

# Chapter 11

## Tolerance

**Abstract** Tolerance is the ability of organisms to cope with stress, in this case to environmental pollutants. It appears to be a widespread phenomenon, and can be achieved by physiological acclimation or genetic adaptation. It can be assessed by comparing responses (lethal or sublethal) of individuals from different populations to the same degree of stress, e.g. the same concentration of a toxicant would produce less of an effect in a tolerant population. The phenomenon is well documented for metals and organic contaminants (for example, resistance of insect populations to insecticides is well-known). There are also cases in which tolerance has been looked for but not found in chronically exposed populations; probably more cases than have been reported in the literature, as this can be viewed as “negative data” and not reported. When enhanced tolerance does not occur in polluted populations, the reasons may be difficult to ascertain; it may be because detoxification mechanisms are adequate to cope with elevated exposures, or that dispersal and mixing between contaminated and reference populations obscures any observation of tolerance, or that the fitness costs counteract the selective advantage of the tolerance, or other reasons.

Compensatory responses to pollutants at the physiological level are referred to as “acclimation.” Pre-exposure to chemicals can induce or enhance detoxification processes, discussed in the previous chapter, which reduces toxicity in pre-exposed organisms, either in the lab or at field sites. These responses (e.g. synthesis of MTs, CYPs) can mitigate effects on individuals experiencing moderate stress. Developing tolerance is energetically expensive, however, and may have deleterious effects on energy allocation. Thus exposure over extended periods may reduce fitness and increase selection pressure for genetically resistant individuals in populations that have variation and heritability for the response. The development of these populations that have adapted and become more resistant to pollution can be considered an evolutionary compensatory mechanism.

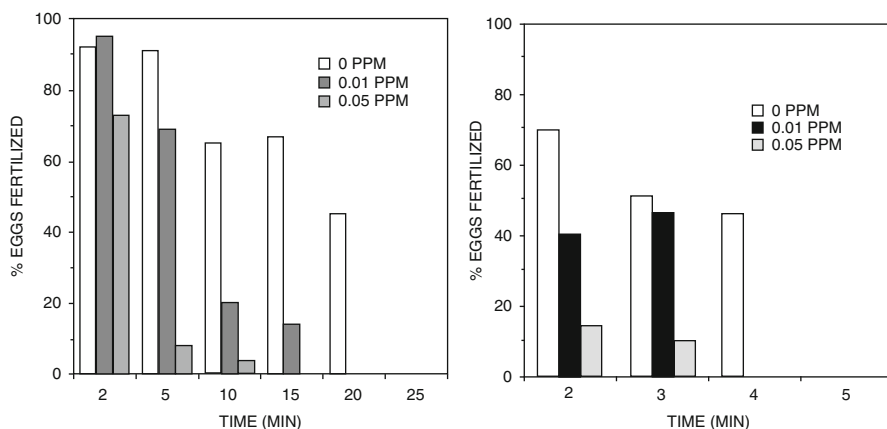
**Keywords** Costs • Cytochrome P450 • CYP • Energetics • Evolution • Food web • Metallothioneins • Sediment • Trade-offs

## 11.1 Metals

Some metals (Zn, Cu) are essential for life, so organisms have developed ways to optimize their exposures and respond to higher levels. Metal tolerance is widespread, and is often associated with the storage of metals in a non-available form, rather than reduced uptake or increased elimination, although these mechanisms have also been observed. Metallothioneins (MTs) play important roles in the metabolism of essential metals and can be utilized in dealing with metal contaminants. For example, some contaminated populations have developed multiple copies of the gene for MT, enabling them to tolerate high metal concentrations (Soskine and Towkik 2010).

### 11.1.1 Fishes

While there are numerous papers about MTs and other protective mechanisms (see previous chapter) and many studies documenting enhanced metal tolerance in fish populations living in contaminated freshwater environments, reports on metal tolerance in marine species are less frequent. Early life stages of the killifish, *Fundulus heteroclitus*, inhabiting Piles Creek (PC), a contaminated industrialized estuary in New Jersey, U.S., have tolerance to Hg, one of the contaminants in the environment (Weis 2002). However, tolerance is manifested only in early life stages. Tolerance to meHg was seen in gametes (both sperm and eggs) and in developing embryos, which were less affected by meHg (Weis et al. 1981; Khan and Weis 1987a, b, c, d) (Fig. 11.1). A possible mechanism for tolerance of the embryos was reduced uptake through the chorion. While PC embryos were more tolerant to



**Fig. 11.1** (a) Effect of meHg on Piles Creek sperm mean fertilization success. (b) Effect of meHg on Long Island (reference) sperm mean fertilization success (Reprinted from Khan and Weis 1987a: 501, courtesy Springer Publishing Co)

meHg, they were less tolerant than embryos of reference populations to inorganic Hg. Tolerance was not exhibited in larvae or adults from the PC population, which showed signs of stress in terms of reduced growth and longevity (Weis and Weis 1989).

Mechanisms of copper tolerance in black-banded rainbowfish (*Melanotaenia nigrans*) were investigated by Gale et al. (2003). One population has been exposed to elevated Cu for over 40 years, due to mine leachate. The 96 h EC<sub>50</sub> of exposed [E] fish was 8.3 times higher than that of reference [R] fish. Both E and R fish were exposed to low (L<sub>Cu</sub>, 30 µg Cu l<sup>-1</sup>) and elevated (E<sub>Cu</sub>, 300 µg Cu l<sup>-1</sup>) Cu for 24 and 48 h, respectively. Copper uptake in most tissues was less (up to 50 %) in E fish. Thus, the mechanism of tolerance was considered to be reduced uptake in the gills, rather than increased binding or elimination. Allozyme electrophoresis was performed and allozyme frequencies at the AAT-1 and GPI-1 loci were significantly different between E and R populations. Also, there was less heterozygosity in the E population. These results suggest that genetic selection may have occurred in the E population: selection for allozymes less sensitive to Cu may be another mechanism of tolerance.

Klerks and Lentz (1998) investigated resistance of mosquitofish *Gambusia affinis* to lead and zinc. Fish collected from highly contaminated Bayou Trepagnier (LA, USA) and exposed to Zn in a laboratory bioassay did not differ in their sensitivity from conspecifics from a control site. In contrast, Bayou Trepagnier fish did show increased resistance to lead. This difference, however, disappeared when both groups were kept for 34 days in clean water. This suggests that the elevated Pb resistance is due to physiological acclimation rather than adaptation at the population level. Annabi et al. (2009) reported resistance to Cd in a population of *G. affinis* from a polluted site in Tunisia. A genetic basis was inferred from assays performed on the F1 generation raised in the laboratory.

In the sheepshead minnow (*Cyprinodon variegatus*) heritability was estimated for tolerance to individual contaminants (phenanthrene, zinc) and to contaminant mixtures (phenanthrene plus zinc, and a complex mixture with three metals and three PAHs) (Klerks and Moreau 2001). Estimates were obtained from resemblances between relatives, parent-offspring pairs, and families of sibs and half-sibs. Heritabilities for resistance to these chemicals were low (with the high resemblances among sibs being due to common environmental and dominance genetic variation rather than additive genetic variation). Results were interpreted to mean that *C. variegatus* in contaminated environments is not likely to become resistant to these contaminants rapidly, and that resistance may develop even more slowly as more contaminants become involved. Adeyami and Klerks (2013) investigated Cu acclimation in the least killifish, *Heterandria formosa* using both lethal and sublethal endpoints, as well as potential mechanisms. Fish were exposed to either a background Cu level or to 15 µg l<sup>-1</sup> Cu for 7 days and then exposed to a lethal level (150 µg l<sup>-1</sup>) and monitored until all fish had died, and (during the first 8 h of this exposure) for whole-body Na levels and lipid peroxidation (LPO). Pre-exposed fish had a significantly longer time-to-death than controls. Neither whole-body Na nor LPO changed in the pre-exposed fish during the acute exposure, while both decreased in the control fish. Thus, acclimation was seen in both time-to-death

and sublethal endpoints, and Cu toxicity may involve both Na loss and LPO. Acclimation may be brought about by prevention of these effects. A follow-up study on potential mechanisms used a similar pre-exposure/exposure design and quantified Cu accumulation,  $\text{Na}^+/\text{K}^+$ -ATPase activity, MT levels, and catalase activity. While Cu levels were higher in pre-exposed fish at  $T_0$ , net accumulation was faster in the controls during the high-level Cu exposure. Therefore, changes in accumulation dynamics may play a role in resistance. Cu-acclimated fish also had higher MT levels. There was no evidence of involvement of  $\text{Na}^+/\text{K}^+$ -ATPase.

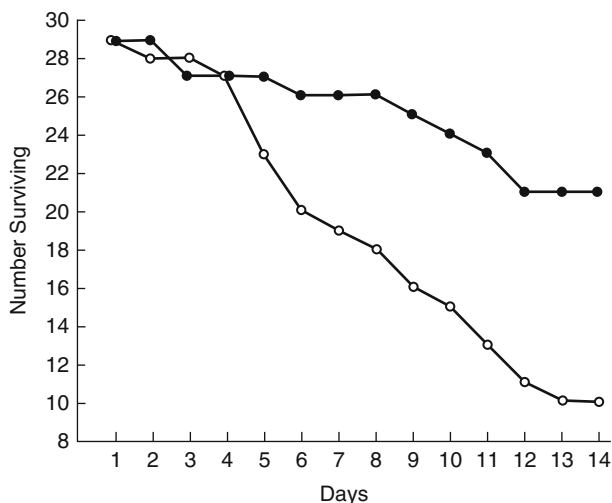
Noel-Lambot (1981) observed in various species of unfed fishes (*Anguilla anguilla*, *Myoxocephalus scorpius*, *Serranus cabrilla*, *Moena chryselis*, *Scorpaena* sp.), white mucus corpuscles in the intestinal lumen that contained high concentrations of Ca and Mg. In fish treated with Cd, Zn, or Cu, these corpuscles contained very high concentrations of these metals, potentially much of the body burden. It appeared that metals were accumulated in the corpuscles directly from ingested sea water and that the corpuscles limited their entry through the intestinal wall, thus protecting the fish against potentially hazardous concentrations of metals.

### 11.1.2 Crustaceans

In some classic studies, Bryan and colleagues (Bryan and Hummerstone 1971, 1973; Bryan and Gibbs 1983) studied invertebrates including the amphipod, *Corophium volutator* in Restronguet Creek (RC), Cornwall, England, an estuary with high metal concentrations, particularly Cu, from mining operations upstream. These amphipods were more tolerant to Cu than conspecifics from reference sites, as was the crab, *Carcinus maenas*, which was also more tolerant to Zn. Since crab larvae have a pelagic phase that distributes them widely, it is likely that the larvae settling in RC are derived from parents from far away with no history of metal exposure. Thus, selection would operate on individual crabs that settle in the contaminated area, with the more resistant ones able to survive due to physiological acclimation rather than genetic adaptation of the population. Investigators found that Zn resistance increased with crab size, supporting the idea of selection on individuals and physiological acclimation. The tolerant crabs accumulated less metal or excreted more, reducing their body burdens.

Fiddler crabs (*Uca pugnax*) from highly contaminated Piles Creek (PC) N.J., U.S.A. were more tolerant to meHg than crabs from a reference site, as indicated by the degree of growth inhibition in regenerating limbs and the degree of delay in time to molt (Callahan and Weis 1983). However, tolerance could not be induced by short-term pre-exposure to meHg in either *U. pugnax* or *U. pugilator*. An adaptive mechanism seen in PC fiddler crabs was the ability to move significant amounts of the toxic Hg and Pb from their soft tissues into their exoskeleton shortly before ecdysis, thus reducing their body burden (Bergey and Weis 2007).

