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.

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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

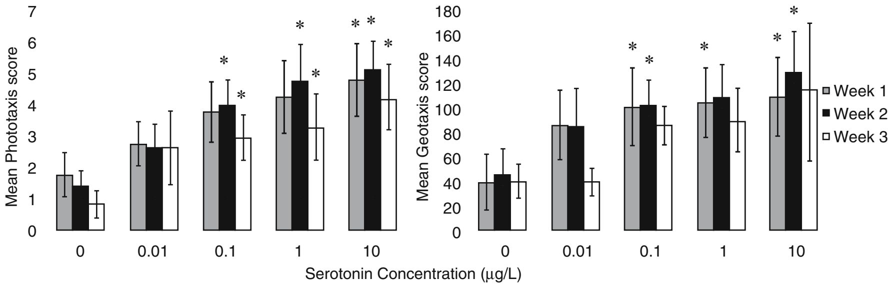

Fig. 9.1 Mean average phototaxis and geotaxis of *E. marinus* exposed to serotonin over 3 weeks. Error bars = 1 SD. * Significantly different from control (Reprinted from Guler and Ford 2010: 400, reprinted courtesy of Elsevier Publishing Co)

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*).

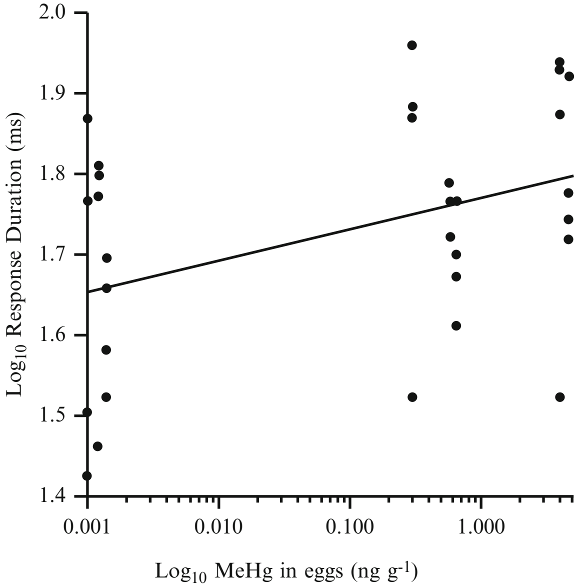

Fig. 9.2 Effect of maternally derived meHg on response duration to a vibratory startle stimulus in Atlantic croaker larvae. Behaviors were log transformed to attain normality. Data points are mean value for each spawn and developmental age (Reprinted from Alvarez et al. 2006: 334, courtesy Elsevier Publishing Co)

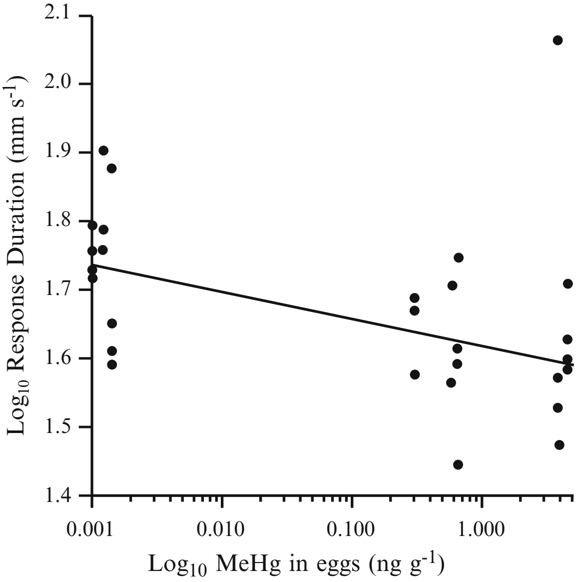

Fig. 9.3 Effect of maternally derived meHg on response speed to a vibratory startle stimulus in Atlantic croaker larvae. Behaviors were log transformed to attain normality. Data points are mean value for each spawn and developmental age (Reprinted from Alvarez et al. 2006: 334, courtesy Elsevier Publishing 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).

