kraus M, Weis JS, Weis P (1988) Effects of mercury on larval and adult grass shrimp (*Palaemonetes pugio*). Arch Environ Contam Toxicol 17:355–363

Kurochkin IO, Ivanina AV, Eilers S, Downs CA, May LA, Sokolova IM (2009) Cadmium affects metabolic responses to prolonged anoxia and reoxygenation in eastern oysters (*Crassostrea virginica*). Am J Physiol Regul Integr Comp Physiol 297:R1262–R1272

Lai JCC, Kakuta I, Mok HOL, Rummer JL, Randall D (2006) Effects of moderate and substantial hypoxia on erythropoietin levels in rainbow trout kidney and spleen. J Exp Biol 209:2734–2738

Lake JL, Rubinstein NI, Lee H II, Lake CA, Heltshe J, Pavignano S (1990) Equilibrium partitioning and bioaccumulation of sediment-associated contaminants by infaunal organisms. Environ Toxicol Chem 9:1095–1106

Lavie B, Nevo E (1986) Genetic selection of homozygote allozyme genotypes in marine gastropods exposed to cadmium pollution. Sci Total Environ 57:91–98

Leung KM, Taylor AC, Furness RW (2000) Temperature-dependent physiological responses of the dogwhelk *Nucella lapillus* to cadmium exposure. J Mar Biol Assoc UK 80:647–660

Levinton JS, Suatoni E, Wallace W, Junkins R, Kelaher B, Allen BJ (2003) Rapid loss of genetically based resistance to metals after the cleanup of a Superfund site. Proc Natl Acad Sci 100:9889–9891

Lohbeck KT, Riebesell U, Reusch TB (2012) Adaptive evolution of a key phytoplankton species to ocean acidification. Nat Geosci. 5:346–351

Love JW, Rees BB (2002) Seasonal differences in hypoxia tolerance in Gulf killifish*, Fundulus grandis* (Fundulidae). Environ Biol Fish 63:103–115

Luoma SN, Cain DJ, Ho K, Hutchinson A (1983) Variable tolerance to Copper in two species from San Francisco bay. Mar Environ Res 10:209–222

Mandic M, Todgham AE, Richards JG (2009) Mechanisms and evolution of hypoxia tolerance in fish. Proc R Soc 276:735–744

Marshall DJ, McQuaid CD (1993) Differential physiological and behavioural responses of the intertidal mussels, *Choromytilus meridionalis* (Kr.) and *Perna perna* L., to exposure to hypoxia and air: a basis for spatial separation. J Exper Mar Biol Ecol 171:225–237

Maxime V, Pichavant K, Boeuf G, Nonnotte G (2000) Effects of hypoxia on respiratory physiology of turbot, *Scophthalmus maximus*. Fish Physiol Biochem 22:51–59

McCulloch M, Falter J, Trotter J, Montagna P (2012) Coral resilience to ocean acidification and global warming through pH up-regulation. Nat Clim Change 2:623–627

McKenzie L, Brooks R, Johnston EL (2011) Heritable pollution tolerance in a marine invader. Environ Res 111:926–932

McMillan AM, Bagley MJ, Jackson SA, Nacci DE (2006) Genetic diversity and structure of an estuarine fish (*Fundulus heteroclitus*) indigenous to sites associated with a highly contaminated urban harbor. Ecotoxicology 15:539–548

Meyer JN, DiGiulio RT (2002) Patterns of heritability of decreased EROD activity and resistance to PCB126-induced teratogenesis in laboratory-reared offspring of killifish (*Fundulus heteroclitus*) from a creosote-contaminated site in the Elizabeth River, VA. USA Mar Environ Res 54:621–626

Meyer JN, DiGiulio RT (2003) Heritable adaptation and fitness costs in killifish (*Fundulus heteroclitus*) inhabiting a polluted estuary. Ecol Appl 13:490–503

Meyer JN, Nacci DE, DiGiulio RT (2002) Cytochrome P450 a (CYP1A) in killifish (*Fundulus heteroclitus*): heritability of altered expression and relationship to survival in contaminated sediments. Toxicol Sci 68:69–81

Miliou H et al (2000) Influence of life history adaptations on the fidelity of laboratory bioassays for the impact of heavy metals (Co^{2+} and Cr^{6+}) on tolerance and population dynamics of *Tisbe holothuridae*. Mar Pollut Bull 40:352–359

