Chapter 7
Larval Development

Abstract After hatching, an organism leaves a relatively closed and protected system for life in a larger environment. Many studies have been performed exposing larval stages directly to contaminants or examining larvae after embryonic exposure. Larvae may be more sensitive than embryonic stages of the same organism, since embryos are protected by an outer membrane that may reduce contaminant uptake (e.g. chorion) that is no longer present in larvae. Larvae also must usually swim and obtain food. Most benthic invertebrates have planktonic larvae, which at a certain stage of development settle to the bottom to metamorphose into a juvenile stage in an appropriate habitat. Larval exposures to contaminants can lead to impaired settlement in the benthic environment and/or to delayed physiological disturbances as juveniles or adults.

Keywords Abnormalities • Delay • Development • Growth • Molting • Morphology • Settlement • Stages

Most marine animals hatch out as small planktonic organisms with little resemblance to the adult form that they will eventually become. Planktonic larvae are common even in taxa that are benthic as adults, such as decapod crustaceans, echinoderms, corals, and most bivalve and gastropod mollusks. The larvae of many species have some yolk, so they need not feed immediately, but most larvae eventually do need to acquire food, (generally smaller phytoplankton) which is a critical point in development. The transition to feeding requires the maturation of a number of organ systems – not only the digestive system, but the nervous system for detecting food and the musculo-skeletal system for moving to the food. There are some species, however, such as deep sea king crabs, in which the larvae are provided with enough yolk that they don't have to feed during their entire larval life (which may be as long as a year). For species that are benthic as adults, planktonic larvae enable greater dispersal. However, being small and floating, they are very vulnerable to predation and only a tiny percentage survive long enough to undergo metamorphosis. Metamorphosis is another critical stage; larvae must

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find an appropriate substrate on which to metamorphose. Contaminants are yet additional stresses on larvae, and the process of metamorphosis is very sensitive. In addition, pollution is probably a greater threat at the time when larvae are settling to metamorphose, as they are likely to encounter higher concentrations of contaminants at the bottom than in the water column. Delayed metamorphosis is a common response to environmental stressors.

7.1 Crustaceans

Since crustaceans go through a series of several larval stages (e.g., nauplius, zoea, megalopa) that undergo a series of molts as they develop, exposures can be performed on particular larval stages that may have differential sensitivity to particular contaminants. In general, later stage larvae are more tolerant than earlier ones. One very common response is a delay in molting from one stage to another and in metamorphosis to the juvenile form.

Molting and its hormonal regulation will be discussed in greater detail in the next chapter.

Deformities are also be produced by some contaminants. Delayed metamorphosis can itself produce "carry-over" effects in juveniles. Simith et al. (2013) found that delayed metamorphosis affected early juvenile survival and growth of mangrove crabs, *Ucides cordatus*. After delayed metamorphosis, survival of juveniles was 11–31 % lower and intermolt periods were 1.5–4.2 days longer than in controls. They also were smaller and had lower growth rates than juveniles derived from non-delayed megalopae. Most effects were observed in all five crab stages studied, indicating that the costs of delayed metamorphosis may persist throughout early juvenile stages.

7.1.1 Metals

Stage II nauplii of the barnacle *Balanus improvisus* were exposed to Cu and Cd by Lang et al. (1981). Cu in concentrations as low as 10 $\mu g\ l^{-1}$ caused a dose related delay in molting to stage III nauplii and these had deformed appendages and loss of setae. Cd at 100 $\mu g\ l^{-1}$ caused a molting delay but no abnormalities. Similar retarding effects of Cu on the shrimp *Metapenaeus ensis* were reported by Wong et al. 1995 (Fig. 7.1).

Rosenberg and Costlow (1976) exposed blue crab megalopae (the last larval stage of crabs) to 50 and 150 $\mu g\ l^{-1}$ Cd and found delayed development from the megalopa to third crab stage. The effect was more pronounced at lower salinities, those preferred by the species. These authors also found that mud crab *Rhithropanopeus harrisii* zoea were more susceptible to Cd than the (older) megalopae. Lopez Greco et al. (2001) found differential responses of different larval stages of the pea crab *Tunicotheres moseri* to Cu (0.5–1,000 $\mu g\ l^{-1}$). The zoea I

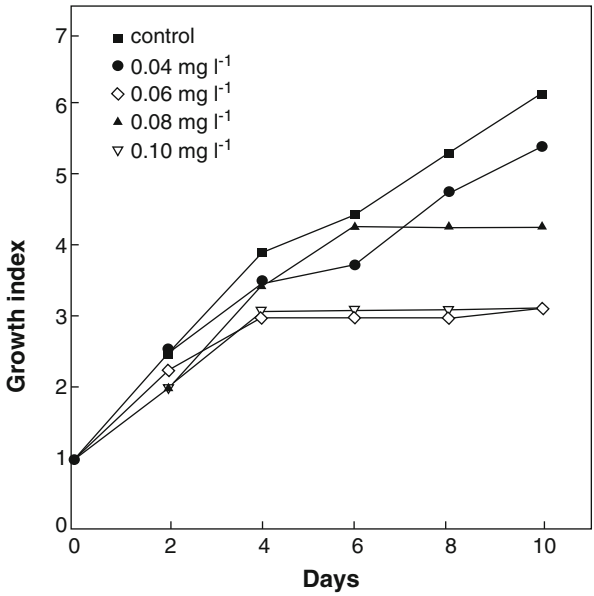

Fig. 7.1 Growth index of *Metapenaeus ensis* larvae during a 10 day exposure to copper. (Reprinted from Wong et al. 1995: 417, courtesy Elsevier Publishing Co.)

stage was the most sensitive, and those that molted to zoea II in 100 $\mu g\ l^{-1}$ had abnormal setae causing reduced swimming. However, the abnormality could be reversed if they were maintained in clean water for subsequent molts to megalopa. Cu at 100 $\mu g\ l^{-1}$ retarded the duration of zoea I stage but did not affect the duration of zoea II, and accelerated the molt from megalopa to first crab.

Developmental rates of blue crab megalopae were prolonged by exposure to Hg or to low salinity. While optimum time was 8 days, the period was extended to 10 days by salinity of 10 psu, and to 13 days at a salinity of 10 psu plus 20 $\mu g\ l^{-1}$ Hg (McKenney and Costlow 1981). After metamorphosis, the first crab stage was more resistant. Exposure of *R. harrisii* and *Callinectes sapidus* larvae to hexavalent chromium slowed zoeal development from hatching to megalopa or to first crab stage (Bookhout et al. 1984). Swimming speed of *R. harrisii* larvae was modified; speed was elevated at low sublethal concentrations (7.2 mg l^{-1}) but depressed at higher concentrations (14 mg l^{-1}). Mortimer and Miller (1994) reported Cr, Ni Zn, and Cu effects on larvae of the crab *Portunus pelagicus* were inhibition of molting, increase in duration of developmental stages, and reduced size. Relative toxicities were $Cu^{2+} > Cd^{2+} \geq Zn^{2+} > Ni^{2+} > Cr(V1)$.

7.1.2 Organics

Pesticides

Effects of the chlorinated hydrocarbon mirex on zoeae of the crabs *Menippe mercenaria* and *Rhithropanopeus harrisii* included retardation and production of an

extra (6th) zoeal stage (Bookhout and Costlow 1974). The duration of zoeal stages and development to 2nd crab stage of *R. harrisii* increased significantly with an increase in concentration of mirex from 0.01 to 10.0 $\mu g\ l^{-1}$. The organophosphate malathion at 0.02–0.08 mg l^{-1} also prolonged development time in these species (Bookhout and Monroe 1977). In contrast, Key et al. (1998) found that 30.0 $\mu g\ l^{-1}$ malathion reduced the number of larval instars in the grass shrimp, *Palaemonetes pugio*.

Snyder and Mulder (2001) exposed lobster, *Homarus americanus*, larvae for 24 h to heptachlor (333 $\mu g\ l^{-1}$), a known endocrine disruptor, on different days of the 1st larval instar and followed the larvae for effects on timing of ecdysis to 2nd stage, molting hormone titers, and alterations in the levels of cytochrome P450 CYP45 and HSP70 proteins. Control larvae molted on Day 8–9 with 96 % survival. Larvae treated with heptachlor for 24 h on Day 3 molted successfully (92 %) on Day 10, a delay of 2 days. Molting days for other 24 h heptachlor treatments were: Day 1 treatment – molt on Day 11, 90 % success, Day 2 treatment – molt on Day 12, 85 % success, and Day 4 treatment – molt on Day 13, 65 % success. Larvae treated on days 5–6 never molted successfully, while those treated during the late premolt on Day 7 molted on Day 8 with the same survival percentage as controls. The effects of 24 h heptachlor treatment were thus very different, depending on the day of exposure and the sensitive period was days 4–6.

On days 1 or 2, heptachlor treatment caused a significant elevation in ecdysteroid levels the day after treatment, which corresponded to a delay in the premolt ecdysteroid peak prior to ecdysis. Larvae treated on day 3 had no immediate effect on ecdysteroid levels, although the premolt peak was delayed. Those treated on days 4 or 5 had diminished ecdysteroid levels for several days. Day 4 larvae had a 4–5 day delay in the premolt peak, while day 5- or day 6-treated larvae never showed any premolt ecdysteroid peak and all had died by day 16. CYP45 and HSP70 levels were elevated for several days following exposure. Delays in molting were correlated with alterations in ecdysteroid levels, suggesting that this pesticide may function as an endocrine disruptor in crustaceans.

Osterberg et al. (2012) investigated toxicity of a number of pesticides to megalopae of blue crabs. LC_{50} values ranged from 0.22 $\mu g\ l^{-1}$ for megalopae in lambda-cyhalothrin to 316,000 $\mu g\ l^{-1}$ for juveniles exposed to Roundup®. Treatment of intermolt megalopae with LC_{20} levels of Roundup® (5,500 $\mu g\ l^{-1}$) reduced the time to metamorphosis, but no effects resulted from treatment with the four active ingredient insecticides (lambda-cyhalothrin, imidacloprid, aldicarb, and acephate). Acephate, aldicarb, imidacloprid, and Roundup® increased mortality of juveniles shortly after molting. The sensitivity of molting crabs to these pesticides indicates that frequently molting animals are particularly vulnerable.

It is not surprising that insect growth regulators should affect crustacean larvae. Exposure to the juvenile hormone mimic methoprene caused crabs in suboptimal salinity to be unable to complete metamorphosis (Bookhout and Costlow 1974). The time required for development was not altered, however, by sublethal concentrations. Methoprene exposure of grass shrimp (*P. pugio*) larvae (McKenney and Matthews 1990) reduced completion of larval metamorphosis at 100 $\mu g\ l^{-1}$.