Comparing grass shrimp (*P. pugio*) from PC and a reference site, Tuckerton (TK) N.J., U.S.A. Kraus et al. (1988) found no significant difference in larval tolerance to



**Fig. 11.2** Number adult shrimp from Piles Creek (PC) (black circles) and Big Sheepshead Creek (BSC, reference population) surviving in  $0.025 \text{ mg l}^{-1}$  meHg over 14 days (Reprinted from Kraus et al. 1988: 358, courtesy Springer Publishing Co)

$\text{HgCl}_2$ . However, PC larvae were more tolerant to meHg than TK larvae. In contrast, PC adults were more tolerant than TK shrimp to both forms of Hg, suggesting that tolerance to meHg may be inherited, while tolerance to inorganic Hg had to be first induced by exposure earlier in life (Fig. 11.2).

Similarly, TK adults exposed to meHg or inorganic Hg (at  $0.01 \text{ mg l}^{-1}$ ) had impaired regeneration of the telson, but PC shrimp were unaffected (Kraus and Weis 1988). Kraus and Kraus (1986) investigated effects of inorganic Hg and meHg ( $0.01 \text{ mg l}^{-1}$ ) on predator avoidance by PC and TK shrimp and found that while exposed TK shrimp were more vulnerable to predation, PC shrimp were unaffected by  $\text{HgCl}_2$  and were less affected by meHg than TK shrimp. PC shrimp were also more tolerant to Cd, and pre-treatment of TK shrimp enhanced their tolerance to higher concentrations of Cd, showing acclimation (Khan et al. 1988).

A metal-tolerant population of the copepod *Tisbe holothuridae* collected from a polluted site was maintained for over 40 generations in the laboratory, after which time they remained more tolerant to  $\text{Co}^{2+}$  and  $\text{Cr}^{6+}$  than those from a clean area (Miliou et al. 2000). This suggests that under laboratory conditions there was little cost in maintaining tolerance, or that the tolerant phenotype had been genetically fixed.

Mangrove crabs *Ucides cordatus* and *Callinectes danae* were sampled from metal-polluted and clean mangroves in Brazil. Crabs from the polluted site showed greater ability to regulate blood osmotic concentrations at low salinity, but *U. cordatus* had reduced hypo-regulatory ability in seawater (Harris and Santos 2000). Differences in ion regulation were also seen. Differences in osmoregulation were considered possible adaptive changes after long-term exposure to contamination.

### 11.1.3 Mollusks

Bryan and Gibbs (1983) studied the bivalve, *Scrobicularia plana* from RC (see above), and found the clams to be resistant to Cu. Populations of the gastropod *Cerithidium rupestre* from Hg-polluted sites were much more tolerant to Hg than those from clean sites, presumably due to selection (Baker et al. 1985). Luoma et al. (1983) found tolerance to soluble Cu in the bivalve, *Macoma balthica*, varied substantially among populations within San Francisco Bay (US). Tolerance differed ten-fold or more over relatively small distances, suggesting geographical isolation of populations is not required for the development of differences in tolerance. Among five species of gastropods exposed to Cd in the laboratory, there was an association of allozyme genotypes with resistance; PGI genotypes of the dead animals had a higher proportion of heterozygotes than genotypes of the animals that survived the Cd treatment (Lavie and Nevo 1986).

Since larvae are generally more susceptible to contaminants than adults, it is of interest to see if they exhibit tolerance as well. Larvae were obtained from oysters (*C. gigas*) from a clean area (Arcachon Bay) and a polluted one (Bidassoa estuary) and exposed to Cu in the laboratory; their MT concentration was measured as well as biomarkers of oxidative stress. Biomarker responses and sensitivity to Cu for larvae from Arcachon Bay oysters were higher than for those from Bidassoa (Damiens et al. 2006).

Hoare et al. (1995) investigated effects of Cu on embryo development in different populations of *Mytilus edulis*. Concentrations of Cu that increased the rates of abnormality in populations from an unpolluted site (Menai Straits, Wales, UK) and to a lesser extent in an intermediately polluted site (Oosterschelde, The Netherlands) did not affect development of embryos from a polluted-site (Westerschelde, The Netherlands). Crosses between populations indicated that tolerance was mostly maternally determined, but there was also evidence of some paternal effect. An appreciable quantity of background embryo abnormality appeared to be sperm-mediated.

Metal exposure may induce specific metal-binding ligands. Metallothionein-like proteins appear to play an important role. Unger and Roesijadi (1996) investigated the effect of sublethal Cd on metallothionein (MT) mRNA accumulation in *Crasostrea virginica* preexposed to Cd. Initial treatments of control, 0.0044, 0.044, and 0.44  $\mu\text{M}$  Cd for 21 days were followed by challenge with 0.44  $\mu\text{M}$  Cd. MT mRNA accumulation during preexposure was concentration- and time-dependent. During the challenge, MT mRNA increased in all oysters and the concentration-dependence of MT mRNA in relation to preexposure concentration was maintained. MT mRNA during the challenge was significantly increased in oysters pre-exposed to the two highest Cd concentrations in comparison with the controls. These data demonstrate greater MT synthesis in individuals pre-exposed to low Cd concentrations and implicate new MT synthesis in acquired tolerance to Cd.

### 11.1.4 Other Taxa

Annelids, which tend to live in (and often eat) sediments, which are sinks for contaminants, can develop resistance. One of the earliest studies of tolerance was that of Bryan and Hummerstone (1971), who found that *Nereis diversicolor* in Restronguet Creek (RC, UK), had enhanced tolerance to Cu. Bryan and Hummerstone (1973) investigated potential mechanisms and found that tolerance to Zn was related to reduced uptake or greater excretion, so RC worms did not accumulate as much in their tissues. Uptake was studied further by Rainbow et al. (2009) who found that RC worms could balance increased uptake with detoxification, and had greater rates of storage detoxification of Zn in the form of crystals in the gut wall. Mouneyrac et al. (2003) tested tolerance of this species from metal-contaminated vs clean areas with a range of metals, and found increased tolerance to Cd, Cu, and Zn but reduced tolerance to Ag. Since tolerance to Cu and Zn in *N. diversicolor* was found to be inherited, it is possible to use the occurrence of tolerant individuals to map the ecological impact of contamination. In RC, the effect of Zn was restricted to an area within 1 km of the head of the estuary, and the impact of Cu was also greatest near the head of the estuary and became negligible by the estuary mouth. Sediment levels of  $>1,000 \mu\text{g}^{-1}$  Cu and  $3,500 \mu\text{g}^{-1}$  Zn are not toxic to this tolerant population (Grant et al. 1989). To evaluate tolerance, Burlinson and Larwrence (2007) developed a behavioral bioassay for *Hediste (Nereis) diversicolor* subjected to Cu. Under metal stress worms showed attempts at burrowing, eversion of the proboscis and abnormal crawling. The bioassay consisted of exposing worms to increasing concentrations of Cu and recording the concentration at which the stress response was elicited. The behavioral end-points were shown to be a good predictor of time of death of Fal estuary worms under acutely toxic conditions. The bioassay would therefore allow the separation of tolerant phenotypes without mortality to the worm. Worms were not affected by consecutive bioassays and it was proposed that tolerance to more than one metal could be determined for individual worms.

However, some negative results have been seen also (Zhou et al. 2003). *N. diversicolor* from other differentially polluted sites in the UK had no difference in Zn or Cd tolerance. There were slight differences in Cu tolerance, but the most tolerant population was not the one from the most contaminated site.

It is also possible to increase tolerance in the laboratory by pre-exposing individual non-tolerant worms, *Neanthes arenaceodentata*, to Cu. Pre-exposed worms had lower uptake of Cu (Pesch and Hoffman 1982), an example of physiological acclimation. *Neanthes virens* was used to study effects of Ag on ion and water balance. Worms from a clean site showed an increase in  $\text{K}^+$  and decrease in  $\text{Ca}^{2+}$  in coelomic fluid after accumulating  $88 \text{ mg l}^{-1}$  Ag, but worms from a Ag-contaminated site had effects only on  $\text{Ca}^{2+}$  (Pereira and Kanungo 1981). Exposed worms showed edema and a curled posture, effects which were more severe in the clean population, indicating that the exposed population had some Ag tolerance.

As tolerance comes with energetic and other costs, one would predict that when the contaminant stress decreases, the degree of tolerance might eventually decrease as well. A population of the oligochaete, *Limnodrilus hoffmeisteri*, in a highly Cd-polluted site in the Hudson River, Foundry Cove, (FC, where there had been a battery factory), was found to be tolerant to Cd. The elevated resistance in FC worms was genetically determined, as it was still present after two generations in clean sediment. Resistance had evolved rapidly (within 30 years). A laboratory selection experiment and estimates of the heritability of this resistance in *L. hoffmeisteri* from the control site, indicated that the resistance could have evolved in 1–4 generations. The laboratory selection resulted in a large increase in resistance after two generations of selection (Klerks and Levinton 1989). The resistant worms had significantly higher levels of a Cd-binding, MT-like protein than control worms. The elevated protein level was shown to be genetically determined and was considered partly responsible for the resistance. In addition, high levels of Cd were found in sulfur-rich granules, possibly in the form of cadmium sulfide (Klerks and Bartholomew 1991). As a result of U.S. Superfund legislation, the site was remediated and most of the Cd was removed, thereby removing the selection pressure for resistance. Following the cleanup, there was a rapid loss of resistance in ~9–18 generations, showing that the resistant worms were at a selective disadvantage relative to non-resistant ones after the pollution was no longer present, possibly reflecting a cost of resistance (Levinton et al. 2003). In contrast, *Nereis diversicolor* from Oued Souss, a highly contaminated site in Morocco, which had acquired tolerance to Cu and Zn due to a long-term exposure, maintained this tolerance after cessation of wastewater discharges in this site (Ait-Alla et al. 2006). This is different from what was observed by Levinton et al., (above). Higher catalase, GSTs and TBARs were observed in worms from Oued Souss than in those from reference sites.

At the community level, nematode communities from RC had increased Cu resistance due to a combination of three factors: (1) evolution of tolerance in some species, (2) an increase in abundance of tolerant species, and (3) the disappearance of more sensitive species. This combination of factors is called Pollution Induced Community Tolerance (PICT) (Millward and Grant 1995).

Non-indigenous species introduced via hull fouling may be able to adapt readily to metal-polluted environments, since they had previously adhered to a metallic boat hull that was probably coated with Cu-based antifouling paints. McKenzie et al. (2011) investigated Cu tolerance in the non-indigenous bryozoan *Watersipora subtorquata* from four populations from sites near Sydney, Australia. Colonies were collected, raised in the laboratory, and their offspring exposed to a range of Cu concentrations. Settlement and metamorphosis were measured, as were larval sizes for each colony. While there was no difference in tolerance among sites, there was a significant genotype  $\times$  environment interaction, with a large variation in the response of colony offspring within sites. The variation in Cu tolerance suggests that there is potential within populations to adapt to elevated Cu, as tolerance is heritable. Larval size also differed significantly and was positively correlated with tolerance.