Miller GM, Watson SA, Donelson JM, McCormick MI, Munday PL (2012) Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. Nat Clim Change 2:858–861

Millward RN, Grant A (1995) Assessing the impact of copper on nematode communities from a chronically metal-enriched estuary using pollution-induced community tolerance. Mar Pollut Bull 30:701–706

Mouneyrac C et al (2003) Trace-metal detoxification and tolerance of the estuarine worm *Hediste diversicolor* chronically exposed in their environment. Mar Biol 143:731–744

Mouneyrac C, Perrein-Ettajani H, Amiard-Triquet C (2010) The use of fitness, reproduction, and burrowing behaviour of the polychaete *Nereis diversicolor* in the assessment of estuarine sediment quality. Environ Pollut 158:121–128

Munday PL, McCormick MI, Meekan M, Dixson DL, Watson S-A, Chivers DP, Ferrari MC (2012) Selective mortality associated with variation in CO_2 tolerance in a marine fish. Ocean Acidification 2012:1–5. doi:10.2478/oac-2012-0001

Nacci D, Coiro L, Champlin D, Jayarman S, McKinney R, Gleason T, Munns WR Jr, Specker JL, Cooper KR (1999) Adaptations of wild populations of the estuarine fish *Fundulus heteroclitus* to persistent environmental contaminants. Mar Biol 134:9–17

Nacci D, Huber M, Champlin D, Jayarman S, Cohen S, Gauger E, Fong A, Gomez-Chiarri M (2009) Evolution of tolerance to PCBs and susceptibility to a bacterial pathogen (*Vibrio harveyi*) in Atlantic killifish (*Fundulus heteroclitus*) from New Bedford (MA, USA) harbor. Environ Pollut 157:857–864

Nacci DD, Champlin S, Jayaraman S (2010) Adaptation of the estuarine fish *Fundulus heteroclitus* (Atlantic killifish) to polychlorinated biphenyls (PCBs). Estuar Coast 33:853–864

Nilsson GE, Östlund-Nilsson S (2008) Does size matter for hypoxia tolerance in fish? Biol Rev 83:173–189

Noel-Lambot F (1981) Presence in the intestinal lumen of marine fish of corpuscles with a high cadmium-, zinc- and copper-binding capacity: a possible mechanism of heavy metal tolerance. Mar Ecol Prog Ser 4:175–181

Parker L, Ross1 PM, O'Connor WA, Borysko1 L, Raftos DA, Pörtner H-O (2012). Adult exposure influences offspring response to ocean acidification in oysters. Presentation at the ocean in a high CO_2 world: third international ocean acidification conference, Monterey Sept 2012

Parrin AP, Harmata KL, Netherton SE, Yaeger MA, Bross LS, Blackstone NW (2012) Within-colony migration of symbionts during bleaching of octocorals. Biol Bull 223(2): 245–256

Peña-Llopis S, Peña JB, Sancho E, Fernández-Vega C, Ferrando MD (2001) Glutathione-dependent resistance of the European eel *Anguilla anguilla* to the herbicide molinate. Chemosphere 45:671–681

Pereira JJ, Kanungo K (1981) Effects of silver on respiration and ion and water balance in *Neanthes virens*. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Biological monitoring of marine pollutants. Academic Press, New York

Pesch CE, Hoffman GL (1982) Adaptation of the polychaete *Neanthes arenaceodentata* to copper. Mar Environ Res 6:307–317

Prince R, Cooper KR (1995a) Comparisons of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on chemically impacted and nonimpacted subpopulations of *Fundulus heteroclitus.* I. TCDD toxicity. Environ Toxicol Chem 14:579–588

Prince R, Cooper KR (1995b) Comparisons of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on chemically impacted and nonimpacted subpopulations of *Fundulus heteroclitus.* II. Metabolic considerations. Environ Toxicol Chem 14:589–596

Purcell KM, Hitch A, Klerks PL, Leberg PL (2008) Adaptation as a potential response to sea-level rise: a genetic basis for salinity tolerance in populations of a coastal marsh fish. Evol Adapt 1:155–160

Purcell KM, Hitch A, Martin S, Klerks PL, Leberg PL (2012) The role of genetic structure in the adaptive divergence of populations experiencing saltwater intrusion due to relative sea-level rise. J Evol Biol 25:2623–2632