The first two larval stages and the final premetamorphic larval stage were more sensitive than intermediate stages. The total number of larval stages was not affected. Dimilin, a chitin synthesis inhibitor, was highly toxic to larvae of *R. harrisii*, *Sesarma reticulatum*, and *C. sapidus*, causing morphological abnormalities that became apparent at molt (Costlow 1979). Significant increases were seen in swimming speed of Stage I, II and III *R. harrisii* zoeae, with 0.3 $\mu g\ l^{-1}$ being the lowest effective concentration. However, it did not delay larval development. Walker et al. (2005) found that low levels of methoprene had adverse effects on lobster larvae. It was toxic to stage II larvae at 1 $\mu g\ l^{-1}$. Stage IV larvae were more resistant, but had significant increases in molt frequency at 5 $\mu g\ l^{-1}$. Environmental concentrations of methoprene inhibited almost all protein synthesis in the hepatopancreas.

Crustacean larvae are often more sensitive than embryos. Larval development of *P. pugio*, was over two orders of magnitude more sensitive to disruption by methoprene and fenoxycarb than was embryonic development (McKenney 2005). Mud crab larvae *R. harrisii*, exhibited reduced metamorphic success at lower concentrations of methoprene and pyriproxyfen than grass shrimp larvae, suggesting that the more rigidly controlled metamorphic process in crabs is more sensitive to endocrine disruptors than the more flexible metamorphic pattern in shrimp. The final crab larva, the megalopa, was more sensitive to methoprene and fenoxycarb than earlier zoeal stages.

PCBs

Roesejadi et al. (1976) tested PCBs (Aroclor 1254) on larvae of *Palaemonetes pugio*. At concentrations above 3.2 $\mu g\ l^{-1}$, there was significant mortality. At 3.2 and 1.0 $\mu g\ l^{-1}$, there was not significant mortality but the duration of development increased and metamorphosis was delayed.

Oil

Zoea larvae of *R. harrisii* were exposed to low concentrations of the water soluble fraction (WSF) of jet fuel for the first 5 days of development. At some low levels there were no negative effects but increased megalopal weight (Laughlin et al. 1981). This was an early reported example of hormesis, a phenomenon that is now widely reported in diverse groups of organisms. The PAHs, phenanthrene and naphthalene, were also tested at 100–200 $\mu g\ l^{-1}$. Phenanthrene-exposed larvae had a decreased development rate, while naphthalene-exposed larvae developed faster than controls (Laughlin and Neff 1979).

Lee et al. (1970) studied effects of freshly prepared WSF of Number 2 fuel oil and WSF exposed to air for 48 h on larval shrimp *Lucifer faxoni*. Based on survival for 14 days, critical levels of toxicities of fresh oil were about 0.2 $mg\ l^{-1}$, while in weathered oil they were around 2 $mg\ l^{-1}$. A similar trend was found in studies of

feeding and activity – fresh extracts were more toxic and effects on feeding were immediate and irreversible, while air-exposed WSF was less toxic, and the effect on feeding was delayed and reversible. Alkyl benzenes, indans, and naphthalenes were rapidly lost from the exposed solution, with negligible concentrations remaining after 24 h. The data suggest that the higher toxicity of fresh WSF was due to volatile aromatic hydrocarbons and there is reduced toxicity following evaporation. This finding is the opposite of that found with fish embryos in Alaska following the oil spill, discussed in the previous chapter. Respiration rates during an 8 h exposure to fresh WSF rose with increasing concentrations up to 30 % of WSF, then fell with further increases of WSF. This may have been hormesis, as later described by Laughlin et al. (1981).

The water accommodated fraction of Number 2 fuel oil (0.1 mg l^{-1}) was tested on *Cancer irroratus* zoea larvae by Johns and Pechenik (1980). Along with decreased survival to megalopae, larvae exhibited reduced food consumption and growth, while metabolic costs of maintenance increased. Larvae of the coonstripe shrimp (*Pandalus hypsinotus*) and king crab (*Paralithodes camtschaticus*) were exposed to solutions of the water-soluble fraction (WSF) of Cook Inlet crude oil in bioassays on intermolt stages I and II and the molt period from stage I to stage II (Mecklenberg et al. 1977). Molting larvae were more sensitive than intermolt larvae, and molting coonstripe shrimp larvae were more sensitive than molting king crab larvae. When molting larvae were exposed to high concentrations of the WSF for as little as 6 h, molting success was reduced by 10–30 % and some deaths occurred. When larvae were exposed to these high concentrations for 24 h or longer, molting declined 90–100 % and the larvae usually died. The lowest concentrations tested did not inhibit molting, but many larvae died after molting.

7.1.3 Contaminants of Emerging Concern

Various hormones and known endocrine disruptors were tested for effects on larval development in the copepod *Acartia tonsa* (Anderson et al. 2001). Tamoxifen (EC50 of 0.049 mg l^{-1}), 17α-ethinylestradiol (EC50 of 0.088 mg l^{-1}) and *p*-octylphenol (EC50 of 0.013 mg l^{-1}) were potent inhibitors of naupliar development. Other estrogens, 17β-estradiol, estrone, and bisphenol A, had little effect. Testosterone and progesterone did not inhibit development, but the antiandrogen flutamide (EC50 of 0.48 mg l^{-1}) had inhibitory effects. Juvenile hormone III (EC50 of 0.102 mg l^{-1}) was a potent inhibitor, as would be expected, but 20-hydroxyecdysone had no effect. Six of the 17 test compounds had LC50: EC50 ratios higher than 10, suggesting that naupliar development, as a parameter, is very sensitive to hormonal disrupters.

Key et al. (2008) examined the effects of a polybrominated diphenyl ether (flame retardant) compound, PBDE-47, on adult and larval stages of grass shrimp (*P. pugio*). The 96-h LC50 was 23.60 μg l^{-1} for larvae and 78.07 μg l^{-1} for adults. Four physiological biomarkers: glutathione (GSH), lipid peroxidation (LPx),

cholesterol (CHL) and acetylcholinesterase (AChE) were assessed. GSH, LPx and AChE were not affected at concentrations up to 50 $\mu g\ l^{-1}$ for 96 h. CHL levels were elevated at the lowest exposure concentrations tested, but significant differences were found only in adults. Effects thus were observed only at levels well above those reported in the environment, but these investigators did not examine development rate, which has been shown to be very sensitive. Breitholtz and Wollenberger (2003) examined effects of PBDE- 47, -99, and -100s on the larval development of the particle-feeding copepod *Nitocra spinipes*. Larval development rate significantly decreased in copepods exposed for 6 days to nominal concentrations of 0.013 $mg\ l^{-1}$ BDE-47 and 0.03 $mg\ l^{-1}$ BDE-99. Partitioning experiments showed that the major fractions were associated with particulate material, showing that development and reproduction in *N. spinipes* are sensitive to PBDEs and that ingestion of particle-adsorbed PBDEs is most likely the major route of exposure.

Chiu et al. 2012 investigated responses of barnacle larvae *Balanus amphitrite* to PBDEs, and demonstrated that chronic exposure to BDE-47 (up to 1,000 $ng\ l^{-1}$) throughout the entire larval stage did not affect settlement, development or growth, despite documented bioaccumulation.

7.1.4 Hypoxia

The ability to regulate O_2 uptake during declining DO co-occurred with metamorphosis from a planktonic to a benthic existence in the Norway lobster *Nephrops norvegicus*. The onset of this regulation appears to be related to the development of hypoxia-related ventilation by pleopods of exchange surfaces on the telson and uropods and a shift of hemocyanin from low to high O_2 affinity (Spicer and Eriksson 2003). This is experimental evidence for the use of uropods/telson by larval lobsters as supplemental gas exchange surfaces. The change occurred with calcification of the exoskeleton at metamorphosis, which restricts gas exchange over the general body surface. Pre-exposure of larvae to reduced DO resulted in the "adult" pattern of regulation being established before metamorphosis. Accelerating ontogeny of this regulation was a result of a shift from a low to a high hemocyanin O_2 affinity before metamorphosis and an increase in the magnitude of the hyperventilatory response in the planktonic larval stages. Thus, the development of respiratory regulation can be influenced by ambient O_2 levels.

7.1.5 Ocean Acidification/Climate Change

Pansch et al. (2012) investigated responses of the barnacle *Amphibalanus improvisus* to simulated warming and ocean acidification (OA) during early development. Nauplii and cyprids were exposed to 12, 20 and 27 °C and pCO_2 of 400 (current),

1,250 and 3,250 μatm for 8 and 4 weeks, respectively. Warming affected larvae more than OA. Increased temperatures favored survival and development of nauplii but decreased survival of cyprids, the subsequent stage. Acidification had no effect upon survival of nauplii but enhanced their development at low (12 °C) and high (27 °C) temperatures. At intermediate temperature (20 °C), nauplii were not affected, even by 3,250 μatm pCO_2. No treatments affected settlement success, showing tolerance of *A. improvisus* larvae to OA predicted for the end of the century. Effects of warming and acidification were studied on larvae of the spider crab *Hyas araneus* from two locations, Svalbard (farther north) and Helgoland (Walther et al. 2010). Larvae were exposed at 3, 9 and 15 °C to present day conditions (380 mg l^{-1} CO_2) and to pCO_2 conditions predicted for the near or medium-term future (710 and 3,000 mg l^{-1}). Enhanced pCO_2 levels extended the duration of larval development and reduced larval growth and fitness, decreasing C/N ratio, a proxy of lipid content. Effects were greatest in the zoeal stages of Svalbard larvae, and during the megalopa stage of Helgoland larvae. The high sensitivity of megalopae from Svalbard to warming and of those from Helgoland to enhanced CO_2 suggests that this larval stage is a sensitive bottleneck within the life cycle of *H. araneus*. Arnold et al. (2009) investigated effects of acidified sea water (pCO_2 approx. 1,200 mg l^{-1}) on early larval stages of the European lobster *Homarus gammarus*. Acidified water did not significantly affect carapace length or development, but reduced carapace mass during the final stage of larval development was seen along with reduced mineral (calcium and magnesium) content of the carapace. These alterations were considered the result of acidosis or hypercapnia interfering with normal homeostatic function.

7.1.6 Polluted Environment

Béguer et al. (2008) reported on morphological deformities in a population of *Palaemon* shrimp in the Gironde estuary (France). The most frequent abnormalities were of the cephalothorax and rostrum, and to a lesser extent scaphocerites and uropods; few cases of antenna or telson deformities were observed. Reports of morphological abnormalities of crustaceans were found in the literature, but previously described only isolated individuals, while in the Gironde estuary up to 40 % of individuals were affected. Authors considered the deformities to be due to pollutants, such as metals and PCBs.