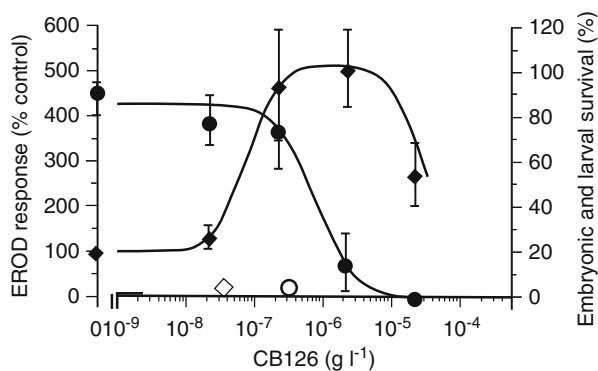


## 11.2 Organics

Tolerance to organic contaminants has also been noted frequently. Possible mechanisms of resistance include reduced uptake, increased sequestration, enhanced metabolism and excretion, or reduced sensitivity at the target sites. Mechanisms of transformation and elimination can be employed. CYPs are ubiquitous proteins that are used in normal metabolic transformations, and can be utilized to detoxify contaminants. Stress proteins are also highly conserved, and can protect organisms against both heat and chemical stressors. In many of the tolerance studies, CYP1A is involved. The mechanism by which TCDD, PCBs, and related chemicals cause induction of these enzymes is through the aryl hydrocarbon receptor (AHR), which is a transcription factor involved in regulation of genes for xenobiotic-metabolizing enzymes, including CYPs. The AHR is a receptor for chlorinated dioxins and other halogenated aromatic hydrocarbons. There is considerable evidence for its role in fishes, and some evidence that it plays a role in invertebrates as well. One might think AHR and CYPs would be enhanced in tolerant populations. However, in many of the populations that exhibit enhanced tolerance, the normal induction of CYP1A in response to exposure does not occur and/or the AHR has greatly reduced sensitivity to binding the chemicals – thus, if the receptors are impaired, the organism doesn't "see" the contaminants and toxic effects are not produced.

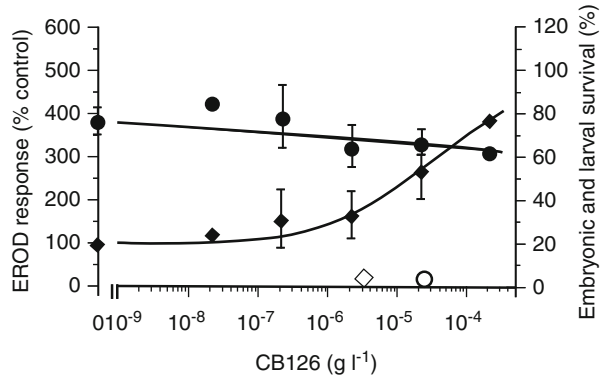
### 11.2.1 Fishes

Many studies have been performed by Nacci and colleagues on the evolution of tolerance in killifish (*F. heteroclitus*) to PCBs in New Bedford Harbor (NB), a highly contaminated site in Massachusetts, U.S. Nacci et al. (1999) compared embryos and larvae from reference sites and NB for sensitivity to dioxin-like compounds (DLCs); fish from NB were much more tolerant (Figs. 11.3 and 11.4).



**Fig. 11.3** Survival (*circles*), and EROD (*diamonds*) responses ( $\pm$ SD) to PCB 126 of West Island (reference population) killifish embryos (Reprinted from Nacci et al. 1999: 13, courtesy Springer Publishing Co)

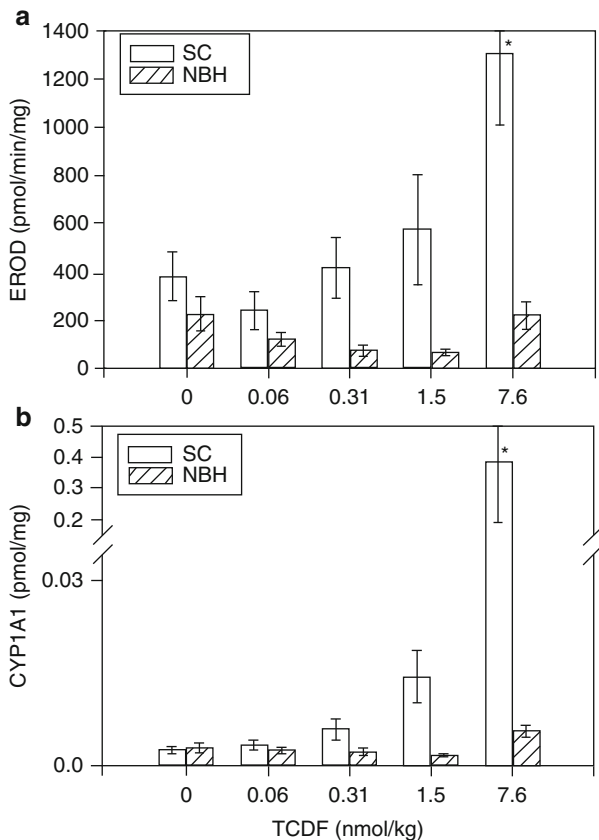
**Fig. 11.4** Survival (circles), and EROD (diamonds) responses ( $\pm$ SD) to PCB 126 of New Bedford Harbor killifish embryos (Reprinted from Nacci et al. 1999: 13, courtesy Springer Publishing Co)



Concentrations of DLCs similar to those measured in NB-collected fish eggs were lethal to reference embryos. Responsiveness was inherited and independent of maternal contaminant contributions. CYP1A was found to have very low inducibility in NB fish (Fig. 11.4). Thus, PCB contamination has selected for fish that are resistant. This adaptation may be a critical way that populations persist in this contaminated site. The NB population is also resistant to dioxin, which could not induce normal levels of CYP1A, showing that the Ah receptor signal pathway was altered in these fish (Bello et al. 2001) (Fig. 11.5).

Examining the possibility that the resistant NB population had reduced genetic diversity, McMillan et al. (2006) used AFLP markers, and found that genetic diversity did not differ among populations from contaminated vs reference estuaries. A possible reason that diversity may have been preserved could be because of large effective population sizes or because the mechanisms for adaptation to the contaminants affected only a small number of loci. Examining the issue of trade-offs, Nacci et al. (2009) investigated whether the NB population might be more susceptible to bacterial infection. They performed bacterial challenges of the marine pathogen *Vibrio harveyi* and found comparable survival by NB and reference fish, and improved survival by NB males. These results were inconsistent with hypothesized trade-offs of adaptation, and suggest that evolved tolerance in NB fish may include mechanisms that minimize the immunosuppressive effects of PCBs. Nacci et al. (2010) investigated many mummichog populations and found that they varied over four orders of magnitude in sensitivity to PCB126 and that this variation reflected the degree of contamination at the population's home site and was heritable between the F1 and F2 generations. The four most tolerant populations were from NB, Bridgeport, CT, Newark, NJ, and Norfolk, VA (all US), all highly contaminated sites (Fig. 11.6). The investigators found some similarities among the four highly tolerant populations, but they did not respond identically, and in at least one population, tolerance appeared to decrease in subsequent generations raised in the laboratory.

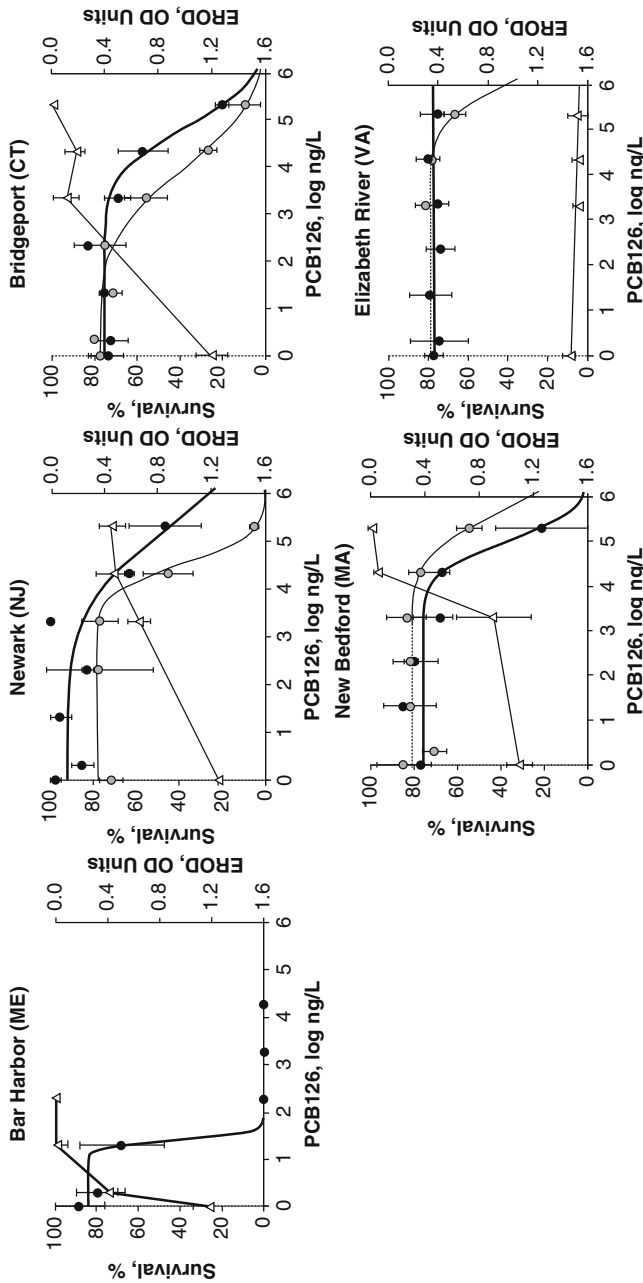
Roark et al. (2005) studied genetic adaptation in populations of the silversides, *Menidia menidia*, a migratory species residing seasonally in reference sites or



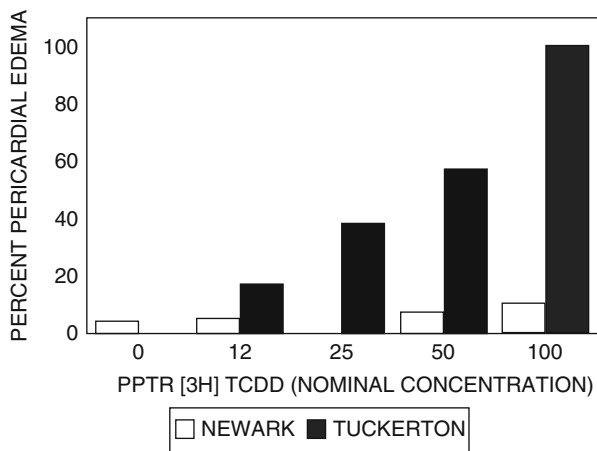
**Fig. 11.5** Induction of hepatic CYP1A1 (EROD) activity (a) and protein (b) in SC (open bars, reference population) and NBH (resistant population, hatched bars) fish injected with corn oil or doses of TCDF. In (a) EROD activities were significantly different between the two populations. \* = Significantly different from control from the same site (Reprinted from Bello et al. 2001: 81, courtesy Oxford University Press)

in NB. Offspring of *M. menidia* from NB were significantly less sensitive to embryonic exposure to the dioxin-like PCB 3,3',4,4',5-pentachlorobiphenyl (PCB 126) than offspring of reference fish. Analysis of ten polymorphic enzymatic loci indicated that juveniles from NB and an adjacent site had significant deviations from Hardy–Weinberg equilibrium at the phosphoglucomutase (*PGM\**) locus. Survivors of embryonic laboratory exposure to PCB 126 indicated that *PGM\** genotypes were associated with survival. Although a relationship was seen between tolerance and *PGM\** genotype, mixing of populations during migration and the absence of multigeneration exposure at contaminated sites may limit adaptation in this species.