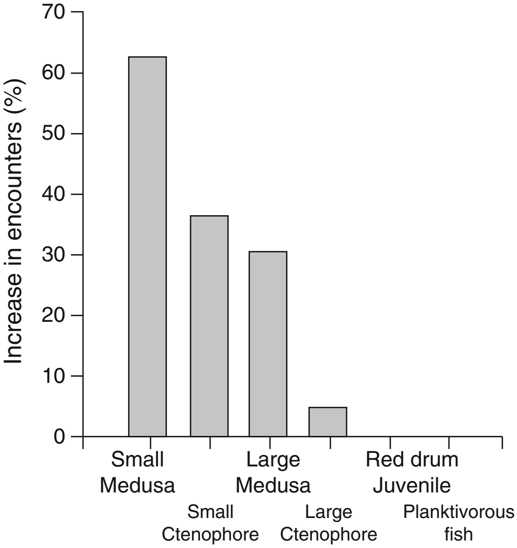

Fig. 9.4 Increase in encounter rates relative to controls between red drum larvae and their predators as a result of 4 days atrazine exposure (Reprinted from Alvarez and Fuiman 2005: 237, courtesy Elsevier Publishing Co)

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 μg g^{-1} dry sediment Cu, 35 μg g^{-1} Cd, or 299 μg 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 μg 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.

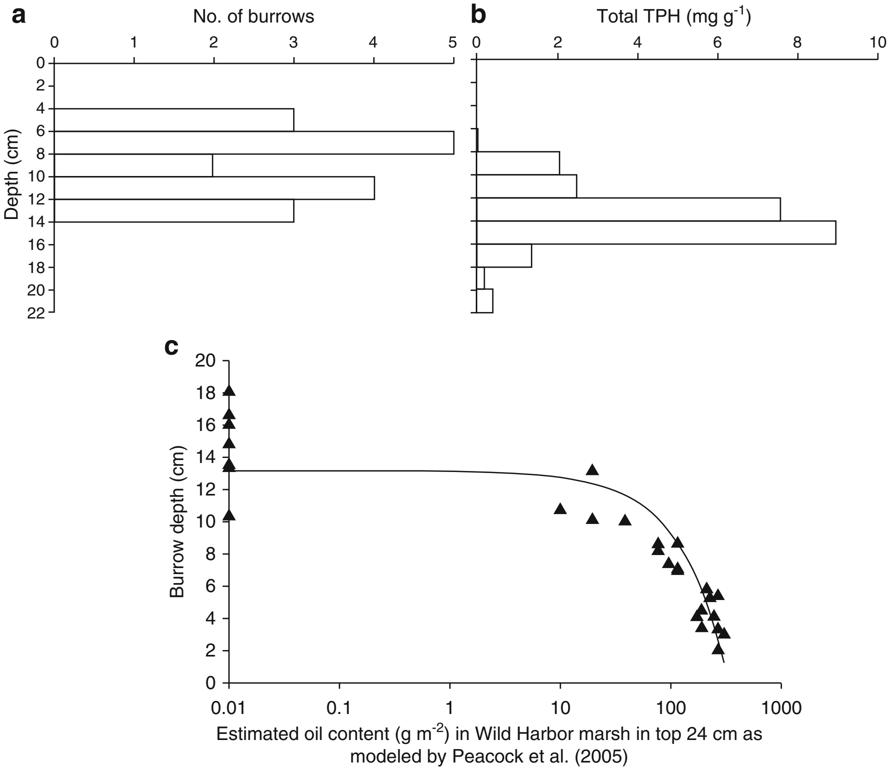

Fig. 9.5 (**a**) number of *U. pugnax* burrows at different depths, (**b**) concentration of total PAH at different depths in sediments from oiled area, (**c**) burrow depth compared to estimated PAH content (g oil m^{-2}) using modeled data (Reprinted from Culbertson et al. 2007: 957, courtesy Elsevier 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 $\mu g\ g^{-1}$ (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).