Fiddler crabs (*Uca pugnax*) from a highly industrialized site contaminated with metals, PCBs, and PAHs near the Arthur Kill in Northern New Jersey (U.S.) produced many eggs, but had proportionately fewer larvae return to settle than in populations from a reference site (Bergey and Weis 2008). Larval life in highly contaminated waterways appeared to be the bottleneck in reducing population density at this site.

7.2 Mollusks

The basic molluscan larva is a trochophore, which feeds with two bands of cilia around its "equator" to sweep food into its mouth. The trochophore stage is often followed by a veliger stage in which the prototroch, the band of cilia nearest the apical tuft, develops into the velum ("veil"), a pair of cilia-bearing lobes with which the larva swims. Eventually, larvae sink to the bottom and metamorphose into the adult form. While most gastropods and bivalves undergo metamorphosis, cephalopods have direct development and hatch as a small form of the adult. There has been hardly any work on effects of toxicants on cephalopods, perhaps because it is not easy to raise them in the laboratory. In contrast, oysters have been used in standardized embryo-larval bioassays (His et al. 1997). In contrast with bivalves, which spawn, releasing eggs and sperm into the water column, gastropods (which are sometimes hermaphroditic) typically mate and have eggs develop in a sheltered site before larvae are released into the water. Growth retardation and abnormalities are common responses of larval mollusks to a variety of toxicants.

7.2.1 Metals

Calabrese et al. (1977) studied a suite of metals (Cu, Zn, Cd) on *Crassostrea virginica* and *Mercenaria mercenaria* larvae. The order of toxicity for oysters was Hg > Ag > Cu > Ni > Zn, while that for clams was Hg > Cu > Ag > Zn > Ni. All retarded shell growth at their LC_{50} concentrations, but Ni retarded shell growth at the LC_5 concentration of 1.1 mg l^{-1}. Toxicities were significantly altered by changing the salinity and temperature (MacInnes and Calabrese 1979). Fertilized eggs of the abalone *Haliotis rubra* were exposed to a range of concentrations of Cd, Cu, Fe, Pb, Hg, and Zn for 48 h after which survival and morphological abnormalities of veliger larvae were recorded (Gorski and Nugegoda 2006). The effective median concentrations affecting morphological development in decreasing order of toxicity were: Cu (7 μg l^{-1}), Hg (21 μg l^{-1}), Zn (35 μg l^{-1}), Fe (4,102 μg l^{-1}), Cd (4,515 μg l^{-1}), and Pb (5,111 μg l^{-1}). It is surprising that Cu was more toxic than Hg, and that Zn was more toxic than Cd and Pb. Effects of Zn on development of oyster (*C. gigas*) larvae were reported by Brereton et al. (1973). At 0.05 mg l^{-1} growth rates of 48-h veliger larvae were slower than controls but development was normal. Increasing concentrations decreased growth and increased the incidence of abnormality and mortality. At concentrations of 0.15 mg l^{-1} veligers were abnormal and had no umbo development.

Exposure of *Mytilus edulis* to 8 μg l^{-1} Cu during the veliger larval or post-larval stages had no significant effects on survivorship or shell growth (Hoare et al. 1995). However, previous exposure to 8 μg l^{-1} Cu during embryonic stages significantly

increased veliger growth rate and decreased spat survivorship in a mussels from the Menai Strait, Wales, UK. In this population, Cu caused a significant increase in embryo abnormalities. The embryo exposure effects outweighed the influence of copper during later stages.

Effects of Hg on survival, growth and metamorphosis of *Crassostrea gigas* larvae were examined (Beiras and His 1994). Growth, the most sensitive process, was significantly retarded at 4 $\mu g\ l^{-1}$. Metamorphosis was significantly reduced when competent pediveligers were exposed to 64 $\mu g\ l^{-1}$ for 48 h. Larval clams *Mercenaria mercenaria* were exposed to dissolved Cu (LaBreche et al. 2002). Clams exposed to 5 μg Cu l^{-1} and fed *Isochrysis galbana* had similar survival to controls, but those in 14 and 29 μg Cu l^{-1} had increased mortality. Swimming activity decreased exponentially. Dissolved Cu was taken up by *I. galbana*, and ingested algae were a source of Cu toxicity for clams.

7.2.2 Organics

Wang et al. (2012) examined effects of PAHs (benzo[*a*]pyrene – BaP) and PCBs (Aroclor 1254) on embryogenesis and larval development of the bivalve *Meretrix meretrix*. Even at 1,600 $\mu g\ l^{-1}$ of BaP and Aroclor1254 only minor reductions in embryo development rates were produced. The most sensitive endpoint was larval metamorphosis, with an EC_{50} value of 20 $\mu g\ l^{-1}$ for BaP and 35 $\mu g\ l^{-1}$ for Aroclor1254. These results indicate that BaP and Aroclor1254 are not highly toxic to *M. meretrix* embryos and larvae.

The toxic effects of different types of gasoline formulations on *Crassostrea rhizophorae* embryos and larvae were studied by Paixão et al. (2007). Oyster embryos were exposed to water-soluble fractions (WSF) of different gasoline formulations at a range of concentrations (0, 4.6, 10.0, 22.0, 46.0, and 100 %), for 24 h. The EC50-24h (concentration causing abnormalities in 50 % of the exposed embryos) was evaluated. The results showed gasoline formulations with high concentrations of monoaromatic hydrocarbons to be the least toxic, while formulations having higher content of aromatic hydrocarbons of 9 carbon atoms and naphtha were the most harmful.

Ryan et al. (2001) examined hard clam (*Mercenaria mercenaria*) larvae exposed to PCBs. Aroclor 1254, at concentrations near environmentally relevant levels. A dose–response relationship was observed for larval development; at higher concentrations, fewer larvae developed to the normal straight-hinge, or D-shaped stage, relative to the controls, while the number of abnormally shaped larvae increased.

Hanson et al. (1997) investigated effects of the detergent linear alkylbenzene sulphonate (LAS) (0–39 mg l^{-1}), which is found in nearshore areas receiving wastewater from urban treatment plants. They examined effects on swimming, grazing, and growth of mussel larvae, *Mytilus edulis* in the laboratory, and effects on settling and population development in field mesocosms. In the laboratory the

larvae had 50 % mortality at 3.8 mg LAS l^{-1} after 96 h. Swimming was affected at 0.8 mg l^{-1} (i.e. smaller diameter of swimming tracks, reduced speed). Feeding was reduced 50 % at 1.4 mg l^{-1}, and specific growth rate was reduced by half at 0.82 mg l^{-1} in 9 days. In mesocosms, the larval population decreased dramatically in 2 days at concentrations as low as 0.08 mg l^{-1}, due to increased mortality and to settling. Settling success was reduced at the same LAS concentration as that which increased mortality. Treated larvae had delayed metamorphosis and reduced shell growth. Authors felt that the larval ciliary apparatus, crucial for swimming, orientation, settling behaviors, and feeding, was damaged by LAS. Data on grazing and growth agreed with video observations of larvae. These effects occurred at LAS levels found in some estuaries. This is an unusually comprehensive study with many ecologically important endpoints evaluated as well as a proposed mechanism for the effects. More studies like this should be done.

Contaminated sediments can also affect mollusk development. Geffard et al. (2003) studied bioavailability and toxicity of sediment-associated PAHs to embryos and larvae of *C. gigas*, exposed to whole sediment and elutriate. Percentages of abnormal larvae and contaminant accumulation were measured. Sediment-associated PAHs were available, as indicated by accumulation in larvae and by abnormalities induced during larval development. The critical body burden of various PAHs was 0.3 μg g^{-1}, above which abnormalities were observed. The bioavailability of PAHs is determined by their solubility; only the soluble fraction is accumulated by the embryos.

Toxicity of glyphosate herbicides to embryo-larval development of oysters (*C. gigas*) was studied by Mottier et al. (2013). Embryo-larval development was quite insensitive to the pure chemical and commercial formulations, but commercial formulations were considerably more toxic, with EC_{50} values of 1,133 and 1,675 μg l^{-1} for Roundup Express® (R_{EX}) and Roundup Allées et Terrasses® (R_{AT}).

Since both oysters and sea urchins are used in embryo-larval bioassays, the respective sensitivity of oyster (*Crassostrea gigas*) and sea urchin (*Paracentrotus lividus*) embryos and larvae to various pollutants were compared (His et al. 1999). *C. gigas* embryos and larvae were more sensitive to copper and to the herbicide Dinoterbe; the sensitivity of both species to TBT was practically the same, and *P. lividus* was more sensitive to lead and mercury. Authors felt the oyster bioassay is more suitable for estuarine waters, because of the broader salinity tolerance of estuarine bivalve larvae than sea urchin larvae.

7.2.3 Contaminants of Emerging Concern

The effects of the endocrine disruptors nonylphenol (NP) and bisphenol A (BPA), on embryonic and larval development of the abalone *Haliotis diversicolor supertexta*, were investigated by Liu et al. (2011). The respective 96-h EC_{50} values based on completion of metamorphosis were very high, 11.65 and 1.02 g l^{-1}, suggesting very

low sensitivity. However, when abalone were exposed for a longer time, or if the benthic diatoms that are both a food source and a settlement substrate for abalone had been exposed and accumulated the chemicals, then there was much greater sensitivity at metamorphosis.

Crassostrea gigas was exposed to a range of concentrations of 4-nonylphenol (0.1, 1, 10, 100, 1,000 and 10,000 μg/l) by Nice et al. (2000). Development to the D-shape larval stage was monitored. This endocrine disruptor delayed development to D-shape, produced abnormalities, and decreased survival rate. Thus oyster larvae are much more sensitive than abalone larvae.

Chiu et al. (2012) investigated effects of the flame retardant PBDE on the gastropod *Crepidula onyx*, and demonstrated that chronic exposure to BDE-47 (up to 1,000 ng l^{-1}) throughout the entire larval stage did not affect settlement, development, or growth despite bioaccumulation.

7.2.4 Hypoxia

Anoxia tolerance of *M. edulis* larvae, (median mortality time) increased from 14 h in early prodissoconch larvae to 38 h in later veliconch larvae. Both embryos and early larvae developed and grew normally at pO_2 values ≥3.16 kPa (Wang and Widdows 1991). Feeding activity of early larval stages was maintained or enhanced under hypoxia, but feeding and growth of later stages was depressed at all hypoxic levels examined. Hypoxia had little influence on settlement, but larvae developed eye-spots at a smaller size, indicating an uncoupling of growth and morphogenesis. These responses were supported by calorimetric and respirometric measurements showing that early larvae could maintain their energy metabolism at reduced O_2, while later stages suppressed heat dissipation in moderate hypoxia.