Prince and Cooper studied the *F. heteroclitus* population in Newark Bay NJ, highly contaminated with PCBs, metals, and dioxins. CYP1A was not inducible



**Fig. 11.6** Responses following lab exposure to PCB 126 for *F. heteroclitus* populations (mean + SE). (Lines = model predictions) for early life stage survival of progeny of field-collected parents (black circles and lines) or laboratory reared F2 progeny (gray circles and lines). Bar Harbor is the reference population, all others are contaminated. EROD activity, measured by optical density. (Reprinted from Nacci et al. 2010: 858, courtesy Springer Publishing Co)



**Fig. 11.7** Percentage of pericardial edema in Newark and Tuckerton *F. heteroclitus* embryos exposed to 0.05 % acetone (vehicle), 12, 25, 50, and 100 ppt [3H]TCDD (nominal), from fertilization to d 27 post-fertilization (Reprinted from Prince and Cooper 1995a, courtesy John Wiley & Sons)

in this population, which was highly tolerant to TCDD exposure. The Newark embryos, when exposed to 12–100 parts per trillion ( $\text{ng l}^{-1}$ ) TCDD did not exhibit pericardial edema or death, seen in the reference population (Fig. 11.7). Resistance could not be attributed to reduced absorption of TCDD. Newark adults were also tolerant to TCDD (unlike the meHg tolerance of nearby PC fish discussed earlier, which was seen only in early life stages). When dermally treated, NB adults did not die or exhibit lesions seen in the reference population. Adults had elevated CYP450 activity, but induction of EROD (ethoxyresorufin-O-deethylase, a catalytic measurement of cytochrome P4501A induction) by TCDD exposure was much lower than in the reference population, suggesting an alteration in the AH receptor, similar to the NB fish.

Adults and laboratory-reared offspring of resistant NB fish showed decreased inducibility of CYP1A mRNA, CYP1A protein, and EROD enzyme activity, compared to fish from cleaner sites (Prince and Cooper 1995b; Elskus et al. 1999; Nacci et al. 1999). The laboratory-reared offspring had increased tolerance to PCBs as well as TCDD. Teratological effects of coplanar PCBs and TCDD in sensitive populations included pericardial edema, craniofacial malformations, yolk sac edema, and spine curvature, which probably were a result of altered vascular modeling, decreased blood flow and altered heart size and function (Antkiewicz et al. 2005), which were not seen in the tolerant populations. Blocking the expression of AHR2 protects against the cardiotoxicity of TCDD (Antkiewicz et al. 2006).

*F. heteroclitus* from a creosote-contaminated site (wood treatment facility) in the Elizabeth River, VA, US (ER) which has extremely high levels of PAHs, have liver neoplasms in moderate to high frequencies (see Chap. 8) (Vogelbein et al.

1990), but are more tolerant to PAHs than reference fish. Similar to the other tolerant populations, CYP1A was not inducible when exposed to PAHs or PCB126 (van Veld and Westbrook 1995; Meyer and DiGiulio 2002; Meyer et al. 2002).

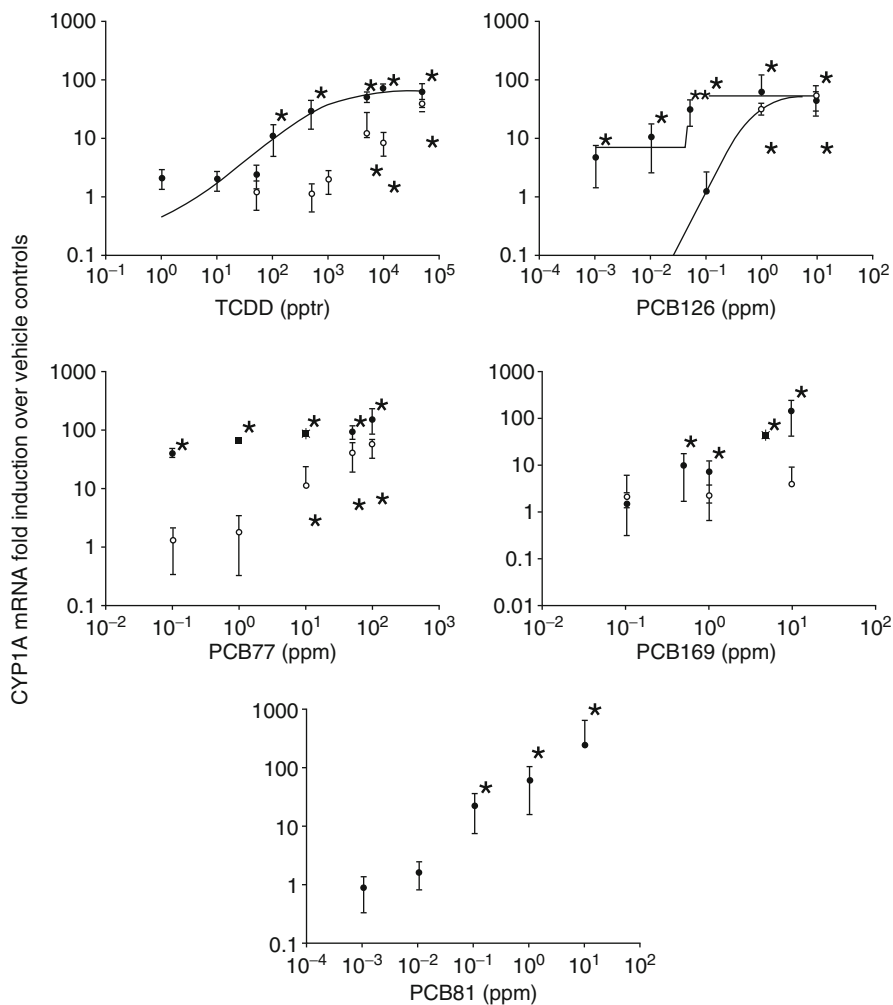
The tolerant ER fish have elevated glutathione S-transferases, enzymes involved in biotransformation of xenobiotics, which could be involved in resistance (Armknrecht et al. 1998). However, when reared in clean water or exposed to other contaminants, the F1 from ER had reduced fitness, indicating evolutionary costs of tolerance (Meyer and DiGiulio 2003). However, ER fish were tolerant to chlorpyrifos, permethrin, and carbaryl, suggesting that the adaptive phenotype was multi-faceted and that aspects other than CYP are likely to affect responses to contaminants (Clark and diGiulio 2012).

Wirgin and colleagues have studied Atlantic tomcod (*Microgadus tomcod*) from the Hudson River (HR), which is contaminated with PCBs and dioxins. HR tomcod have liver cancers (see Chap. 8), but have undergone evolutionary changes and become resistant to PCBs. They accumulate high levels of PCBs, PCDDs and PCDFs, but not PAH. The toxicity of these compounds and induction of CYP1A is mediated through the aryl hydrocarbon receptor (AHR), which has two forms, of which AHR2 is more active in fishes. HR fish have higher levels of CYP1A mRNA and bile metabolites of PAH than fish from four cleaner estuaries (Wirgin et al. 1994), but in the laboratory they had very reduced inducibility of CYP1A mRNA after treatment with PCBs and TCDD (Fig. 11.8) (Yuan et al. 2006a, b).

The reduced inducibility of CYP 1A mRNA and increased resistance to PCBs and TCDD in HR tomcod persisted in the F1 and F2 generations reared in the laboratory (Wirgin and Chambers 2006; Wirgin et al. 1992). Wirgin et al. (2011) investigated the mechanistic basis for the resistance and found that HR fish had variants in AHR not found elsewhere – there were deletions in the AHR2 gene. The fish are missing six base pairs of DNA of the AHR2 gene, and the two amino acids each triplet would code for. The mutated gene reduces the binding of molecules like TCDD to the receptor by fivefold, which lessens the toxicity. These changes were considered an evolutionary response to contaminant exposure. Authors felt AHR2 is a sensitive target for selection because of its regulatory role in the metabolism of contaminants. Evolutionary change probably resulted from selective pressure against sensitive phenotypes at susceptible early life stages.

Pollution resistance in populations of North American fishes, focusing on organic contaminants and genetic mechanisms, was reviewed by Wirgin and Waldman (2004).

Peña-Llopis et al. (2001) investigated the genetic basis of herbicide tolerance in eels in a laboratory study. *Anguilla anguilla* were exposed to 41.8 mg l<sup>-1</sup> of the herbicide molinate in a time to death (TTD) test. Glutathione content (GSx, GSH, GSSG), glutathione reductase (GR) and  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GT) activities were determined in the liver and muscle of dead and surviving animals and compared with non-exposed eels. TTD was positively correlated to hepatic GSH, GSH:GSSG ratio, hepatic and muscular GR, but negatively correlated to muscular GSH. Eels that were able to induce GR activity, increase GSH and maintain the GSH:GSSG ratio in the liver showed greater survival than those that lost glutathione



**Fig. 11.8** Comparison of hepatic CYP1A mRNA expression in Miramichi River (*filled circles*, reference site) and Hudson River (*open circles*) tomcod injected with graded doses of TCDD or different PCBs. \* = Significant difference from vehicle control for that population (Reprinted from Yuan et al. 2006a: 313, courtesy of Elsevier Publishing Co)

homeostasis. It would be interesting to follow up and see if these responses are greater in eels collected from herbicide-contaminated field sites than in those from clean sites.

In contrast to the above studies, resistance was not found in darter gobies (*Gobionellus boleosoma*) from a coastal marsh with a long history of PAH contamination (Klerks et al. 1997). A 2-week pre-exposure at the polluted site resulted in a decreased rather than an increased resistance in a subsequent laboratory

exposure to polluted sediment; also fish from the contaminated sites did not show increased resistance to polluted sediment, confirming the lack of acclimation and of adaptation. No differences were detected in frequencies of allozyme genotypes between gobies from polluted vs a control site, and heterozygosity was similar in both populations. Authors suggested four explanations for the lack of resistance: (1) bioavailability of contaminants could have been low, although other evidence contradicts this. (2) The contaminated marsh has many different chemicals and development of resistance is less likely when more contaminants are involved. (This can also be ruled out since PC, NB, NBH, HR and other polluted sites discussed above also have multiple contaminants). (3) The hydrocarbon distribution at the contaminated marsh is very patchy, such that fish may avoid exposure to the highly-contaminated sediment. (4) Gene flow may be sufficiently high in this mobile species to prevent local adaptation. It would seem likely that explanation three or four (or some other) is correct. Another goby species, (*Gillichthys mirabilis*) from three differentially polluted southern California estuaries was studied by Forrester et al. (2003). Fish from each estuary were transplanted to cages in each estuary in reciprocal transplant experiments. The growth rates of caged fish, and the size-distribution of natural populations, showed the same pattern of difference among estuaries. The total pollutant burden at the site was related to the growth of caged fish. Fish in the field caging experiments, and other fish held in the laboratory under constant conditions, showed no difference in growth due to their estuary of origin. These results thus also suggest a lack of genetic adaptation or physiological acclimation. It is interesting that of a limited number of reports of lack of tolerance, gobies seem to be a group that does not exhibit tolerance. It is likely that many other taxa do not develop tolerance but publications reporting such are relatively rare.

### 11.2.2 Crustaceans

While there is considerable literature on development of tolerance to pesticides by freshwater crustaceans, reports of tolerance in marine species are limited. Grass shrimp (*P. pugio*) in the laboratory could be acclimated to specific metals and PAHs, but not to polluted sediments that contained a variety of contaminants. Similarly, shrimp from Pass Fourchon in Louisiana, a site with elevated PAHs and metals did not exhibit PAH tolerance (Klerks 1999). The explanation offered for the lack of tolerance in the shrimp was that acclimation is less likely when there are numerous contaminants; one contaminant may inhibit the detoxification of others, and energetic requirements of exposure to one contaminant may be offset by decreases in resistance to other types of contaminants. However, sites like PC, NB, HR and NB, discussed above, also have numerous contaminants, yet have tolerant animals (including grass shrimp at PC).