Putnam HM et al (2012) Endosymbiotic flexibility associates with environmental sensitivity in scleractinian corals. Proc R Soc B. doi:10.1098/rspb.2012.1454

Rainbow PS, Luoma SN (2011) Biodynamic parameters of the accumulation of toxic metals, detoxification, and the acquisition of metal tolerance. In: Amiard-Triquet C, Rainbow PS, Romeo M (eds) Tolerance to environmental contaminants. CRC Press, Boca Raton, pp 127–151

Rainbow PS, Poirier L, Smith BD, Brix KV, Luoma SN (2006) Trophic transfer of trace metals from the polychaete worm *Nereis diversicolor* to the polychaete *N. virens* and the decapod crustacean *Palaemonetes varians*. Mar Ecol Prog Ser 321:167–181

Rainbow PS, Amiard J-C, Amiard-Triquet C, Cheung M-S, Zhang L, Zhong H, Wang W-X (2007) Trophic transfer of trace metals: subcellular compartmentalization in bivalve by prey, assimilation by a gastropod predator and in vitro digestion simulations. Mar Ecol Prog Ser 348:125–138

Rainbow PS, Smith BD, Luoma SN (2009) Differences in trace metal bioaccumulation kinetics among populations of the polychaete *Nereis diversicolor* from metal-contaminated estuaries. Mar Ecol Prog Ser 376:173–184

Rice CA, Myers MS, Willis ML, French BL, Casillas E (2000) From sediment bioassay to fish biomarker – connecting the dots using simple trophic relationships. Mar Environ Res 50:527–533

Roark S, Kelble MA, Nacci D, Champlin D, Coiro L, Guttman SI (2005) Population genetic structure and tolerance to dioxin-like compounds of a migratory marine fish (*Menidia menidia*) at polychlorinated biphenyl–contaminated and reference sites. Environ Toxicol Chem 24:726–732

Romeo M, Wirgin I (2011) Biotransformation of organic contaminants and the acquisition of resistance. In: Amiard-Triquet C, Rainbow PS, Romeo M (eds) Tolerance to environmental contaminants. CRC Press, Boca Raton, pp 175–208

Rossi SS, Anderson J (1978) Petroleum hydrocarbon resistance in the marine worm *Neanthes arenaceodentata* (Polychaeta: Annelida), induced by chronic exposure to No. 2 Fuel oil. Bull Environ Contam Toxicol 20:513–521

Schöttler U, Grieshaber M (1988) Adaptation of the polychaete worm Scoloplos armiger to hypoxic conditions. Mar Biol 99:215–222

Silverstein RN, Correa AM, Baker AC (2012) Specificity is rarely absolute in coral-algal symbiosis: implications for coral response to climate change. Proc R Soc B Biol Sci 279:2609–2618

Smith KJ, Able KW (2003) Dissolved oxygen dynamics in salt marsh pools and its potential impacts on fish assemblages. Mar Ecol Prog Ser 258:223–232

Soskine M, Towkik D (2010) Mutational effects and the evolution of new protein functions. Nat Rev Genet 11:572–582

Spicer J, Baden S (2001) Environmental hypoxia and haemocyanin between-individual variability in Norway lobsters *Nephrops norvegicus* (L.). Mar Biol 139:727–734

Stickle WB, Kapper MA, Liu L-L, Gnaiger E, Wang SY (1989) Metabolic adaptations of several species of crustaceans and molluscs to hypoxia: tolerance and microcalorimetric studies. Biol Bull 177:303–312

Sze PW, Lee SY (1995) The potential role of mucus in the depuration of copper from the mussels *Perna viridis* (L.) and *Septifer virgatus* (Wiegmann). Mar Pollut Bull 31:390–393

Thresher RE, Tilbrook B, Fallon S, Wilson NC, Adkins J (2011) Effects of chronic low carbonate saturation levels on the distribution, growth and skeletal chemistry of deep-sea corals and other seamount megabenthos. Mar Ecol Prog Ser 442:87–99

Ueda N, Ford C, Rikard S, Wallace R, Boettcher A (2009) Heat shock protein 70 expression in juvenile Eastern Oysters, *Crassostrea virginica* (Gmelin, 1791), exposed to anoxic conditions. J Shellfish Res 28:849–854

Unger M, Roesijadi G (1996) Increase in metallothionein mRNA accumulation during Cd challenge in oysters preexposed to Cd. Aquat Toxicol 34:185–193