Effects of hypoxia (1.5 mg O_2 l^{-1}, 20 % sat) and anoxia (<0.07 mg O_2 l^{-1}, <1 % sat) on oyster (*Crassostrea virginica*) larval settlement, juvenile growth, and juvenile survival were studied. Settlement was reduced significantly in hypoxic treatments, and almost no settlement took place in anoxia (Baker and Mann 1992). After 96 h, 38 and 4 % of the larvae in hypoxic and anoxic treatments had settled, while 79 % settled in control normoxic treatments. After settlement, juveniles in hypoxia grew one third as much as those in normoxia, while those in anoxia did not grow at all. In response to hypoxic treatments, post-settlement oysters with shell heights of >469 μm maintained normal rates of ingestion but oysters with shell heights of 436 μm reduced ingestion rates to 54–61 % of control rates (Baker and Mann 1994). These oyster sizes differed in the extent of gill development, which may have been responsible for the differential responses. In response to microxic treatments, (<0.4 mg O_2 l^{-1}, <5 % sat) ingestion rates were 1–14 % of normoxic rates and decreased with body size. Authors concluded that oysters have the ability to feed at nearly all stages of settlement and metamorphosis, that hypoxia affects the feeding of only the youngest post-settlement oysters, while microxic conditions will affect all post-settlement oysters.

The embryo/larval development of bay scallop (*Argopecten irradians*) was inhibited at a DO of 1.38–3.64 mg l^{-1} at 23 °C (Wang and Zhang 1995). Tolerance to anoxia increased with larval sizes and was related to their oxygen debt (accumulation of lactic acid).

Gastropod larvae may be more sensitive than the bivalves discussed above. Effects of low DO on early development and swimming behavior of veliger larvae of the snail, *Nassarius festivus* were studied (Chan et al. 2008). Embryonic development was significantly delayed when DO was reduced to 3.0 mg O_2 l^{-1}. Veligers that hatched at 4.5 mg O_2 l^{-1} had smaller velar lobes, shell length and shell width and lower swimming speeds than those in normoxia. The percentage that developed into juveniles was reduced and metamorphosis was delayed at 4.5 mg O_2 l^{-1} while all larvae at 3.5 mg O_2 l^{-1}died before metamorphosis. Juveniles that developed at 4.5 mg O_2 l^{-1} were smaller than those at 6.0 mg O_2 l^{-1}, indicating that DO levels well above hypoxic levels (2.8 mg O_2 l^{-1}) have significant impacts on hatching and larval development in these gastropods.

7.2.5 Ocean Acidification/CO_2

Acidification leads to thinner shells in mollusks, which can make them more susceptible to predation. Kurihara et al. (2007) exposed eggs of the oyster, *C. gigas* for 48 h to seawater at pH 7.4, and examined the larval morphology and shell mineralization. Only 5 % of the low pH group developed into normal D-shaped veligers compared with 68 % of the controls, although no difference was seen up to the trochophore stage. Control D-shaped veligers had greater shell length and height 24–48 h after fertilization, while the few D-shaped veligers of the experimental group had no shell growth during that period (Fig. 7.2). Calcification appeared to be particularly affected by low pH and/or the low $CaCO_3$ saturation state of high-CO_2 seawater.

Mussel embryos (*M. galloprovincialis*) were incubated for 6 days in control and high-CO_2 (2,000 mg l^{-1}, pH 7.4) seawater (Kurihara et al. 2008). While embryogenesis was unaffected, development at the trochophore stage was delayed when the shell began to form. Veligers of the high-CO_2 group showed morphological abnormalities, reduced height and length, consistent with the previous findings on the oyster, although the severity of CO_2 effects was less in the mussels, possibly due to differing spawning seasons. In contrast, Gazeau et al. (2007) found that mussels, in this case *M. edulis*, were more sensitive than oysters – calcification rates of *M. edulis* and *C. gigas* declined linearly with increasing pCO_2, but mussels declined more, and projections were that mussel and oyster calcification may decrease by 25 and 10 %, respectively, by the end of the century. Gazeau et al. (2010) found impacts on mussel calcification with a decrease of ~0.5 pH unit during the first 2 days of development. Hatching rates were 24 % lower while D-veliger shells were 13 % smaller at pH 7.6 than at control pH of 8.1. Although larvae developed a shell at this pH, lower hatching and growth could lead to a significant decrease in settlement success.

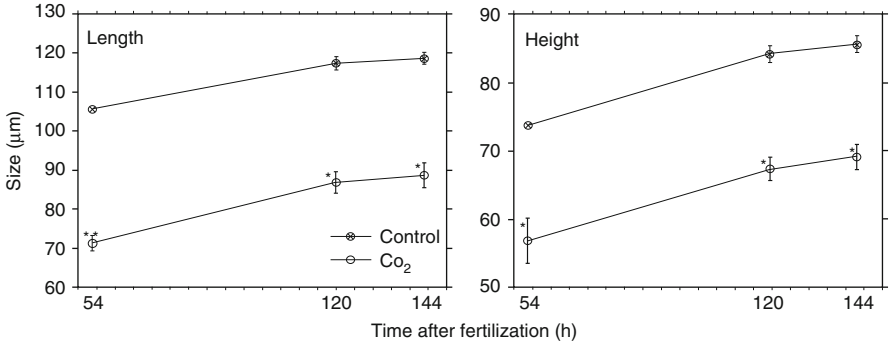

Fig. 7.2 Comparison of shell length and height of D-shaped larvae of *M. galloprovincialis* in control and high CO_2 at 54, 120, and 144 h after fertilization. Error bar = SD, * = significant difference between control and high CO_2 groups (Reprinted from Kurihara et al. 2008: 229, courtesy of Inter-Research)

Hettinger et al. (2012) investigated consequences of decreased pH for early stages of the Olympia oyster (*Ostrea lurida*). Oysters were raised through their larval period and into early juvenile stages at control pH (8.0) as well as 7.9 and 7.8. Larvae at pH 7.8 had a 15 % decrease in shell growth rate, and a 7 % decrease in shell area at settlement. Impacts were greater 1 week after settlement; juveniles that had been larvae in low pH had a 41 % decrease in shell growth rate. Importantly, this was seen in juveniles kept at control pH as well as those that were still in reduced pH, indicating a strong delayed effect from the larval stage. Impacts of early exposure to low pH persisted for at least 1.5 months after juveniles were transferred to control pH. Delayed effects appear to be very important and are overlooked in short-term larval tests that end at metamorphosis.

Endangered northern abalone (*Haliotis kamtschatkana*) larvae were exposed to various levels of CO_2: 400 (ambient), 800, and 1,800 mg l^{-1} (Crim et al. 2011). Larval survival decreased by 40 % in elevated CO_2, but the percent of surviving larvae to metamorphose was unaffected. Shell abnormalities occurred in 40 % of the larvae at 800 mg l^{-1} CO_2 and almost all larvae at 1,800 mg l^{-1} had abnormal or no shells. Zippay and Hoffman (2010) examined the effect of pH on larvae of the red abalone, *Haliotis rufescens*. Low pH (7.87) decreased thermal tolerance of pretorsion and late veliger stages, but not post-torsion and premetamorphic veligers. However, the expression pattern of shell formation genes was not affected in any of the stages.

Since acidification will be accompanied by increasing temperature, Talmadge and Gobler (2011) studied responses of larvae and juveniles of *M. mercenaria*, *C. virginica*, and *Argopecten irradians* to temperatures (24 and 28 °C) and CO_2 levels (~250, 390, and 750 mg l^{-1}). Increased temperature and CO_2 each depressed survival, development, growth, and lipid synthesis of *M. mercenaria* and *A. irradians* larvae and effects were additive (Figs. 7.3 and 7.4). Juvenile

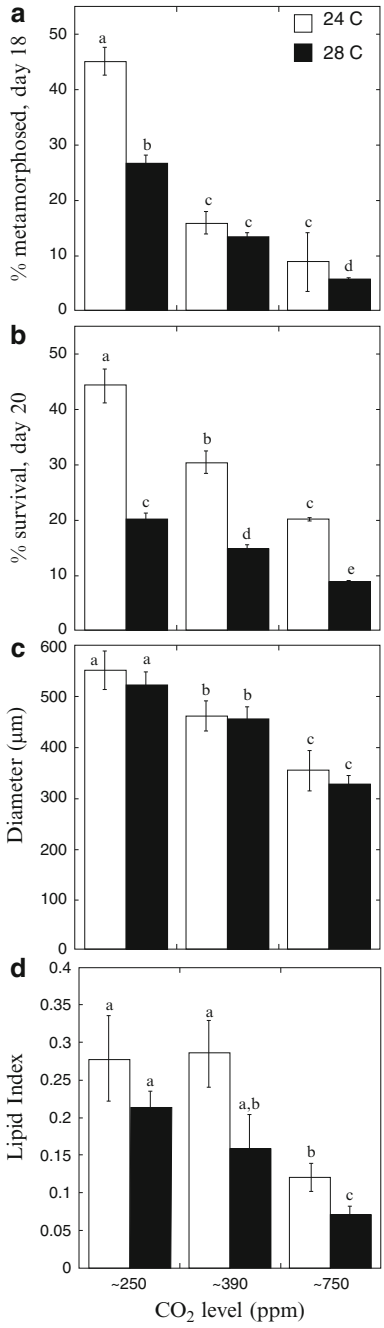

Fig. 7.3 Performance of *M. mercenaria* larvae grown under 250, 390, and 750 ppm CO_2, and 24 and 28 °C. *Letters* represent significant differences (Reprinted from Talmage and Gobler 2011)

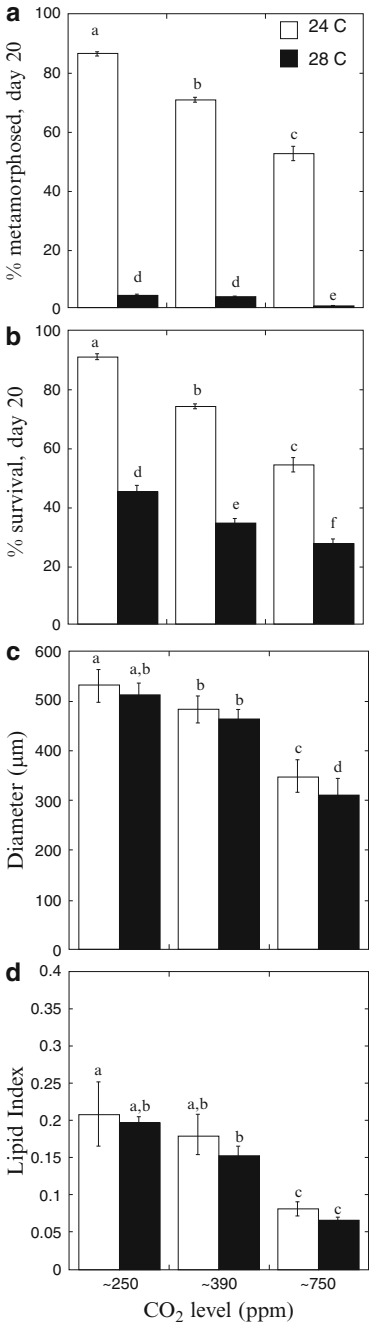

Fig. 7.4 Performance of *Argopecten irradians* larvae grown under 250, 390, and 750 ppm CO_2, and 24 and 28 °C. Letters represent significant differences (Reprinted from Talmage and Gobler 2011)

M. mercenaria and *A. irradians* were negatively affected by higher temperatures while *C. virginica* juveniles were not. *C. virginica* and *A. irradians* juveniles were negatively affected by higher CO_2, while *M. mercenaria* was not. Larvae were more vulnerable to elevated CO_2 than juveniles. Increases in temperature and CO_2 will have combined negative consequences for coastal bivalves.