Harper-Arabie et al. (2004) investigated whether the allozymes of the enzymes glucose phosphate isomerase (GPI), phosphoglucomutase (GPM), or mannose phosphate isomerase (MPI) were related to survival of *P. pugio* during acute



endosulfan, fluoranthene, and chromium(VI) exposures. *Palaemonetes pugio* were exposed in the laboratory to  $6.3 \mu\text{g l}^{-1}$  endosulfan,  $100 \text{ mg l}^{-1}$  chromium(VI), or  $0.6 \text{ mg l}^{-1}$  fluoranthene. Dead shrimp were removed at approximately 15–30 m intervals and the individual's genotypes for the *Gpi*, *Mpi*, and *Pgm* enzymes were determined. Results indicated individuals that were heterozygous for the *Gpi* allozyme survived longer than the homozygous MM genotype when exposed to chromium(VI) or fluoranthene. No allozyme genotypes were related to tolerance to endosulfan. The results support the hypothesis that there is a genetic basis for tolerance in *P. pugio* during acute exposures to chromium(VI) and fluoranthene.

Carman et al. (2000) compared responses of meiofaunal communities from contaminated vs reference sediments to diesel oil. Responses at the two sites were generally similar, but several variables, including abundance of total nauplii, ostracods, and copepods were affected to a greater degree in the reference community. *Pseudostenhelia wellsi* (a benthic copepod) nauplii from the reference site showed greater adverse effects of diesel oil, suggesting increased tolerance of copepods from the contaminated site. However, no differences in tolerance were noted in meiobenthic copepods (*Microarthridion littorale*) populations exposed to a highly contaminated sediment mixture vs unexposed animals (Kovatch et al. 2000), despite the fact that significant genetic differences were found. The absence of enhanced tolerance may be because detoxification mechanisms are adequate to cope with elevated exposures, or that mixing between contaminated and reference populations obscures any observation of tolerance, or that the fitness costs counteract the selective advantage of tolerance.

### 11.2.3 Mollusks

Reduced uptake appears to be a common method by which mollusks acquire tolerance to organic pollutants. Mussels from the vicinity of the *Prestige* oil spill on the coast of Spain were investigated 4–8 years after the spill. The results showed that PAH pollution was still present, but bioaccumulation in *M. galloprovincialis* was low, compared to reference mussels. This suggests that the mussels in the area of the spill had been able to reduce their uptake of PAHs, as a method of tolerance (Fernández-Tajes et al. 2011). Reduced accumulation of PCBs by mollusks in contaminated sites has also been noted. Accumulation factors (AFs) for PCBs (the lipid normalized PCB concentration in organisms divided by the organic carbon normalized PCB concentration in sediments) were measured for PCBs in infaunal mollusks at field sites with a range of sediment Aroclor (A-1254) and total organic carbon (TOC) concentrations. The average AFs for A-1254 were higher at sites with lower contaminant concentrations ( $15.0\text{--}48.3 \text{ ng g}^{-1}$  dry sediment) than at more contaminated sites ( $328\text{--}9,200 \text{ ng g}^{-1}$ ) (Lake et al. 1990).

Metabolic mechanisms of tolerance are also seen in mollusks. Yawetz et al. (2010) investigated defense mechanisms in mussels, *Donax trunculus*, from differentially polluted sites. Mussels from an oil-polluted site showed increased

activity of the system of active transport of organic anions (SATOAs) and the multidrug resistance transporter (MDR) in the gills. In contrast, those collected near a PVC factory showed a decrease in SATOA activity and no increase in the activity of MDR in the gills. Those from the reference site demonstrated equilibrium between energy production and utilization, while in *Donax* from both the oil polluted and the PVC-polluted sites, the mitochondrial redox state reflected intensive consumption of energy. No significant changes were found in the activity of reduced glutathione s-transferase (GST) in the cytosolic fraction of the digestive gland of *Donax* from any of the three sites. These data demonstrate an increase in the anti-chemical defense systems and an intensification of energy metabolism in the mussels at polluted sites.

#### 11.2.4 Other Taxa

Polychaete worms (*Neanthes arenaceodentata*) chronically exposed to petroleum hydrocarbons had developed enhanced tolerance (Rossi and Anderson 1978). However, tolerance could not be induced in the laboratory. In successive generations raised in WSF of fuel oil, F3 adults were no more tolerant than F1 animals. It appears that resistance was due to a selection process that selected over many generations for animals with genetic characteristics conferring resistance.

Physiological tolerance can arise from developing a way to metabolize the contaminant and excrete it. The opportunistic polychaete, *Capitella* sp. I accumulated fluoranthene from sediment in a concentration-dependent manner, but body burdens began to decrease after 2 days and were undetectable by 7 days, despite continued exposure to fluoranthene. Worms pre-exposed to PAH-contaminated sediment for 1 week excreted much more ingested fluoranthene than control worms and retained significantly less in their tissues than control worms (Forbes et al. 1996), indicating they had increased their metabolism and excretion of the hydrocarbon.

### 11.3 Hypoxia

As summarized by Hochachka et al. (1993) organisms use various defense strategies when confronted with low oxygen: (1) switching toward other metabolic pathways including anaerobic metabolism, (2) suppressing energy metabolism in order to balance ATP production and utilization, (3) minimizing the damage caused by physiological changes such as may occur by loss of ion homeostasis, and (4) using metabolic or other protective adaptations that are needed for recovery after hypoxia.

Marine animals initially respond to hypoxia by attempting to maintain oxygen delivery (e.g. increasing respiration rate, number of red blood cells, or oxygen binding capacity of hemoglobin or hemocyanin), then later by conserving energy (metabolic depression, reduced activity, down-regulation of protein synthesis

and down-regulation/modification of certain regulatory enzymes). Upon exposure to prolonged hypoxia, animals must eventually resort to anaerobic respiration. Hypoxia reduces growth and feeding, which eventually affects fitness. Many marine organisms can detect and actively avoid hypoxia. Some benthic animals leave their burrows and move up to the sediment surface, making them more vulnerable to predation. Under chronic hypoxic conditions, there is a general tendency for benthic suspension feeders to be replaced by deposit feeders; demersal fish by pelagic fish; and macrobenthos by meiobenthos. Microflagellates and nanoplankton also tend to dominate in the phytoplankton community (Wu 2002).

### 11.3.1 Fishes

Some fish can engage in aquatic surface respiration (ASR), getting their oxygen from the air. Active swimmers can avoid hypoxic areas, but some are more likely to remain in hypoxic water than others due to differences in physiological tolerance and movement responses. Determining avoidance responses is important for identifying the species most susceptible to impacts of hypoxia. A trawl survey was used to examine avoidance responses of blue crabs (*C. sapidus*) and several fish: pinfish (*Lagodon rhomboides*), spot (*Leiostomus xanthurus*), Atlantic croaker (*Micropogonias undulatus*), bay anchovy (*Anchoa mitchilli*), and flounders (*Paralichthys dentatus* and *P. lethostigma*) to chronic hypoxia and episodic hypoxic upwelling events in the Neuse River Estuary, NC, USA (Bell and Eggleston 2005). Trawl collections quantified changes in distribution and abundance patterns during normoxia, chronic hypoxia, and hypoxic upwelling events. Pinfish, anchovies, blue crabs, and flounder abundance increased with increasing DO. The two taxa most closely associated with the bottom (blue crabs and flounder) showed the strongest avoidance response to hypoxia. All taxa showed a stronger avoidance response to chronic hypoxia than to episodic hypoxic upwelling, which was attributed to reduced ability to avoid the rapid intrusions of hypoxic water during episodic events, or to increased risks of predation in shallow habitats, which may force some individuals back into hypoxic water. Froeschke and Stunz (2012) used mesocosm choice experiments to examine how habitat selection in pinfish (*Lagodon rhomboides*) and Atlantic croaker (*M. undulatus*) was affected by substrate, DO, food availability, and predation risk. Both species strongly avoided low DO, but at moderate levels of DO, substrate and food influenced selection. Both species avoided predators even when the alternative habitat had low DO, suggesting that predation risk is more important than DO for habitat selection. Swimming speed and indicators of stress (blood cortisol and lactate) were measured in Atlantic cod to assess if a shift in physiological homeostasis preceded changes in behavior or vice versa (Herbert and Steffenson 2005). Swimming speed increased when DO was reduced rapidly, an avoidance response. However, swimming speed was reduced at moderate DO and continued to drop under progressively deep hypoxia. Elevations in plasma cortisol and blood lactate indicated physiological stress but only at

levels near the critical oxygen tension. Reduced activity is adaptive for survival in low DO. Mandic et al. (2009) noted that sculpins with higher hypoxia tolerance inhabited the O<sub>2</sub> variable intertidal zone, while species with lower hypoxia tolerance inhabited the more stable subtidal zone. Hypoxia tolerance is associated with enhanced O<sub>2</sub> extraction capacity, which has three principal components: routine O<sub>2</sub> consumption rate; mass-specific gill surface area; and hemoglobin O<sub>2</sub>-binding affinity. Differences in the concentration of ATP and GTP provide a system with plasticity for survival in a highly O<sub>2</sub> variable environment.

Inheritable tolerance and acclimation to sulfide (related to eutrophication and low DO in salt marshes) was found in California killifish *F. parvipinnis* exposed to dissolved sulfide (Bagarinao and Vetter 1993). The high sulfide tolerance, particularly of concentrations typical of salt marshes, was explained by mitochondrial sulfide oxidation. Sulfide tolerance and mitochondrial sulfide oxidation did not diminish in fish held in the laboratory in sulfide-free water for 1–2 months. Seasonal differences in capacity of gulf killifish, *F. grandis*, to tolerate hypoxia were studied by Love and Rees (2002), who measured the frequency of aquatic surface respiration (ASR) during gradual reduction in DO and survival time during severe hypoxia, and found both to be significantly affected by season. Fish collected in summer did not engage in ASR until the DO concentration dropped below that required to cause ASR during other seasons. Laboratory acclimation to low DO did not change the relationship of ASR and DO, suggesting that the seasonal effect on ASR was not due simply to previous exposure. Fish collected in the summer and winter had longer survival times in severe hypoxia than fish collected in the fall. Seasonal variation in ASR and survival suggest that tolerance may be subject to acclimatization. Increased tolerance in summer could increase survival in low DO, which prevails during the summer. In comparison to other marsh fishes, *F. heteroclitus* is highly tolerant of low oxygen (D'Avanzo and Kremer 1994; Smith and Able 2003), with little or no mortality occurring until DO drops below 1 mg l<sup>-1</sup>. *F. heteroclitus* does not avoid water of 1 mg l<sup>-1</sup> in laboratory choice experiments, whereas other marsh fish do (Wannamaker and Rice 2000). During chronic exposure to oxygen tensions near their  $P_{crit}$ , the oxygen-binding capacity of blood increases due to increased hematocrit and hemoglobin oxygen affinity (Greaney et al. 1980). The increased hematocrit is likely due to several factors, including splenic contraction and synthesis and possibly maturation of red blood cells stimulated by erythropoietin (Lai et al. 2006). As oxygen drops below  $P_{crit}$ , *F. heteroclitus* and *F. grandis* utilize anaerobic metabolism. Increased glycolysis is reflected by the accumulation of lactate in blood and tissues after both acute and chronic hypoxic exposure (Cochran and Burnett 1996).

ASR and avoidance are not viable options for sluggish bottom dwelling fishes such as flatfish. Maxime et al. (2000) examined turbot (*Scophthalmus maximus*) during progressive severe hypoxia followed by recovery. Fish initially increased their ventilation amplitude and frequency, which maintained standard O<sub>2</sub> consumption over a broad range of DO, until a critical level of 30 mmHg. The hyperventilation induced a moderate blood alkalosis, compensated by a lactic acidosis. Blood pH did not decrease below control values, which could be explained by the retention of most

of the lactate produced in muscle, and by a high capacity for  $H^+$  excretion. During the recovery period, a marked increase in  $O_2$  uptake (oxygen debt repayment) was related to lactate elimination. When energy contributions of aerobic and anaerobic processes were assessed in terms of ATP, the anaerobic contribution during hypoxia was  $>20\%$  of the total energy budget and compensated for the reduced aerobic metabolism. The high value of  $O_2$  tension in arterial blood in normoxia and during recovery from hypoxia showed high diffusing capacity of gills, also contributing to the high tolerance of turbot for low DO.