Van Veld PA, Westbrook DJ (1995) Evidence for depression of cytochrome P4501A in a population of chemically resistant mummichog (*Fundulus heteroclitus*). Environ Sci 3:221–234

Vogelbein WK, Fournie JW, van Veld PA, Huggett RJ (1990) Hepatic neoplasms in the mummichog *Fundulus heteroclitus* from a creosote-contaminated site. Cancer Res 50:5978–5986

Wallace WG, Lopez GR, Levinton JS (1998) Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. Mar Ecol Prog Ser 172:225–237

Wang JS, Chou HN, Fan J-J, Chen C-M (1998) Uptake and transfer of high PCB concentrations from phytoplankton to aquatic biota. Chemosphere 36:1201–1210

Wannamaker CM, Rice JA (2000) Effects of hypoxia on movements and behavior of selected estuarine organisms from the southeastern United States. J Exp Mar Biol Ecol 249:145–163

Weis JS (2002) Tolerance to environmental contaminants in the mummichog, *Fundulus heteroclitus*. Hum Ecol Risk Assess 8:933–953

Weis JS, Weis P (1989) Tolerance and stress in a polluted environment: the case of the mummichog. Bioscience 39:89–96

Weis JS, Weis P, Heber M, Vaidya S (1981) Methylmercury tolerance of killifish (*Fundulus heteroclitus*) embryos from a polluted vs nonpolluted environment. Mar Biol 65:283–287

Whitely NM (2011) Physiological and ecological responses of crustaceans to ocean acidification. Mar Ecol Prog Ser 430:257–271

Wirgin I, Chambers RC (2006) Atlantic tomcod (*Microgadus tomcod*): a model species for the response of Hudson River fish to toxicants. In: Waldman JR, Limburg KE, Strayer S (eds) Hudson river fishes and the environment. American Fisheries Society, Bethesda, pp 331–364

Wirgin I, Waldman J (2004) Resistance to contaminants in North American fish populations. Mutat Res Fund Mol Mech Mutag 552:73–100

Wirgin I, Kreamer G-L, Grunwald C, Squibb K, Garte SJ, Courtenay S (1992) Effects of prior exposure history on cytochrome P4501A mRNA induction by PCB congener 77 in Atlantic tomcod. Mar Environ Res 34:103–108

Wirgin I, Grunwald C, Courtenay S, Kreamer GL, Reichert WL, Stein JE (1994) A biomarker approach to assessing xenobiotic exposure in cancer prone Atlantic tomcod from the North American Atlantic coast. Environ Health Pers 102:764–770

Wirgin I, Roy NK, Loftus M, Chambers C, Franks D, Hahn ME (2011) Mechanistic basis of resistance to PCBs in Atlantic tomcod from the Hudson River. Science 331:1322–1325

Wu R (2002) Hypoxia: from molecular responses to ecosystem responses. Mar Pollut Bull 45:35–45

Wu RS, Lam PKS, Wan KL (2002) Tolerance to, and avoidance of, hypoxia by the penaeid shrimp (*Metapenaeus ensis*). Environ Pollut 118:351–355

Yawetz A, Fishelson L, Bresler V, Manelis R (2010) Comparison of the effects of pollution on the marine bivalve *Donax trunculus* in the vicinity of polluted sites with specimens from a clean reference site (Mediterranean Sea). Mar Pollut Bull 60:225–229

Yuan Z, Courtenay S, Wirgin I (2006a) Comparison of hepatic and extra hepatic induction of cytochrome P4501A by graded doses of aryl hydrocarbon receptor agonists in Atlantic tomcod from two populations. Aquat Toxicol 76:306–320

Yuan Z, Courtenay S, Chambers RC, Wirgin I (2006b) Evidence of spatially extensive resistance to PCBs in an anadromous fish of the Hudson River. Environ Health Persp 114:77–84

Zhou Q, Rainbow PS, Smith BD (2003) Tolerance and accumulation of the trace metals zinc, copper and cadmium in three populations of the polychaete *Nereis diversicolor*. J Mar Biol Assoc UK 83:65–72

Index

Note: * refers to index terms that have more than 100 citations, and are listed only for their first citation in each chapter.

J.S. Weis, *Physiological, Developmental and Behavioral Effects of Marine Pollution*, DOI 10.1007/978-94-007-6949-6,