Impacts have already been seen on oyster larvae. Researchers studied oysters at the Whiskey Creek Hatchery in Oregon after oyster production failures, examining the coastal waters in which the shellfish were raised. For several years larval production collapsed by up to 80 % at shellfish farms. Production failures were linked to CO_2 levels in the water in which the oysters spawned and spent their early lives when they develop into larvae and build their initial shells. Barton et al. (2012) linked the collapse of oyster seed production to increased CO_2 from seasonal upwelling of low pH water, which inhibited larvae from developing shells and growing at a rate that would make commercial production viable.

However, adaptation may be possible. Parker et al. (2012) found that while elevated CO_2 reduced growth, developmental rate and survival of oyster *Sacostrea glomerata* larvae, exposing adults to elevated CO_2 during reproductive conditioning had positive effects. Larvae spawned from adults at elevated CO_2 were larger, developed faster, and had similar survival as larvae from adults at ambient CO_2. Furthermore, they were more resilient to elevated CO_2 than wild larvae, suggesting that they may be able to acclimate or adapt to elevated CO_2.

7.2.6 Polluted Environments

Money et al. (2011) analyzed responses of *C. gigas* larvae exposed to water from the industrialized Tamar estuary (England). A high level of toxicity (up to 100 % abnormal development) was seen at two stations, particularly during periods of the tidal cycle when the influence of more pristine coastal water was lowest. Competitive ligand-exchange Cu titrations showed that natural organic ligands reduced the free cupric ion concentration to levels that were unlikely to have been the sole cause of the observed toxicity. It is probable that combined effects of Cu and other contaminants contributed to the response.

Effects of contaminants along a well-defined North Sea pollution gradient were assessed by McFadzen (1992) using veliger larvae of *C. gigas* and the Manila clam *Tapes philippinarum*. The results demonstrated that larval survival steadily improved further offshore towards the Dogger Bank, with higher mortalities occurring in the surface microlayer and sediment elutriate samples than in the subsurface bulk waters. Clam larvae were more sensitive to contaminants than the younger oyster larvae.

7.3 Fishes

Fish larvae tend not to be quite as different from adult forms as larvae of invertebrates. As they grow, they gradually change shape, instead of having a specific metamorphosis. Exceptions to this are in the flatfish, whose planktonic larvae resemble those of other fishes, but which undergo a major metamorphosis in which one eye (and accompanying nerves) migrate from one side of the head to join the other eye, as the fish settles to the bottom to lie on one side. Eels also undergo a clear metamorphosis. Large numbers of bioassays of contaminants have been done using fish larvae, of which most have been on freshwater species such as zebrafish, fathead minnows, and medaka.

Based on experimental data with sole (*Solea solea*), a bioaccumulation model was adapted to calculate concentrations of persistent organic pollutants in tissues of developing fish (Foekema et al. 2012). Tissue concentrations were predicted to peak at the time when larvae become free-feeding, when lipid reserves are depleted. This may explain delayed effects on larvae that have been observed after egg and embryo exposures. Effects of embryonic exposures on larval behavior are discussed Chap. 9.

7.3.1 Metals

Larvae of garpike (*Belone belone*) exhibited vertebral flexures, reduced activity and swimming ability after incubation in 0.5 mg l^{-1} Cd (Dethlefsen et al. 1975). Toxicity of Cd, Cr (VI) and Cu to *Cyprinodon variegatus* larvae was evaluated in terms of survival and growth over 7 days (Hutchinson et al. 1994). Concentrations affecting survival and growth after 7 days were 0.75 mg Cd l^{-1}, 24.0 mg Cr^{6+} l^{-1} and 0.16 mg Cu l^{-1}. Effects of 2 h pulse-exposure of Cd or Zn on early life stages of Australian crimson spotted rainbow fish (*Melanotaenia fluviatilis*) were investigated by Williams and Holdway (2000). For Cd and Zn, 9–10-day-old larvae were more tolerant than younger ages and Cd was more toxic than Zn. Pulse-exposed metals (3.3 mg l^{-1} of cadmium and 33.3 mg l^{-1} of zinc on 3 h old embryos) caused reduced hatch, spinal deformities, and toxicity in larvae. Continuous exposure LC50 values for 9–10-day-old larvae were 0.01 and 0.27 mg l^{-1} for cadmium and zinc, respectively. Zn at 0.1, 0.5 and 2.0 mg l^{-1} produced deformations of the jaw, head, optic capsules, otic capsules and vertebral column of yolk-sac larvae of herring *Clupea harengus* (Somasundaram et al. 1984). Authors suggested that larvae with moderate deformations, induced by lower concentrations, may survive and continue development, although while this is possible in a laboratory, it seems relatively unlikely to occur in the field where there are predators. However, some adult fish with mild deformities have been collected from field sites.

LC50 values for larvae of red sea bream *Pagrus major* were higher than those of embryos, indicating that embryos were more sensitive to Cd than larvae (Cao

et al. 2009). Cd concentrations of ≥0.8 mg l^{-1} led to low hatchability, delay in time to hatch, and high mortality, morphological abnormality, and reduced length in the embryos and larvae. Heart beat and yolk absorption of the larvae were significantly inhibited at some high concentrations but they were not as sensitive as other endpoints. Anderson et al. (1991) compared the relative sensitivity of topsmelt (*Atherinops affinis*) sperm, embryos, and larvae to copper chloride. The EC_{50} from 48-h fertilization experiments was 109 μg l^{-1}. The EC_{50} from 12-day embryo development tests was 142 μg l^{-1}, and the mean LC_{50} from 96-h larval mortality tests was 238 μg l^{-1}. Authors concluded that sperm were more sensitive than embryos, and embryos were more sensitive than larvae. However, the larval test was lethality, while the others had sublethal endpoints. It would have been a more valid comparison if sublethal endpoints had been evaluated for larvae as well.

7.3.2 Organics

Pesticides and PCBs

Holdway et al. (1994) studied effects of pulse exposure with two synthetic pyrethroids, fenvalerate and esfenvalerate, on survival of larval Australian crimson-spotted rainbow fish (*Melanotaenia fluviatilis*). Both pesticides were highly toxic with 1-h esfenvalerate pulse-exposure concentrations as low as 0.32 μg l^{-1}, and 1-min fenvalerate pulse-exposure concentrations of 4.5 μg l^{-1}, causing significant mortality. There was a complex relationship between pesticide concentration and time to mortality. At low concentrations of pesticide, most mortality occurred within the first 24 h, while at higher concentrations, mortality continued for 96 h after exposure. The authors suggested that mortality within the first 24 h was due to direct physiological effects on the larvae, while subsequent mortality was primarily due to starvation of larvae unable to recover from the initial insult.

Parental exposure to DDT (2.0 or 10.0 μg per 100 g fish per day in the diet for 1 month) affected behavior of Atlantic croaker, *Micropogonias undulatus*, larvae (Faulk et al. 1999). The proportion of larvae responding to a vibratory stimulus, burst and routine swimming speeds, active duration, and pause duration were affected by parental exposure. Burst speeds in response to the visual stimulus were lower than controls. These changes may decrease survival by increasing predation rates and/or decreasing feeding rates. Additional studies on larval behavior are discussed in Chap. 9.

Monosson et al. (1994) gave adult white perch (*Morone americana*) injections of 3,3′,4,4′-tetrachlorobiphenyl (TCB) at one of three doses (0.2–5.0 mg TCB/kg body weight) approx. 3 months prior to the spawning season and at 3-week intervals. Fewer females receiving the highest dose matured. Those that did mature had a GSI 50 % that of controls. Levels of estradiol-17β, testosterone, and VTG were not altered. By 7 dph survival of larvae from females exposed to 1.0 and 5.0 mg/kg TCB

was reduced compared to controls (0, 1 and 54 %, respectively). Thus decreased larval survival was seen at parental doses less than those that decrease ovarian growth, oocyte maturation, or circulating sex steroid hormone and VTG levels in adults.

Olufsen and Arukwe (2011) studied effects of PCB-77 (3,3′,4,4′-tetrachlorobiphenyl) on vascular and bone development of salmon (*Salmo salar*). PCB-77 (1 or 10 ng l^{-1}) produced concentration-dependent increases in the rate of bone tissue formation, dependent on larval age. Evidence of vascular system disruption by the PCB was observed as cardiac edema, anemia and arrhythmia. Foekema et al. (2008) exposed early life stages of sole (*Solea solea*) to dioxin-like PCB 126 until 4, 8, 10 and 15 days post fertilization (dpf), then raised them in clean seawater. The LC50s at the start of the free-feeding stage (12 dpf) was 39–83 ng PCB 126 l^{-1} depending on exposure duration. After fish had completed metamorphosis, the LC50 values were between 1.7 and 3.7 ng PCB 126 l^{-1} depending on exposure duration. Thus exposure of embryos for only 4 days caused adverse effects during a critical developmental phase 2 weeks later. This study indicates that fish tests that are terminated shortly after the fish become free-feeding underestimate the toxicity of compounds such as PCBs.

Larval and metamorphosing summer flounder (*Paralichthys dentatus*) were exposed to the dioxin-like PCB 126, to document effects on metamorphic development (Soffientino et al. 2010). Median lethal doses ranged between 30 and 220 ng g^{-1} wet mass, indicating that this species is very sensitive. Dose-dependent induction of cytochrome P-4501A (CYP1A) at 4 days post-exposure was observed in liver, stomach, intestine, and kidney of metamorphosing larvae. A single sublethal dose (15 ng g^{-1}) delayed metamorphic progress as determined by the degree of eye migration, and resulted in abnormally high levels of cell proliferation and abnormal gastric gland morphology in late metamorphic stages. These results suggest that larval and metamorphic stages of summer flounder are vulnerable to the effects of dioxin-like compounds, including lethality, developmental delay, and malformations.