Nilsson and Östlund-Nilsson (2008) reviewed the literature on the relation of fish size to hypoxia tolerance and concluded that body size *per se* has little impact on the ability to take up oxygen during low DO conditions, primarily because the respiratory surface area matches the metabolic rate over a wide size range. In cases where size-related differences are seen in a species, these were considered likely to reflect adaptation to different life-styles or habitats. However, during severe hypoxia and anoxia where fish must rely on glycolysis for survival, large individuals have a clear advantage because small ones will run out of glycogen or reach lethal levels of anaerobic end-products (lactate and  $H^+$ ) sooner, due to their higher mass-specific metabolic rate. Those species that have evolved extreme adaptations to hypoxia, such as hemoglobin with very high oxygen affinity or alternative anaerobic end-products, reveal that evolutionary adaptation is more important in developing hypoxia tolerance than physiological acclimation.

### 11.3.2 Crustaceans

Active swimmers like shrimp can actively avoid hypoxic areas. When confronted with a gradient of dissolved oxygen, *Metapenaeus ensis* were able to avoid hypoxic areas and move to oxygenated water. Their ability to detect and avoid hypoxia may enhance their survival (Wu et al. 2002). Behavior and mortality of juvenile Norway lobster (*Nephrops norvegicus*) changed with lowered DO; energetically costly activities were reduced, and general activity declined (Eriksson and Baden 1997). In normoxia, juveniles walked and burrowed, but when exposed to hypoxia they became inactive with occasional outbursts of escape swimming. To increase oxygen availability they raised their bodies. However, oxygen saturations of 25 % were lethal within 24 h. Juveniles were more sensitive than adults. Copepods, *Acartia clausi* from the polluted Elefsis Bay (Greece) were much more tolerant of low DO conditions than ones from reference areas (Kerambrun et al. 1993). A common way of increasing tolerance is to synthesize more hemocyanin (Hc) or increase its oxygen affinity. Blue crabs (*Callinectes sapidus*) were held in hypoxic (50–55 mmHg) water for 7–25 days. Lactate, urate, and  $Ca^{+2}$  all raised the  $O_2$  affinity of blue crab hemocyanin (Hc); by 25 days, blood lactate and urate had risen slightly, and  $Ca^{+2}$  had increased dramatically. Hc concentration had also increased by 25 days. At both 7–25 days there was an increase in hemocyanin- $O_2$  affinity and a change in subunit composition (DeFur et al. 1990).

The extent to which exposure to hypoxia (40, 30, and 20 % sat) produce an increase in the concentration of Hc in Norway lobster *Nephrops norvegicus*, depends on the initial Hc concentration and the intensity of the hypoxia. While Hc did increase with decreasing  $pO_2$ , individuals with relatively high initial Hc levels did not increase Hc further. The greatest hypoxia-related increase in Hc was in individuals with the lowest initial Hc concentrations. The changes in Hc concentration took place over a short time scale (hours rather than days) (Spicer and Baden 2001).

### 11.3.3 Mollusks

Metabolic depression and arrest are common responses among mollusks to low DO. Starved *Crassostrea virginica* and *Thais haemastoma* were anoxia tolerant; their metabolic rates were depressed under anoxia to 75 and 9 % of the normoxic rate (Stickle et al. 1989). Ueda et al. (2009) investigated whether tolerance to low oxygen could be selected for in oysters and examined the expression of heat shock protein 70 (HSP 70) in control and anoxia-challenged juvenile oysters. *C. virginica* were collected from an area (CP) considered to have normoxic conditions and an area (WH) with periodic anoxia. F1 oysters were produced from CP and WH parents that survived anoxia for 96 h and from both parental stocks not exposed to anoxia. The F1 oysters were subsequently exposed to anoxia or normoxia, and expression of HSP 70 was examined. Three HSP 70 isoforms were expressed in both anoxia- and normoxia-exposed oysters from all groups. In general, there were not significant differences in the expression of these proteins between the control and anoxia-treated oysters, suggesting no selection for tolerance. They did not report on the actual anoxia tolerance of the F1 oysters, however.

Two South African mussels show marked differences in their tolerance to low DO. *Choromytilus meridionalis* occurs low on the shore and on rocks associated with sand, while *Perna perna* occurs higher on the shore on rocks that are not usually influenced by sand. *C. meridionalis* withstands prolonged exposure to hypoxia ( $<1.00$  ppm  $O_2$ :  $LT_{50} > 30$  days) longer than *P. perna* with  $LT_{50}$  value of  $\approx 6.5$  days for hypoxia. *C. meridionalis* activates anaerobic pathways more readily than *P. perna* when exposed to hypoxia, and shows a much stronger tendency to close its valves and to reduce its heart rate. With declining oxygen tension it regulates oxygen uptake down to a lower level (2 ppm  $O_2$ ) than *P. perna* (3 ppm  $O_2$ ) (Marshall and McQuaid 1993).

Kurochkin et al. (2009) studied metabolic responses to prolonged anoxia and subsequent recovery in anoxia-tolerant *C. virginica* and effects of Cd on these processes. Anoxia led to anaerobic respiration, as indicated by accumulation of L-alanine, acetate, and succinate. Prolonged anoxia caused a decline in the maximum activity of electron transport chain and oxygen uptake by mitochondria. During reoxygenation, there was a significant overshoot of mitochondrial oxygen uptake ( $<70$  % above normoxic values). Mild mitochondrial uncoupling in anoxic

tissues and a subsequent strong stimulation during recovery may help to restore redox status and protect against formation of damaging reactive oxygen species (ROS). Exposure to Cd inhibited anaerobic metabolism, abolished the stimulation of mitochondrial oxygen uptake, and led to oxidative stress and a loss of mitochondrial capacity during the recovery period.

#### 11.3.4 Other Taxa

Many polychaetes including the lugworm, *Arenicola marina*, live in eutrophic benthic habitats subject to frequent hypoxia. They are able to shift gradually from aerobic metabolism to anaerobic metabolism, accompanied by a drop in ATP demand and production. The activity of the enzyme glycogen phosphorylase plays a major role in switching to anaerobic metabolism (Kemp 1993). The intertidal polychaete *Scoloplos armiger* in anaerobic conditions produces energy in a similar manner, but is less tolerant. Energy is produced from phosphagen stores and from the conversion of glycogen to fatty acids, mainly propionate and acetate. *S. armiger* is able to maintain aerobic metabolism down to a  $P_{wO_2}$  of  $\sim 20$  Torr and even at a  $P_{wO_2}$  of 10 Torr retained some aerobic metabolism. However, *S. armiger* cannot reduce its energy demands to the same degree as *A. marina* and has a relatively small pool of glycogen, which may account for why it is only moderately resistant. Its recovery from anoxia is slower than in *A. marina*. In the field during low tide *S. armiger* goes up into the oxic layer, where it can maintain aerobic metabolism (Schöttler and Grieshaber 1988).

The number of forms of enzymes involved in glycolysis can affect the degree of hypoxia tolerance of a species. Nine polychaete species (*Paraprionospio pinnata*, *Nephtys ferruginea*, *Glycera americana*, *Haploscoloplos* sp., *Lumbrineris composita*, *Sigambra bassi*, *Aricidea pigmentata*, *Cossura chilensis*, and *Pectinaria chilensis*) were assayed for lactic dehydrogenase, octopine dehydrogenase, strombine dehydrogenase and alanopine dehydrogenase. Each species had a characteristic number of the pyruvate oxidoreductases, ranging from 4 in *Paraprionospio pinnata* to 1 in *Pectinaria chilensis*. The pyruvate saturation curves suggest that NADH is oxidized at different rates depending on the amino acid used in the reaction with pyruvate. The data indicate that organisms with more than one pyruvate oxidoreductase have greater metabolic capacity to cope with low DO because these enzymes would better regulate the pyruvate consumption rate during the transition period. Thus, the dominance of *Paraprionospio pinnata* in hypoxic areas, and its worldwide distribution is consistent with its high number of pyruvate oxidoreductases with different pyruvate consumption rates (González and Quiñones 2000).

Low oxygen is also found in deep sea hydrothermal vent habitats (though not due to pollution). The polychaete *Methanoaricia dendrobranchiata* lives at hydrocarbon seeps in a microhabitat that is always very hypoxic and sulfidic. A suite of adaptations to low DO were described by Hourdez et al. (2002). The worms



can regulate their oxygen consumption down to about 870 Pa oxygen, have a large gill surface area, a small diffusion distance from sea water to blood, a very high hemoglobin oxygen affinity and a Bohr effect that is pronounced at high oxygen saturations. They can withstand extended periods of anoxia even though when fully saturated, the hemoglobin binds sufficient oxygen for only 31 min of aerobic metabolism.

## 11.4 Climate Change/Ocean Acidification

A special issue of Proceedings of the Royal Society has been devoted to the idea of “evolutionary rescue” in changing environments (ER) as a way to maintain biodiversity in the face of climate change. In the introductory article, Gonzalez et al. (2013) explain that ER is the idea that evolution might occur sufficiently fast to arrest population decline and allow recovery before extinction results. ER provides a perspective on evolutionary dynamics that focuses on short time-scales, genetic variants of large effects, and absolute rather than relative fitness. Contributions in the issue cover conceptual developments and modeling, as well as theoretical and experimental results. Populations under severe stress may be rescued by natural selection, but its operation has ecological and genetic constraints. Whether evolution will be rapid enough to rescue declining populations will depend upon the population size, the degree of genetic variation, the degree of maladaptation and the evolutionary history. A factor that may limit their ability to adapt is the rate at which beneficial mutations can become established. Population persistence can also be influenced by phenotypic plasticity (acclimation), and by the evolution of plasticity itself. These articles are general and none focus on the oceans or pH.

Kelly and Hofmann (2012) reviewed current literature on the potential for adaptation to elevated  $p\text{CO}_2$  in marine organisms. Although the number of papers is currently quite small, they argued that data on physiological effects, natural variation in pH and lessons learned from previous work on adaptation can all inform predictions and priorities for future research. They argued that selection is one of the most important forces maintaining intraspecific genetic variation. Unlike temperature, pH lacks a strong global gradient, and so selection may maintain less adaptive variation for pH than for temperature. However, long-term data sets for natural pH variation are scarce, so pH gradients may be more common than previously observed. Two important effects of elevated  $p\text{CO}_2$  are reduced calcification and changes in metabolism. Detailed understanding of physiological mechanisms underlying these effects is important for predicting the ability to acclimatize or adapt. They recommended that future research should assess adaptation to local pH conditions and measure the capacity for adaptation to acidified conditions in natural populations.



### 11.4.1 Fishes

Juvenile anemonefish, *Amphiprion melanopus*, which show an increase in metabolic rate and decreases in length, weight, condition and survival in response to acidification, can adjust to the pH expected by 2100 (1,000  $\mu\text{atm CO}_2$  and a temperature rise of 1.5–3.0 °C) if their parents were also raised in more acidic water (Miller et al. 2012). Under those circumstances, juveniles were able to compensate for the change, although it is not known if the tolerance persists throughout their lives. How parent fish pass on the ability to deal with acidity to their offspring is not known. The time interval is too short for it to be genetic adaptation in the normal sense. Thus some fish species have greater capacity to cope than was previously thought. Anemonefish are particularly hardy, however, and may not be representative of all marine fish.