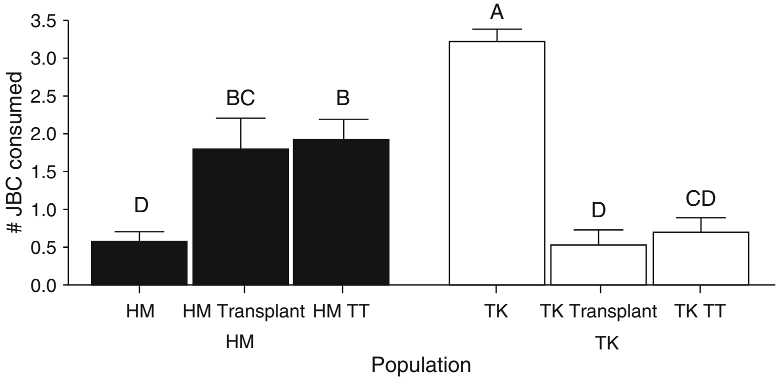

Fig. 9.6 Number of juvenile blue crab prey (*JBC*) consumed in 24 h by native adult blue crabs, transplanted crabs, lab trophic transfer [*TT*] crabs from Hackensack and Tuckerson (reference site). *Black columns* Hackensack [*HM*] crabs, *white columns* Tuckerton [*TK*] crabs. Error bars = 1 SE, letters 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 μg 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 μg l^{-1} of either $HgCl_2$ or meHg for 1 week reduced prey capture. Sticklebacks (*Gasterosteus aculeatus*) exposed to 3 μg 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 μg 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 μg l^{-1} inhibited olfactory-mediated alarm responses in chinook salmon (*Oncorhynchus tshawytscha*) (Scholz et al. 2000). Homing behavior was impaired at 10 μg 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 μg 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 μg l^{-1}) (Tierney et al. 2006).

Embryonic exposure (topical treatment) of mummichog (*F. heteroclitus*) embryos to PCB 126 ($\geq$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 μg 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 μg 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,

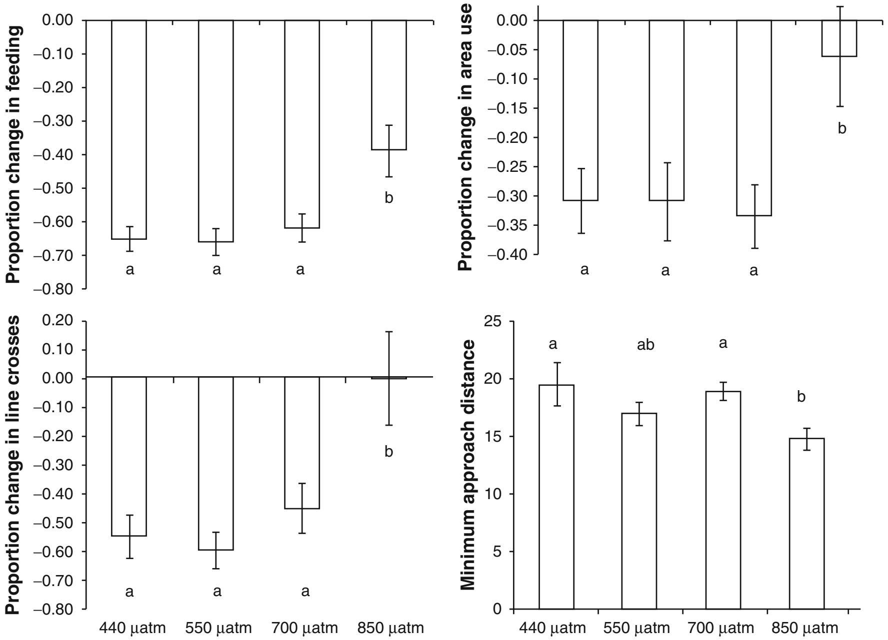

Fig. 9.7 Mean proportion change (±SE) in feeding strikes (*top left*), area use (*top right*), line crosses (*lower left*) and minimum approach distance (*bottom right*) for juvenile damselfish treated with different $p\text{CO}_2$ concentrations and exposed to sight of a spiny chromis. Letters denote 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 $p\text{CO}_2$.

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).