McCarthy et al. (2003) examined effects of PCBs (Aroclor 1254) on Atlantic croaker larvae. Adult fish were given a dietary administration of 0 (control) or 0.4 (dosed) mg Aroclor 1254 kg^{-1} fish day^{-1} for 2 weeks during the final stages of gonadal recrudescence. Fertilized eggs collected from control and dosed adults immediately after spawning contained 0 and 0.66 μg Aroclor 1254 g^{-1} egg, respectively. Growth rate of dosed larvae was significantly lower than that of control larvae, with dosed larvae showing a 4 day delay in attaining a given size. Routine swimming speed and activity were similar, but there was a difference in response to stimulus. While the percentage of control larvae responding to the stimulus and their burst speeds increased with age, dosed larvae had no such increase. Further studies on larval behavior are described in Chap. 9.

Couillard et al. (2008) investigated the interaction between PCBs and pesticides. The effect of diazinon was evaluated in *Fundulus heteroclitus* larvae produced from eggs differentially treated with PCB126. A few hours after fertilization, eggs

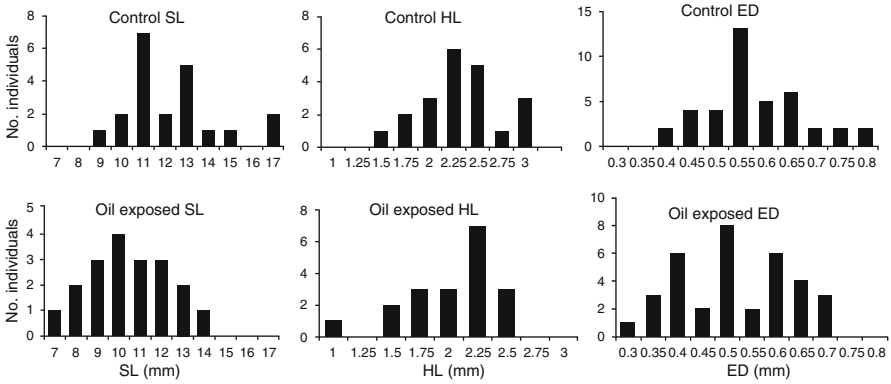

Fig. 7.5 Size distribution of standard length (*SL*) head length (*HL*) and eye diameter (*ED*) of herring larvae in control and oil exposure groups after 8 weeks recovery in clean sea water (Reprinted from Ingvarsdottir et al. 2012: 73, courtesy of Elsevier Publishing Co.)

were treated topically with PCB126 (100 pg ul^{-1}) in dimethyl sulfoxide or not treated. Newly hatched larvae were exposed to diazinon (125–12,900 ng l^{-1}) or seawater alone. Diazinon inhibited cholinesterase activity at 361 ng l^{-1}. Body length was inversely related to diazinon concentration. Embryonic treatment with PCB126 also caused a reduction in body length. The effects of PCB126 and diazinon on body length are cumulative because no significant interactions were observed.

Oil and Dispersants

Atlantic herring (*C. harengus*) larvae were exposed to dispersed Arctic crude oil at 0.129, 0.373, 0.496, 2.486 and 6.019 μg l^{-1} total PAH, and control seawater for 12 days, then transferred to clean water for 8 weeks (Ingvarsdóttir et al. 2012). Higher mortality was found in all oil concentrations after 12 days. There was no difference in mortality during the recovery phase, but after recovery in clean seawater, the oil-exposed larvae exhibited delayed effects including morphological deformities and reduced growth (Fig. 7.5).

Atlantic cod (*Gadus morhua*) larvae were exposed to five concentrations of either artificially weathered dispersed oil containing oil droplets and water-soluble fraction (WSF) or the filtered dispersions containing only the WSF (Olvsvik et al. 2011). The larvae were exposed for 4 days then subjected to transcriptional analysis at 13 days post hatching. The most affected genes were those related to drug metabolism, endocrine system development and function, and lipid metabolism. Oil exposure also increased expression of genes involved in bone resorption, and decreased expression of genes related to bone formation. The altered gene transcription was dominated by the WSF; oil droplets played a smaller role.

Kawaguchi et al. (2011) exposed eggs and larvae of Japanese flounder (*Paralichthys olivaceus*) to heavy oil and investigated neural disorders. In larvae exposed to 8.75 mg l^{-1}, the facial and lateral line nerves partially entered into the incorrect region. Exposed larvae also had abnormal expression of *Sema3A*, an axon guidance molecule, suggesting that the abnormal expression of *Sema3A* caused disruption of the facial nerve scaffolding.

Newly hatched mummichog (*Fundulus heteroclitus*) were exposed to crude oil or water-accommodated fraction (WAF) of dispersed crude oil to evaluate if the dispersant-induced changes in dissolved PAH affected larval survival or body length (Couillard et al. 2005). Weathered Mesa light crude oil (0.05–1 g l^{-1}) with or without Corexit 9500® was used. At 0.2 g l^{-1}, the addition of dispersant caused a two- and fivefold increase in concentrations of total PAH and high-molecular-weight PAH with three or more benzene rings. The highest mortality rates (89 %) were in larvae exposed to dispersed oil. Reduced body length correlated with increased levels of PAH. Thus, dispersion increased total PAH, the proportion of high molecular weight PAH, and overall toxicity.

Contaminants of Emerging Concern

Turbot embryos (*Psetta maxima*) were exposed to BDE-47 and BDE-99 for 6 days. Both compounds caused lethal toxicity as well as non-lethal malformations during embryo development (Mhadhbi et al. 2012). BDE-47 was more toxic than BDE-99 (LC50 values for embryos and larvae, respectively, BDE-47: 27.35 and 14.13 μg l^{-1}; BDE-99: 38.28 and 29.64 μg l^{-1}). PBDEs were teratogenic at concentrations higher than 8.14 and 16.12 μg l^{-1} for BDE-47 and -99 respectively, leading developmental delays and death, as well as malformations and mortality of larvae. Based on environmental concentrations of PBDEs in various aquatic ecosystems, authors concluded that waterborne BDE-47 and BDE-99 pose little risk of acute toxicity to marine fish. However, no sublethal effects on larvae were investigated. More subtle effects of PFOS and PFOA were studied in salmon (*S. salar*) larvae (Arukwe et al. 2013) exposed to100 μg l^{-1} from fertilization for 52 days. PFOS and PFOA body burden increased during the exposure period and affected metabolism and morphometry. PFOA produced increases in heart-, thymus-, liver- and kidney somatic indexes (HSI, TSI, LSI and KSI). PFOA and PFOS decreased whole body dehydroepiandrosterone (DHEA), estrone and testosterone at sampling day 21 and increased cortisol and cholesterol at the end of recovery period (day 56). They observed changes in FA (fatty acid) composition that involved increases in FA methyl esters (FAMEs), mono- and poly-unsaturated FA (MUFA and PUFA) and a decrease in n-3/n-6 PUFA ratio by both PFOA and PFOS. Authors concluded that changes in hormonal and FA profiles may represent cellular and/or physiological adaptation to continuous exposure by increasing membrane fluidity, and/or overt developmental effects.

7.3.3 Hypoxia

Silverside (*Menidia beryllina*) larvae avoid hypoxic water. When larvae sink from an upper normoxic layer into a lower hypoxic layer, they display an avoidance reaction consisting of a burst of fast swimming that ends in an upward direction leading the larva out of the hypoxic region. Each swimming burst lasts for approximately 2–3 s, with a maximum speed of approximately 25 mm s^{-1} (Weltzien et al. 1999). The reaction was seen in larvae from 6 to 64 h post hatching and was correlated with the DO but not with N_2 concentration or salinity. The avoidance response was observed at DO levels from 4.7 to 0.8 mg O_2 l^{-1}.

Tolerance to hypoxic stress and oxygen consumption was studied in the red sea bream, *Pagrus major*, from its early life stage until 42 days post-hatch (Ishibashi et al. 2005). Lethal DO levels and mass-specific metabolic rates increased with larval growth from 2.6 to 5 mm total length (TL), then levels remained high and decreased until about 9.5 mm TL, around the flexion and post flexion stages. In juveniles, lethal DO levels and mass-specific metabolic rates decreased as TL increased. The relationship between lethal DO levels and mass-specific metabolic rates indicated that metabolic rates were highest during metamorphosis, when hypoxia tolerance was lowest. It was presumed that the increasing metabolic rate at metamorphosis decreased the metabolic scope of activity. Similar results were found in bonefish larvae (Pfeiler 2001). Survival times of metamorphosing leptocephali of the bonefish *Albula* sp. placed in hypoxic sea water (0.68 mg O_2 l^{-1}) decreased from 15 to 5 min over the 10 day metamorphic period. Thus they became more sensitive to low DO as they went through metamorphosis. Increased sensitivity to hypoxia again coincided with increased oxygen demand at metamorphosis.

Larvae of the air-breathing teleost *Monopterus* are frequently exposed to periods of hypoxia, which they survive because they have capillary networks in the skin, a small blood-water barrier, pectoral fins that generate a respiratory water current originating from the oxygen-rich surface layer, and a principal flow of blood that runs countercurrent to the water stream. The larva as a whole can be considered functionally comparable to a fish gill lamella (Liem 1981).

7.3.4 Ocean Acidification

Atlantic cod (*Gadus morhua*) larvae were kept at three levels of pCO_2: present day (380 mg l^{-1}), end of next century (about 1,800 mg l^{-1}) and an extreme, coastal upwelling scenario where winds bring CO_2-rich deep water to the surface (about 4,200 mg l^{-1}), in a mesocosm experiment (Frommel et al. 2012). Elevated pCO_2 caused tissue damage in many internal organs, including liver, pancreas, kidney, eye, and gut about 1 month after hatching.

Clownfish *Amphiprion percula* larvae were reared from hatching to settlement at three pH levels (control pH 8.15; intermediate pH 7.8 and extreme: pH 7.6) to test possible effects of ocean acidification on otolith development (Munday et al. 2011a). There was no effect of pH 7.8 on otolith size, shape, chemistry, or symmetry between left and right. However, at pH 7.6 otolith area and maximum length were larger than controls. These results support the hypothesis that pH regulation may cause increased precipitation of $CaCO_3$ in otoliths in elevated CO_2, as suggested by an earlier study, and imply that otolith development is robust to the changes in pH. Newly hatched spiny damselfish *Acanthochromis polyacanthus* were reared for 3 weeks at 4 different levels of pCO_2 from concentrations already experienced in near-reef waters (450 μatm CO_2) to those predicted to occur over the next 50–100 years (600, 725, 850 μatm CO_2). Elevated pCO_2 had no effect on growth, survival or size of skeletal elements (Munday et al. 2011b). Also, otolith size, shape and symmetry between left and right side of the body were not affected by elevated pCO_2, despite the fact that they are composed of aragonite These results suggest that this species is tolerant to increases in environmental CO_2.