Munday et al. (2012) used a field-based experiment to test for differential survival associated with variation in  $\text{CO}_2$  tolerance in a wild population of coral-reef fishes. Juvenile damselfish, *Pomacentrus wardi* exhibited variation in their response to elevated (700  $\mu\text{atm}$ )  $p\text{CO}_2$  when tested in the laboratory and this influenced their behavior in the field. Individuals that were sensitive to elevated  $p\text{CO}_2$  (changing normal response to predator odor) were more active and moved farther from shelter in natural coral reef habitat and consequently had higher mortality from predation than fish that were more tolerant to elevated  $p\text{CO}_2$ . Authors concluded that if individual variation in  $\text{CO}_2$  tolerance is heritable, this selection of phenotypes tolerant to elevated  $p\text{CO}_2$  could potentially help mitigate the effects of ocean acidification.

Adaptation to climate change also involves temperature tolerance. Klerks and Blaha (2009) compared heat tolerance of fish collected from pairs of sites with different thermal regimes, using least killifish (*Heterandria formosa*) and eastern mosquitofish (*Gambusia holbrooki*). They measured heat tolerance as temperature-at-death and time-to-death when field-collected fish were exposed in the laboratory to increasing water temperatures. For the four pairs of populations that were compared, two differed in heat tolerance. Fish from one site with above-normal temperatures had reduced heat tolerance indicative of stress, rather than tolerance. Fish from another site with above-normal temperatures had elevated heat tolerance, which appeared to be due to acclimation rather than adaptation, since it was not maintained in their offspring.

For coastal wetland species, sea level rise results in salt water intrusion, which can be another stress. Populations of the eastern mosquitofish, *Gambusia affinis*, with exposure to saline environments develop adaptations for increased survival in high salinity (Purcell et al. 2008). Fish from brackish and intermediate marshes had increased salinity tolerance compared to fish from freshwater marshes. They tested the descendents of fish from the fresh and brackish marshes that were reared for two generations in fresh water, and found that descendents of fish from brackish

marshes had higher survival in saline water than descendents of fish from freshwater, implying genetic adaptation. Purcell et al. (2012) evaluated the genetic structure of *G. affinis* populations previously shown to have adaptations for increased salinity tolerance. They found that gene flow was higher between populations experiencing different salinity regimes within an estuary than between similar marsh types in different estuaries, suggesting the development of saline-tolerant phenotypes is due to local adaptation.

### 11.4.2 Crustaceans

Crustaceans appear to be very variable in vulnerability to ocean acidification due to differences in lifestyle and in the ability to compensate for environmental change. It is predicted that strong iono- and osmo-regulating species are likely to be the most tolerant to acidification, because they have the compensatory mechanisms to respond to acid–base disruptions. These species tend to inhabit shallow coastal environments with freshwater inputs, where they experience natural variations in seawater  $p\text{CO}_2$ ,  $p\text{O}_2$ , salinity and temperature. The ability to compensate for the effects of ocean acidification can also vary with lifestyle. Decapods with high rates of activity, for example, have a greater capacity for passive compensation of hemolymph acid–base disturbances (i.e. buffering by non-bicarbonate buffers) than slow-moving, relatively inactive species due to differences in respiratory variables (Whitely 2011).

### 11.4.3 Mollusks

Amerala et al. (2011) sampled oyster, gastropod and crab populations at sites close to and far from drains discharging acid sulfate soil (ASS) runoff, hypothesizing that reduced populations of smaller-sized individuals would be found at the more acidified sites. Oysters *Saccostrea glomerata* and gastropods (*Bembicium auratum*) were less abundant at ASS-affected than reference sites in New South Wales, Australia, but impacts were smaller than predicted. Although gastropod populations were dominated by smaller individuals, oyster populations were skewed towards larger individuals at affected sites, and abundances of both species were comparable to reference estuaries. Authors felt that behavior, physiological acclimation and/or genetic selection may be responsible, or that populations may recover between the rain events that bring in acidic runoff. Thus, at the population level, these calcifying organisms showed some resistance to acidic runoff.

Parker et al. (2012) exposed adult Sydney rock oysters, *Saccostrea glomerata* to ambient and elevated  $p\text{CO}_2$  during reproductive conditioning and measured development, growth and survival of their larvae. Elevated  $p\text{CO}_2$  caused a reduction

in growth, development rate and survival. However, exposing adults to elevated  $p\text{CO}_2$  during reproductive conditioning had positive effects on larvae. Larvae spawned from adults exposed to elevated  $p\text{CO}_2$  were larger, developed faster and had similar survival as larvae spawned from adults exposed to ambient  $p\text{CO}_2$ . This suggests that these oysters have the capacity to acclimate or adapt to elevated  $p\text{CO}_2$ .

#### 11.4.4 Other Taxa

##### Corals

Some corals can adapt to high temperatures and/or low pH. Populations that were able to survive a massive bleaching event in 2010 in Southeast Asia were ones that had previously experienced severe bleaching in 1998. In Indonesia, corals responded to higher temperatures in a typical way, with branching species such as staghorn corals suffering severe die-offs. But at sites in Singapore and Malaysia, normally susceptible *Acropora* corals appeared healthy and unbleached. The locations that had less severe bleaching in 2010 had bleached in 1998. In contrast, the site that had severe bleaching in 2010 had not bleached in 1998. It appears that the corals that had bleached in 1998 had subsequently adapted and/or acclimatized to thermal stress (Guest et al. 2012). Genetic changes in the corals may play a role in increasing tolerance to temperature. Some of the *Acropora hyacinthus* corals in the back reef of Ofu Island, American Samoa, thrive in pools with daily heat fluctuations of up to 6 °C. To find the molecular basis of this resilience, Barshis et al. (2013) compared gene activity in heat-resistant and heat-sensitive corals by measuring their ‘transcriptome’ – the RNA molecules transcribed from the genes – under different temperatures. They found a suite of genes that are present in both types of corals but are more highly expressed in the heat-resistant corals, which had 60 stress-related genes turned on even before the experiment began. These genes, which code for a number of antioxidants and heat shock proteins, are “frontloaded” in the resistant corals – already turned on and ready to work even before the stress began.

Corals may also adapt by hosting varieties of algal symbionts that are more temperature tolerant. Increasing numbers of coral species have been found to be able to host multiple algal symbionts. Being capable of hosting symbionts that survive in warmer temperatures suggests they have potential to adapt to warmer temperatures (Silverstein et al. 2012). However, some confounding evidence was found by Putnam et al. (2012) who collected 132 samples of coral from reefs around Moorea, analyzed DNA to determine the types of dinoflagellates present, and reviewed the scientific literature for information on how well each type of coral dealt with environmental changes. Surprisingly, the corals that hosted many diverse dinoflagellates were less resilient. For example, flexible corals such as *Acropora* and *Pocillopora* that hosted a variety of symbionts fared the worst after a mass bleaching in 1994. Corals that were more selective about their symbionts coped

more successfully. It would appear that the symbionts, though less diverse, were more temperature tolerant. Complicating the picture further, Hume et al. (2013) studied heat-tolerant corals in the Persian Gulf, and found that although both the coral host and the associated algae need to withstand the high temperatures, the algae belong to a group not known for its thermal tolerance. The algae found in most of the corals in Abu Dhabi reefs were previously described as a 'generalist strain' that is usually not found in corals exposed to high levels of heat stress.

Bleaching was studied in two species of octocorals, *Phenganax parrini* and *Sarcothelia* sp. both of which had similar responses (Parrin et al. 2012). While symbionts detach or die leaving the polyps bleached, large numbers of the symbionts accumulate in the stolons. The presence of symbionts in the stolons may be a mechanism to help the colonies recover from bleaching if the symbionts subsequently migrate back into the polyps.

Climate change includes both ocean acidification and temperature increase. Edmunds (2011) exposed *Porites* corals to different temperatures and pH for 1 month in the laboratory, and provided them with brine shrimp as food. Increasing the amount of food reduced the effects of acidification. Both calcification and biomass were sustained at high  $p\text{CO}_2$  by increased food, showing that this species can resist effects of 1 month in high  $p\text{CO}_2$  through heterotrophy. This mechanism may play a role in determining the extent to which corals can resist the long-term effects, and it depends on adequate amounts of suitable plankton food being available, which is also necessary to survive bleaching.

Organisms that form calcium carbonate skeletons produce it in one of two forms, aragonite and calcite. McCulloch et al. (2012) suggest that those with aragonite skeletons, such as *Porites* and *Acropora*, have molecular pumps that enable them to regulate their internal acid balance, which buffers them from the changes in seawater pH. This up-regulation of pH at the site of calcification provides them with enhanced resilience to the effects of acidification, while those that use the calcite pathway lack this resilience. The cold-water coral *Lophelia pertusa* can keep growing even in low pH if given time to adjust to the new conditions (Form and Riebesell 2012). Coral branches were kept at a range of  $p\text{CO}_2$  levels while temperature was constant and corals were fed well. In the short term, a pH decrease of only 0.1 unit caused a decline of growth by about one third, but after 6 months the corals seemed to adapt; those in high  $p\text{CO}_2$  grew even faster than controls. However, since acidification is only one aspect of climate change, more studies are needed to investigate interactions of carbon dioxide, temperature, and food availability. Further evidence for adaptation to long-term exposure to very low carbonate concentrations was seen by Thresher et al. (2011) who examined the distribution and skeletal characteristics of corals along a natural deep-sea concentration gradient on seamounts. Carbonate under-saturation had little effect on depth distribution, growth, or skeletal composition of live scleractinians or gorgonians, with corals growing, often abundantly, in waters as much as 20–30 % under-saturated.

## Coccolithophores/Algae

Coccolithophores, phytoplankton with calcite shells, have been shown to be vulnerable to acidification. However, when *Emeliana huxleyi* were cultured under increased CO<sub>2</sub> conditions, after 1 year (500 generations) they grew and calcified better than non-adapted control populations when tested under acidified conditions (Lohbeck et al. 2012).

Collins (2011) used an experimental evolution approach to investigate adaptation in changing environments, using fitness and the total number of reproductive events to quantify adaptation of phytoplankton communities to global change, where environmental variables change continuously. She examined the possible scenario that climate change will not acidify the ocean because microbes will absorb the extra carbon in photosynthesis, store it and release oxygen, a logical scenario because many microorganisms are photosynthesizers. To test this idea, algae were grown in high CO<sub>2</sub>, and 1,000 generations later some surprising responses were seen. Some of the algae in high CO<sub>2</sub> did not store it any more. Others photosynthesized much faster, but were no longer able to use the extra carbon for growth. Thus, the notion that phytoplankton will efficiently absorb all the extra CO<sub>2</sub> would appear to be unlikely.

## 11.5 Costs of Tolerance

### 11.5.1 Energy Costs

Processes involved in developing tolerance are energetically expensive and may have deleterious effects on energy allocation. To adjust to the metabolic costs of a chemical stressor, a shift in allocation of energy occurs at the expense of other energy-demanding processes such as growth. For example, mucus secretion in the gastropod *Patella vulgata* uses almost 25 % of the animal's energy (Davies et al. 1990). In dogwhelks, *Nucella lapillus*, exposed to Cd, reduced oxygen consumption and glycogen level could be linked to the production of mucus and metallothioneins (Leung et al. 2000). The production of enzymes to detoxify organic contaminants also has energy costs (Calow 1991). Many of the studies described in Chap. 3 report decreased metabolic rate, which reflects energy costs. Thus contaminant exposure over extended periods may reduce fitness, which increases selection pressure for more resistant individuals in populations that exhibit variation and heritability for their response. Populations subject to strong selection pressure for a particular stress may show a reduction in genetic variation, since the more susceptible genotypes would have disappeared and only the resistant remain. This may in turn increase their vulnerability to other types of stresses, which is a cost of tolerance. For example, in Cd-polluted environments, the upregulation of heat shock proteins is partially suppressed in the oyster *Crassostrea virginica*, resulting in reduced

thermotolerance (Ivanova et al. 2009). Thus, populations with tolerance to some pollutants may have lower tolerance to others and lower survival ability in the longer term. Grant et al. (1989) demonstrated that the genetically based tolerance of *Nereis* worms in Restronguet Creek to Cu was a disadvantage at sites with low levels of metals. The metabolic costs of the tolerance produced a much lower scope for growth than seen in non-resistant populations. The loss of Cd-tolerance in the Foundry Cove (Hudson River) *Limnodrilus* worms after clean-up of the Superfund site (Levinton et al. 2003) demonstrates that the tolerance had a cost and when the selection pressure for Cd-tolerance was removed, non-tolerant worms were at an advantage.