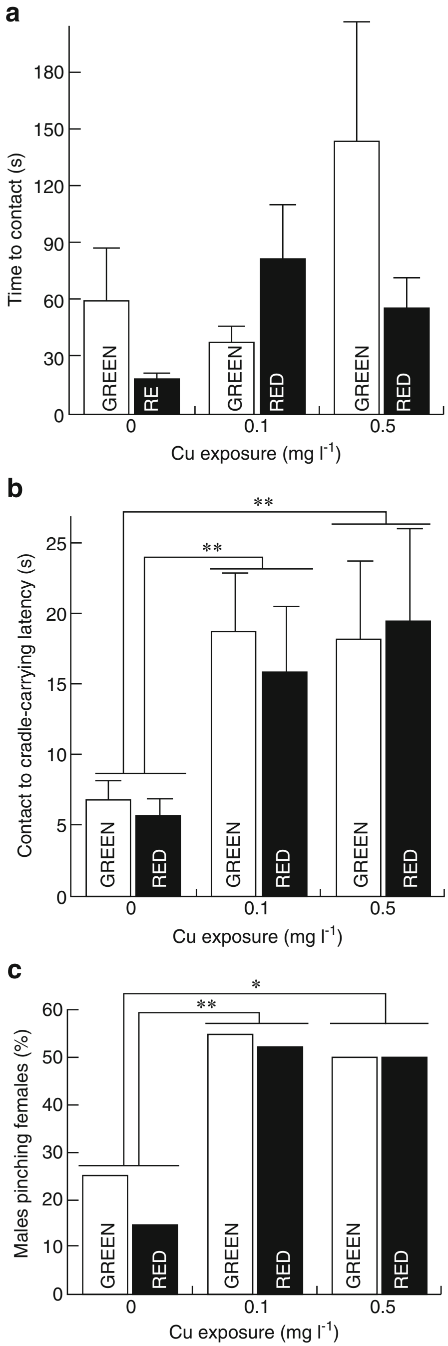

Fig. 9.8 Behavioral response to a pre-molt female of red and green color morphs male *C. maenas* exposed to 0, 0.1 or 0.5 mg l^{-1} Cu for 5 days. (**a**) Time to contact the female, (**b**) time between contact and cradle-carrying, (**c**) proportion of males that pinched female. * and ** = significant (*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 $\mu g\ g^{-1}$) 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 $\mu g\ l^{-1}$ 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).

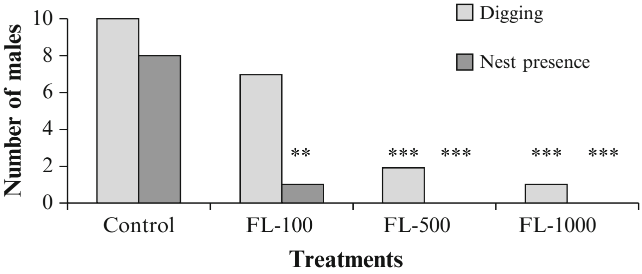

Fig. 9.9 Nest-building activity by male three-spined sticklebacks after 21-days exposure to different concentrations of flutamide. Number of males showing digging activity (*light grey*) or had a nest (*dark grey*). Significant difference from control ** $< p$ 0.01, *** $p < 0.001$ (Reprinted from Sebire 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 μg 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 $\mu g\ l^{-1}$ 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 $\mu g\ l^{-1}$atrazine during the parr–smolt transformation significantly reduced migratory activity in an experimental stream during the period of peak migration in the wild population.

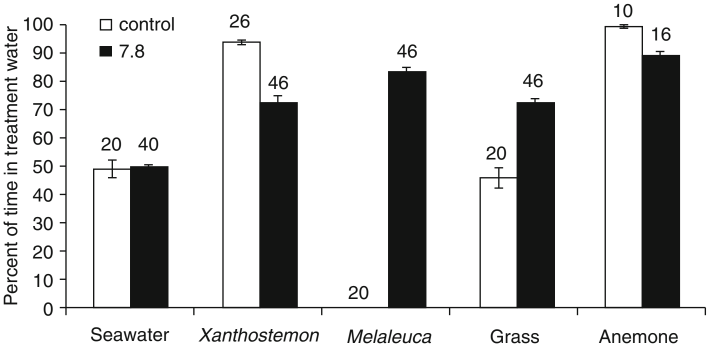

Fig. 9.10 Response of larval clownfish to olfactory cues from three tropical plants and from anemones when reared in current seawater pH (control, *open bars*) and ph 7.8 (*filled bars*). Bars = percentage of time larvae spent on the side of a 2-channel flume chamber which contained the cue. Numbers 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 $\mu g\ l^{-1}$ 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 pCO_2 conditions avoided reef noise, as expected, but this behavior was absent in high pCO_2 (700 and 900 μatm pCO_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 pCO_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 pCO_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 pCO_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 $\mu g\ l^{-1}$ 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 $\mu g\ l^{-1}$) or atrazine (1.0 $\mu g\ l^{-1}$) 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 $<0.004\ \mu g\ l^{-1}$ 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.

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