7.3.5 Polluted Environments

The sea surface is an important habitat for eggs and larvae of many fishes but it also concentrates contaminants. The microlayer generally has higher levels than the water column of anthropogenic substances which frequently occur at concentrations $10^2 - 10^4$ greater than these in the water column. These include plastics, tar lumps, PAHs, hydrocarbons, chlorinated hydrocarbons, and metals, such as, lead, copper, zinc, and nickel. Studies were conducted to determine the toxicity of the sea surface of Puget Sound to planktonic larval stages (Hardy et al. 1987). Three contaminated urban bays and a rural reference bay were studied. Sand sole (*Psettichthys melanostictus*) embryos and larvae of anchovies and kelp bass were exposed in the field and lab to the sea-surface microlayer. Laboratory exposure to surface microlayer from urban bays increased the incidence of chromosomal aberrations and reduced hatching of sole. *In situ* hatching success of sole eggs was reduced to 50 % in urban bays compared to reference sites. Toxicity was correlated with concentrations of PAHs and metals in the sea-surface microlayer, and was similar in sole, anchovy, and kelp bass. Cross et al. (1987) similarly correlated contaminants in the microlayer in coastal waters off California with toxic effects on kelp bass (*Paralabrax clathratus*) eggs and larvae, which were highest in Los Angeles harbor (with high metals: 17 $\mu g\ l^{-1}$ Ag, 0.26 $\mu g\ l^{-1}$ Cd, 32 $\mu g\ l^{-1}$ Cr, 101 $\mu g\ l^{-1}$ Cu, 100 $\mu g\ l^{-1}$ Pb, and 457 $\mu g\ l^{-1}$ Zn; high chlorinated organics such as 30,708 $ng\ l^{-1}$ Aroclor 1242, and 8,141 $ng\ l^{-1}$ Aroclor 1254; and high PAHs, such as 1,260 $ng\ l^{-1}$ phenanthrene, and 2,178 $ng\ l^{-1}$ benz(a)pyrene). Microlayer samples from farther offshore had lower contaminants and lower toxicity.

There have been studies investigating skeletal abnormalities in adult field-collected fish. These likely originated when the fish were larvae, so are discussed

in this chapter. These are relatively subtle abnormalities that allow the fish to survive to adulthood. Fish from metal-polluted waters in the Gulf of Bothnia were examined for morphological anomalies, such as vertebral and spinal deformities and asymmetrical fins and gill rakers (Bengtsson et al. 1985). Fourhorn sculpin (*Myoxocephalus quadricornis*) from polluted sites had high frequencies of skeletal deformities, which decreased as distance from polluted areas increased. Whitefish (*Coregonus lavaretus*) from polluted areas had elevated frequencies of deformed gill rakers and of asymmetrical gill rakers and fins. Kessabi et al. (2013) studied skeletal deformities in natural populations of the Mediterranean killifish *Aphanius fasciatus* from the Tunisian coast. Fish were collected from one reference area and three industrialized polluted areas (S2: industrialized coast of Sfax, S3: coast of Khniss and S4: Hamdoun'Oued), and skeletal deformities were diagnosed with double staining. A total of 1,025 abnormalities were quantified, which were classified into categories of abnormalities on spines, vertebrae, arcs and mandibles. In addition, levels of Cd, Cu and Zn, various PAHs, and estrogenic compounds were measured in water and sediment from the different sites. The frequency of spinal deformities was greatest in fish from S2, the site which had significantly higher levels of metals and PAHs than all the others.

7.4 Other Taxa

Larval studies have been performed with various species of cnidarians, mainly corals. The planula is the free-swimming, ciliated larval form of cnidarians. When ready to metamorphose into a coral, it must find a hard substrate (many may prefer specific substrates) where it anchors and grows into a polyp. Echinoderms (sea urchins) are frequently used in standard embryo-larval bioassays. Sea urchins, sand dollars, and brittle stars have a pluteus larva that uses ciliated bands for swimming and suspension feeding. The larva uses its ciliated arms to sweep food into its mouth as it glides through the water. As the larva develops, it increases the number of arms. The body form changes dramatically with metamorphosis. Many larval structures used during planktonic life are lost, and replaced by appendages adapted to the adult's benthic life. Polychaete worm larvae have also been studied. Polychaetes typically hatch into planktonic trochophore larvae, which eventually metamorphose into the adult form by adding segments.

7.4.1 Metals

Coral larvae are sensitive to metals, especially Cu, which reduced settlement success of *Acropora tenuis* larvae after 48-h compared with controls. The 48-h EC_{50} was 35 $\mu g\ l^{-1}$ (Reichelt-Brushett and Harrison 2000), indicating that moderate Cu concentrations impair settlement of coral larvae.

Brix et al. (2012) tested larvae of the sea echinoderm, *Lytechinus variegatus* in an 18-day study in which larvae were continuously exposed to Ag-laden algae (*Isochrysis galbana*). After 7 days, no significant effects were observed on growth up to the highest concentration tested (10.68 $\mu g\ g^{-1}$ dw Ag in algae) but after 18 days, significant effects were observed in all Ag treatments >0.68 $\mu g\ g^{-1}$ dw Ag in algae (corresponding waterborne Ag concentration of 0.05–0.07 $\mu g\ l^{-1}$). However, the dose–response relationship was quite flat with a similar growth inhibition (15 %) in all Ag treatments.

7.4.2 Organics

Oil and Dispersants

Laboratory experiments were conducted to measure larval settlement and metamorphosis of the polychaete *Streblospio benedicti*, as well as juvenile bioaccumulation and growth rates when exposed to sediment-associated PAHs (Chandler et al. 1997). Larval settlement and metamorphosis was reduced, but not significantly (relative to controls) by IX, 5X and 10X background PAH mixture concentrations of the six most abundant PAHs in urbanized Murrell's Inlet South Carolina (1X = 0.9 μg PAH g dry sediment^{-1}). Bioaccumulation of the most abundant PAH, fluoranthene (FL), was very high in this PAH tolerant species—9.5–13.7X FL sediment concentrations after 28-day exposures. Twenty-eight-day exposures to 0.26 and 2.4 μg FL g^{-1} caused no significant mortality, and produced positive weight gains in *S. benedicti* up to 18 d exposure. However, dramatic weight declines occurred from days 18–28 in both FL treatments. High tolerance of PAH may explain why this species recruits and survives in hydrocarbon-contaminated sediments.

Epstein et al. (2000) investigated short-term effects of five oil dispersants (Inipol IP-90®, Petrotech PTI-25®, Bioreico R-93®, Biosolve® and Emulgal C-100®) on planula larvae of the Red Sea stony coral *Stylophora pistillata* and soft coral *Heteroxenia fuscescens*. Larvae were exposed to WSFs, dispersed oil water accommodated fractions (WAFs) and dispersants dissolved in seawater. While WSF reduced settlement success (Fig. 7.6), dispersants produced a greater decrease in settlement. Dispersed oil showed a dramatic increase in toxicity to both species, suggesting that the low oil: seawater ratio 1:200, that was not lethal to *S. pistillata* larvae in the WSF tests, became highly toxic after dispersion. Dispersants and WAF caused deformation, abnormal swimming and tissue degeneration (Fig. 7.7). Authors suggested dispersants not be used near coral reefs.

Pesticides

Markey et al. (2007) examined effects of two organophosphates (chlorpyrifos, profenofos), an organochlorine (endosulfan), a carbamate (carbaryl), a pyrethroid

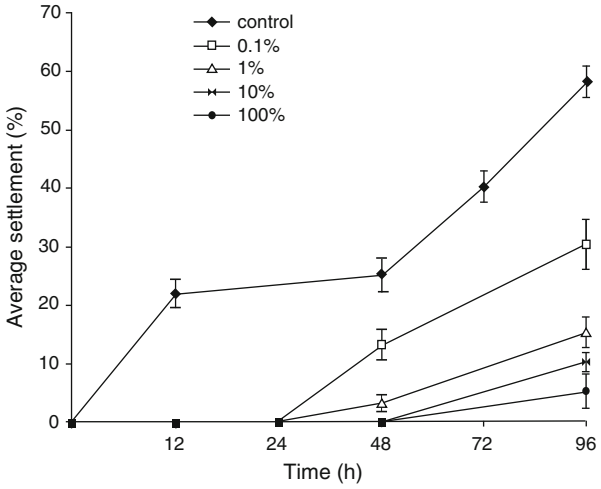

Fig. 7.6 Settlement rates of *S. pistillata* planulae in seawater control and Egyptian crude oil WSF treatments (Reprinted from Epstein et al. 2000: 499, courtesy Elsevier Publishing Co.)

(permethrin), and a fungicide (2-methoxyethylmercuric chloride, MEMC) on embryos and larvae of the coral *Acropora millepora*. Fertilization was not affected by any of the insecticides up to 30 $\mu g\ l^{-1}$, but settlement and metamorphosis were reduced by 50–100 % after 18 h exposure to very low concentrations (0.3–1.0 $\mu g\ l^{-1}$) of each insecticide.

Endosulfan (ES) strongly inhibited larval settlement and early juvenile growth of the polychaete *Streblospio benedicti*. ES concentrations as low as 50 $\mu g\ kg^{-1}$ sediment reduced settlement by >50 % relative to control sediments (Chandler and Scott 1991). Higher concentrations closer to actual field levels suppressed colonization completely. Early growth of newly metamorphosed juveniles was depressed 36 and 40 % in 50 and 100 $\mu g\ kg^{-1}$, respectively. This is in contrast with the tolerance of this species for PAHs (above) and tolerance of harpacticoid copepods. When the benthic harpacticoid copepod *Pseudobradya pulchella* was exposed to sediment ES < 200 $\mu g\ kg^{-1}$, survival and egg production were unaffected. Of the *P. pulchella* tested, >95 % survived 200 $\mu g\ kg^{-1}$, and over 98 % of the females produced normal clutches of eggs. Similarly, survival of another common benthic copepod, *Nannopus palustris*, was not affected below 200 $\mu g\ kg^{-1}$ ES, but 200 $\mu g\ kg^{-1}$ reduced survival.

Contaminants of Emerging Concern

Chiu et al. (2012) investigated effects of flame retardant polybrominated diphenyl ethers (PBDEs) on larvae of the polychaete *Hydroides elegans*, and demonstrated that chronic exposure to BDE-47 (up to 1,000 ng l^{-1}) throughout the entire larval stage did not affect settlement, development or growth despite bioaccumulation.