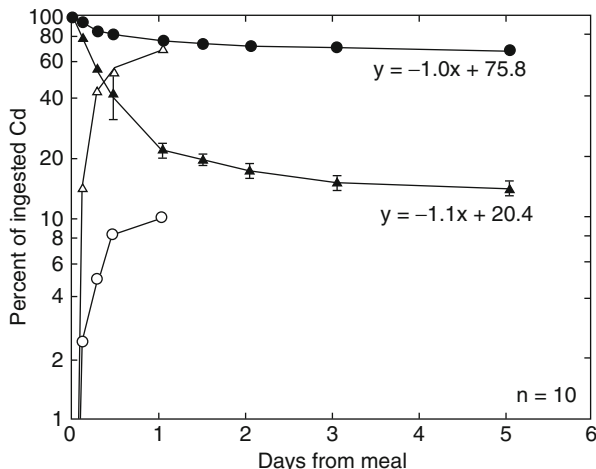
Meyer and DiGiulio (2003) reported that F1 and F2 offspring of PAH-resistant killifish from the creosote-contaminated Elizabeth River were less tolerant of low DO than the F1 and F2 from fish from reference sites. They also found that the F1 but not F2 offspring from ER had reduced growth and survival in clean conditions. The F1 offspring were more susceptible to phototoxicity and oxidative stress. Frederick et al. (2007) similarly found that adult ER fish had lower antibody responses and increased susceptibility to bacterial pathogens. However, since these tests were on fish taken from ER, rather than their offspring raised in the laboratory, it cannot be established if this is a “cost” of tolerance or a direct effect of PAHs and other contaminants on the immune system.

It is often difficult to separate out costs of tolerance from direct or indirect sublethal effects of the contaminants. For example, metal-tolerant *Nereis diversicolor* from the Seine River exhibited lower physiological and population status than those from a reference site (Mouneyrac et al. 2010). Reduced feeding (as described in many studies) is a common response to contaminants, and will also have negative effects on the energy budget. This could be responsible for the observations of Mouneyrac et al. (2010). Also, killifish from Piles Creek that are resistant to meHg in early life stages show reduced feeding, predator avoidance, growth, and longevity as adults (Weis and Weis 1989). These may be costs of tolerance, but are more likely to be direct effects of the contaminants. It is necessary to study laboratory-raised F2 and F3 generations to distinguish true costs of tolerance from direct effects of the contaminated environment.

### **11.5.2 Food web Costs**

#### **Metals**

Mechanisms that allow organisms to tolerate contaminants could be a risk to consumers if tolerant prey accumulate high concentrations of contaminants and transfer them to less well-adapted predators. In addition, for organisms that produce mucus in response to contaminants (e.g. mussels), the secreted mucus that has high contaminant levels (Sze and Lee 1995) may then be consumed by other species. Trophic transfer can have major, even lethal impacts. For example, shrimp that



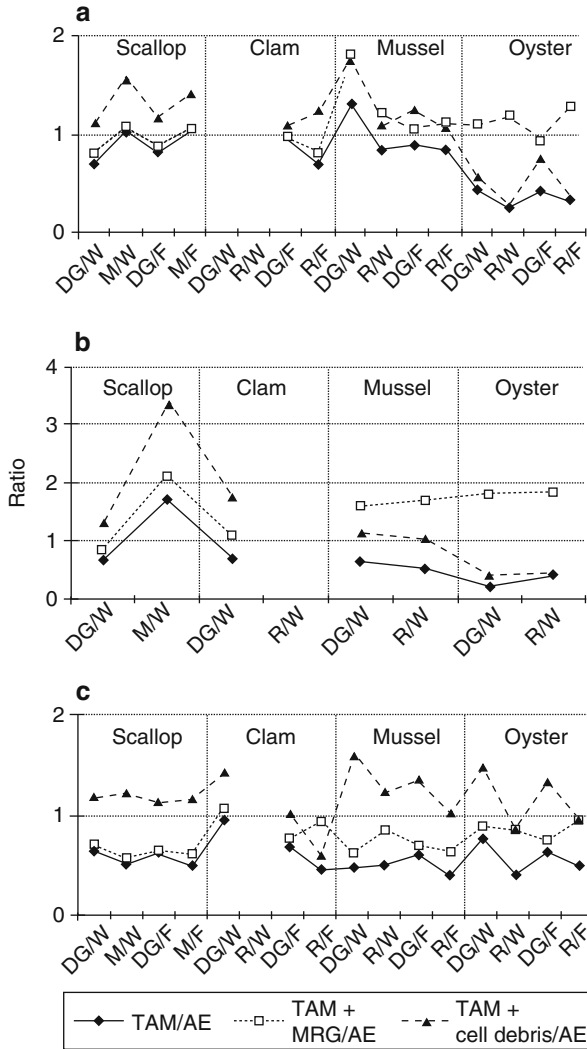
**Fig. 11.9** Percentage retention (filled symbols, mean + SE) and egestion (open symbols) of  $^{109}\text{Cd}$  by grass shrimp fed  $^{109}\text{Cd}$ -labelled South Cove (reference site, circles) or Foundry Cove (triangles) *Limnodrilus hoffmeisteri* (Reprinted from Wallace et al. 1998: 232, courtesy Inter-Research)

consumed metal-rich *N. diversicolor* from Restronguet Creek had elevated mortality (Rainbow et al. 2006), suggesting that the shrimp were not as tolerant as their polychaete prey. In many invertebrates, metals complexed with MT in the cytosol are transferred to predators, while metals stored in metal-rich granules (MRG) tend to be unavailable to predators (Fig. 11.9) (Wallace et al. 1998). However, some predators may be able to acquire metals from MRG in their food (Rainbow et al. 2007) (Fig. 11.10) so differences in trophic transfer of metals are also affected by the digestive processes of the predator, which will affect the predator's body burden.

## Organics

Despite the ability of many organisms to degrade organic compounds, many xenobiotics accumulate in biota to varying degrees, especially persistent organic pollutants (POPs), such as DDT, PCBs, PBDEs, and dioxins. These are metabolized poorly, and therefore are readily passed up food chains, biomagnifying in the process. Therefore predators (including humans) acquire higher concentrations than their prey. Fish consumption advisories remain in many places, due to unacceptably high levels of chemicals such as PCBs in fish or crab muscle tissue. Tolerant prey can accumulate greater concentrations to pass onto their predators. For example, crustaceans fed PCB-tolerant phytoplankton *Nannochloris oculata* accumulated more PCBs than those fed sensitive *Isochrysis galbana* (Wang et al. 1998).

In contrast, PAHs tend to be better metabolized and do not biomagnify because they are rapidly transformed at higher trophic levels (especially vertebrates), so



**Fig. 11.10** *Nassarius festivus*. Ratio of percentage distributions of accumulated radiolabelled (a) Cd (b) Ag, and (c) Zn in the combined subcellular fractions of trophically available metal (TAM), TAM + metal-rich granules (MRG), and TAM + cell debris in tissues (*DG* digestive gland, *M* adductor muscle, *R* remaining soft tissues) of four bivalves, after uptake from water (W) or food (F), to percentage assimilation efficiency (AE) (Reprinted from Rainbow et al. 2007: 133, courtesy Inter-Research)

their levels may be “trophically diluted” along the food web. But this is not always the case. The oligochaete worm *Monopylephorus rubroniveus* has a high tolerance of and a low ability to metabolize and eliminate PAHs. It bioaccumulates high concentrations of fluoranthene from sediments, which it passes on to consumers



such as grass shrimp (Filipowicz et al. 2007). Prey that do transform PAHs may produce carcinogenic metabolites, which are then passed on to consumers. English sole fed B[a]P-contaminated polychaetes showed slower growth, increased CYP1A, and hepatic DNA adducts (Rice et al. 2000). Bottom-dwelling fish in areas contaminated with PAHs have higher incidence of tumors (see Chap. 8), probably partly due to PAHs acquired from their prey.

## 11.6 Discussion and Conclusions

It is clear that pollutants can cause physiological changes that reduce their toxicity and exert selection pressure on populations in contaminated environments. Reduced accumulation and increased depuration are effective ways to become more resistant to a chemical. Other mechanisms include synthesis of MTs and other metabolic detoxification methods. In addition to physiological acclimation, many species are able to evolve to become more tolerant to the contaminants they are exposed to. Life evolved in the presence of metals, and MTs and other protective mechanisms are found in a diversity of species including microorganisms, invertebrates, plants, and vertebrates, enabling them to resist toxic effects. The most common defense against toxic organic chemicals are the cytochrome P450s (CYPs), highly diverse enzyme systems that may have been used initially to defend animals against natural plant toxins, and subsequently became utilized to defend against the growing numbers of toxic organic chemicals produced by industry.

However, organisms do not have the luxury of coping with one chemical at a time, but must deal with numerous chemicals in some coordinated fashion.

Tolerance is an issue relevant to toxicity testing for choosing organisms for “standard toxicity tests,” since the origin of the animals used in bioassays will affect their responses to chemical stress. It is also relevant to the field of biomarkers. Biochemical biomarkers in organisms from polluted field sites will be different (reduced) if the organisms have developed tolerance.

The acquisition of tolerance to pollutants in the environment allows populations of organisms to sustain themselves in highly contaminated sites, even though they may show negative effects such as reduced abundance, reduced genetic diversity, altered behavior, liver tumors, etc. Thus, while some organisms can develop tolerance to pollutants in their environment, this comes at a cost, often energetic. Also, in cases in which high concentrations of contaminants are accumulated, this can pose risks to non-tolerant consumers, including humans who eat seafood containing elevated levels of methylmercury, PCBs, or other contaminants that biomagnify through food webs. As Klerks et al. (2011) noted in a review, approaches used over the past few decades have limitations, including difficulty in separating the various genetic and environmental variance components, since responses depend on specific populations and test conditions. To improve insights into the long-term consequences of adaptation, it is important to look not only at resistance itself, but also at the fitness consequences and responses in other characteristics.

Developments in molecular genetics have yielded additional insights. Quantitative genetics is benefiting from the use of molecular tools and is becoming an important field for studying evolutionary toxicology.

Other reviews on the overall topic of tolerance have been published recently including Amiard-Triquet (2011, 2013), Johnson (2011), Berthet et al. (2011), Rainbow and Luoma (2011), Romeo and Wirgin (2011) and others in the same volume edited by Amiard-Triquet, Rainbow and Romeo.

As contaminant levels are reduced as a result of environmental clean-ups and reduction of inputs, negative effects on marine species are expected to decline, along with tolerance and bioaccumulation, resulting in a healthier environment for both marine species and humans. Such “good news” has been seen in many areas and is expected to continue for conventional pollutants like metals, oil, and persistent organic compounds. However, with respect to CECs, warming temperatures, and ocean acidification, the future health of the marine environment is likely to worsen, since there has been no progress in curbing these inputs. Whether or not organisms will be able to evolve tolerance to the inevitable increased levels of stressors associated with climate change remains in question. Research to date suggests that coral reefs and some shellfish may face very serious consequences although some species will undoubtedly be able to thrive, while others may disappear. Those who care about the environment can only hope and advocate for international agreements that will result in major reductions in greenhouse gases in the near future.

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