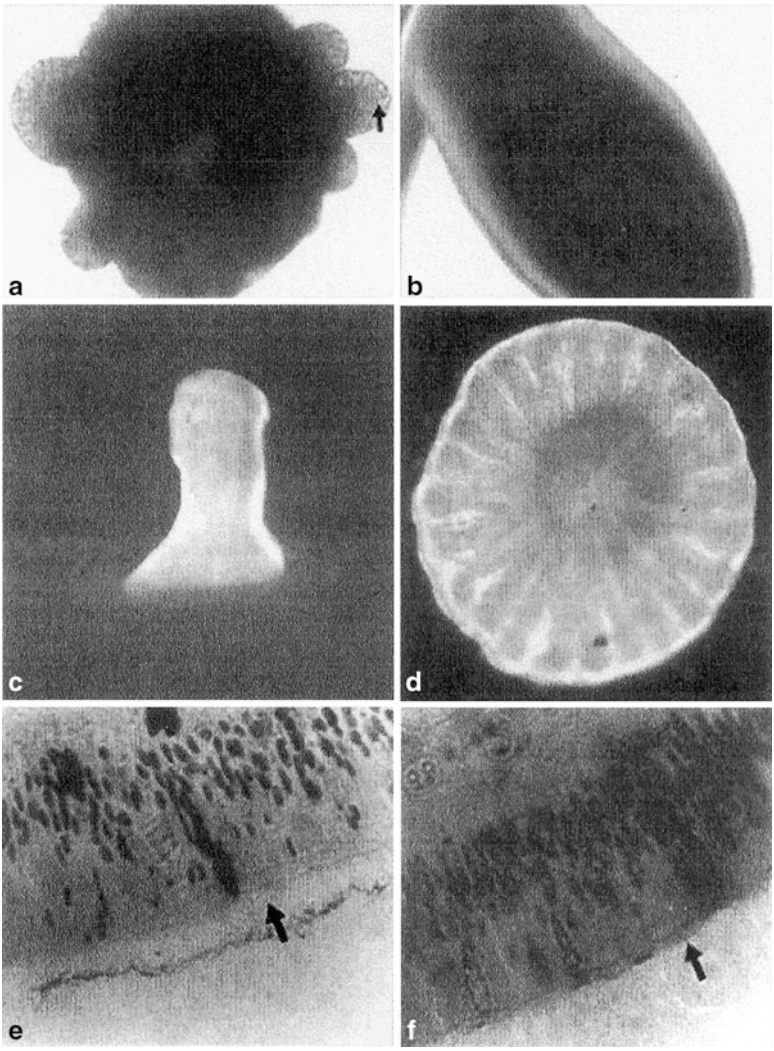

Fig. 7.7 Effects of dispersed oil on *S. pistillata* planulae. (**a**) Disintegration. Release of small spherical bodies through the ectodermal layer (*arrow*) (**b**) Control planula (**c**) deformed primary polyp with no mouth or tentacles (**d**) deformed unattached planula with 12 pairs of septa instead of 6 (**e**) and (**f**) are histological sections from treated larva (**e**) and control (**f**). *Arrows* show ectodermal layer which is damaged in (**e**) (Reprinted from Epstein et al. 2000: 501, courtesy Elsevier Publishing Co.)

7.4.3 Hypoxia

Miller and Graham (2012) made the unexpected finding that low DO had a positive effect on planula larva settlement of the jellyfish *Aurelia* sp. Greatest settlement rates occurred under lowest DO (1.3 mg l^{-1}), indicating that reduced DO promotes settlement. In another set of experiments, they found that survival of scyphistomae (the benthic attached stage) decreased only marginally under prolonged (56 days) hypoxia. Laboratory experiments showed that the normal sessile community coverage was significantly reduced under similar levels of hypoxia when compared to normoxia. Thus, not only was settlement of planulae favored at low DO, but potentially competing species were reduced. These findings support previous ideas that eutrophication and hypoxia can promote outbreaks of jellyfish.

7.4.4 Climate Change/Ocean Acidification

In a field study, artificial collectors were placed for 1 month along pH gradients near CO_2 vents in the Tyrrhenian Sea, Italy to collect newly settled forms (Cigliano et al. 2010). Seventy-nine taxa were identified from six main groups (foraminiferans, nematodes, polychaetes, molluscs, crustaceans and chaetognaths). Calcareous foraminiferans, serpulid polychaetes, gastropods and bivalves were greatly reduced as pCO_2 rose from normal (336–341 mg l^{-1}, pH 8.09–8.15) to high levels (886–5,148 mg l^{-1}) near vent sites (pH 7.08–7.79). The syllid polychaete *Syllis prolifera* was most abundant at the most acidic station, although many polychaetes and small crustaceans could settle and survive. A few taxa (*Amphiglena mediterranea*, *Leptochelia dubia*, *Caprella acanthifera*) were most abundant at sites with intermediate pCO_2 (pH 7.41–7.99), showing that increased pCO_2 can affect the settlement of a wide range of benthic organisms.

Corals

Nakamura et al. (2011) found that the oxygen consumption of planula larvae of the coral *Acropora digitifera* was reduced (but not significantly) with reduced pH (8.0, 7.6, and 7.3). Metamorphosis was significantly decreased under acidified conditions after both short- (2 h) and long- (7 days) term exposure. Larval metabolism and settlement, and post-settlement growth of the coral *Porites astreoides* was studied at ambient seawater, 560, and 800 μatm. Larval metabolism was depressed by 27 and 63 % at 560 and 800 μatm, respectively (Albright and Langdon 2011). Settlement was reduced by 45 % at 560 μatm and 60 % at 800 μatm, but via indirect pathways, i.e. by altering the substrate community composition and reducing settlement cues. Post-settlement growth decreased by 16 and 35 % at 560 and 800 μatm, respectively. Similarly, pCO_2 concentrations of 800 and 1,300 μatm significantly

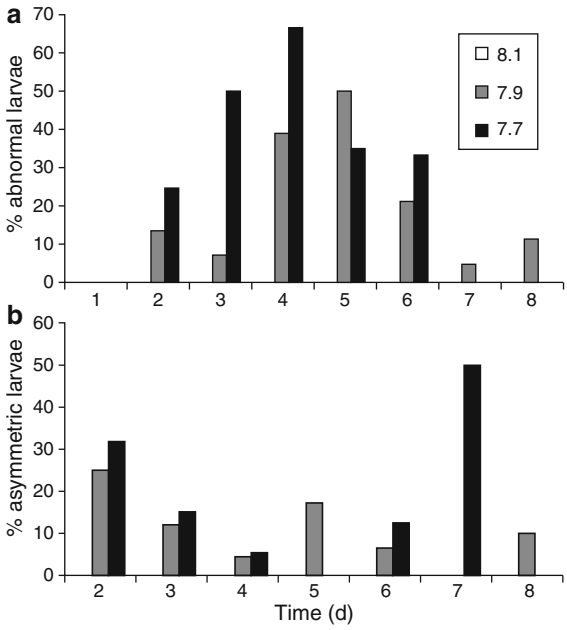

Fig. 7.8 Percentage abnormal (**a**) and asymmetric (**b**) *O. fragilis* larvae at pH 8.1, 7.9, and 7.7. Note absence of abnormalities at pH 8.1 (controls) (Reprinted from Dupont et al. 2008: 293, courtesy Inter-Research)

reduced *Acropora millepora* settlement and crustose coralline algae (CCA) cover by ≥45 % (Doropoulos et al. 2012) (CCA are important for inducing coral settlement). The preferred alga substrate for settlement (*Titanoderma*) of control larvae was avoided by larvae as pCO_2 increased, and other substrates selected. These results suggest acidification may reduce coral populations by reducing coral settlement rates, disrupting larval settlement behavior, and reducing the availability of desirable coralline algal species for coral settlement and recruitment.

Echinoderms

Larval development of the brittlestar *Ophiothrix fragilis* (a keystone species throughout the shelf seas of the eastern Atlantic) was studied by DuPont et al. (2008). A decrease of 0.2 pH units induced 100 % larval mortality within 8 days while control larvae showed 70 % survival. Low pH also resulted in smaller larval size, abnormal development and skeletogenesis including abnormalities, asymmetry, and altered skeletal proportions (Fig. 7.8). The larval development of the sea urchin *Arbacia dufresnei* from a sub-Antarctic population was studied at high (8.0), medium (7.7) and low (7.4) pH (Catarino et al. 2012). Larvae showed a developmental delay at low pH but without increases in abnormalities. Even at calcium carbonate saturation states <1, skeletal deposition occurred. Thus, some polar and sub-polar sea urchin larvae are resilient to acidification.

Larval sand dollars *Dendraster excentricus* have calcified skeletal rods supporting their bodies, and propel themselves with ciliated bands looped around projections called arms. The ciliated bands are used in food capture, and filtration rate is correlated with band length. Therefore, swimming and feeding performance are sensitive to morphological changes. When reared at elevated pCO_2 (1,000 mg l^{-1}), larvae developed significantly narrower bodies at the four and six-arm stages and had significantly smaller stomachs and bodies, suggesting reduced feeding ability (Chan et al. 2011).

Sea urchins (*Heliocidaris erythrogramma*) were reared by Byrne et al. (2011) in varying temperature and pH: +2–4 °C, and pH 7.6–7.8. Treatments resulted in unshelled larvae and abnormal juveniles, with the percentage of normal juveniles decreasing in response to both stressors. The number of spines decreased with increasing acidification, and the interactive effect between stressors indicated that +2 °C warming reduced the negative effects of low pH, which may be good news for the future of this species, since warming and lower pH will happen together. To investigate effects of acidification on calcification at the molecular level, Kurihara et al. (2012) evaluated the expression of biomineralization-related genes in the sea urchin *Hemicentrotus pulcherrimus* at control, 1,000, and 2,000 mg l^{-1} CO_2 from egg to pluteus larva. They found that the expression of the gene *msp130*, which is proposed to transport Ca^{2+} to the calcification site, is suppressed by increased CO_2. This suggests that OA suppression of the expression of skeletogenesis-related genes is responsible for impaired biomineralization of sea urchins.

7.5 Conclusions/Discussion

Larvae are very sensitive to contaminants, in some cases more so than embryos. The studies that have been done are numerous, and are a "mixed bag." While many studies to date focus solely on measurements of mortality (i.e. LC50 in standard toxicity tests), other (more interesting) studies examine sublethal effects on growth, development, behavior, physiological processes, etc. Studies of sublethal effects on these processes enable one to understand the ways in which a particular stressor affects the larvae, and may give insight and understanding into possible mechanisms of larval mortality. The taxa that have been used in these studies tend to be ones for which techniques have been developed and standardized for raising them in the laboratory, but techniques can be developed for culturing additional species. This should be encouraged and would be a significant advance for the field, as responses and sensitivities vary considerably among different taxa. Settlement and metamorphosis to juvenile stages is critical in many taxa, and is often the stage most sensitive to stressors. Furthermore, some effects of larval exposures are not apparent until after larvae have metamorphosed and have become juveniles or adults. This emphasizes the point that long-term studies are far more useful and important than short term larval toxicity tests.

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