CHAPTER 14

Latent Effects: Surprising Consequences of Embryonic and Larval Experience on Life after Metamorphosis

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14.1 Introduction and Definitions

Metamorphosis is typically a time of rapid transition—in body plan, physiology, lifestyle, and habitat. An old world and lifestyle are left behind, and a new world is opened. That is certainly how metamorphosis was presented to me as a student: a new life, a new beginning. As Lahiri (2015, p. 35) wrote, "Metamorphosis is a process that is both violent and regenerative, a death and a birth." But, as it turns out, there is growing recognition of the links between experiences in one life history stage and phenotypes exhibited in later stages (Marshall et al., 2003; Allen et al., 2006; Padilla and Miner, 2006; Marshall and Morgan, 2011; Flores et al., 2013; Burggren, 2014; Burton and Metcalfe, 2014; Ross et al., 2016). In some cases, for example, juvenile growth, survival, or other performance criteria are relatively poor following short-term, sublethal stresses experienced by larvae, even when the larvae seem to have completely recovered from the stress before metamorphosing (e.g., Pechenik, 2002; Chiu et al., 2008). Additional examples of such "latent effects" (Pechenik, 2006) are given in the material that follows. There is growing evidence that such effects are not simply artifacts of laboratory rearing: latent effects have now been documented for juveniles transplanted into the field, for a number of species in a number of taxonomic groups (e.g., bryozoans: Ng and Keough, 2003; ascidians: Marshall et al., 2003; barnacles: Emlet and Sadro, 2006; Thiyagarajan et al., 2007; gastropods: Chiu et al., 2007; bivalves: Hettinger et al., 2013a). Such responses are of growing importance as the aquaculture industry expands, and as the quality of life in the marine environment changes due to global warming, ocean acidification, changing rainfall patterns, and increased pollutant input. They may also help to explain at least some of the variation seen in growth rates among individuals of the same species recruiting to the same locations, and give us new incentive to determine the degree to which parental care (e.g., brooding) can protect against such impacts. As discussed later, brooding can sometimes expose developing embryos to some environmental stresses even while protecting them from exposure to others (Chaparro et al., 2014; Segura et al., 2014).

The delayed responses just referred to have been defined as "latent effects" because they show up later in development, after metamorphosis (reviewed by Pechenik, 2006). To the extent that stress-induced poor performance in the larval stage simply carries over to poor performance of the same sort (e.g., growth rates) in juveniles, these can also be considered to be "carryover effects" (Marshall et al., 2003; Wendt and Johnson, 2006; Hettinger et al., 2012), although such terminology has been used for a very different purpose both in the medical literature (reviewed by Pechenik, 2006) and the ecological literature (reviewed by O'Connor et al., 2014).

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But what do we call the effects of stress that cause reduced larval growth throughout the larval stage and also result in reduced juvenile growth under ideal conditions after metamorphosis, when metamorphosis involves a pronounced switch in the food-collecting machinery—for example, when the food-collecting velum of a gastropod larva is replaced after metamorphosis by a food-collecting gill, or a food-collecting radula? If the reduced juvenile growth rates are caused by flawed or slowed development of the new feeding apparatus, I would refer to these as latent effects as well. If they are caused by flaws (e.g., in digestive physiology) initiated in the larval stage and maintained in the juvenile, I would refer to those as carryover effects. In all of these cases, the responses could also be called "legacy effects" (Padilla and Miner, 2006), as they represent cases of juvenile or adult performance being influenced by experiences in previous stages of the life history: the juveniles or adults bear the legacy of earlier experience. The term "legacy effects" could also apply to the effects of maternal or paternal experience—often relating to nutritional stress or to the presence of predators or predator cues—on offspring performance (Allen et al., 2006; Burggren, 2014; Soubry, 2015; Long et al., 2016; Ross et al., 2016); studies concerning such transgenerational effects (e.g., Marshall, 2008) will not be discussed in this review.

This review primarily concerns latent effects—effects that show up after metamorphosis but that result from stresses experienced before metamorphosis—and focuses exclusively on the marine invertebrate literature. Related findings have also been reported for birds, lizards, amphibians, insects, and even humans (Bresnahan and Susser, 2007; Stamper et al., 2009; Anderson et al., 2013; Zambonino-Infante et al., 2013; Jonsson and Jonsson, 2014; Bouchard et al., 2015; Whiteside et al., 2015; Wang et al., 2016a). I reviewed the literature on this topic about ten years ago (Pechenik, 2006). Of the 45 entries listed in that paper's Table 1, nearly one-third (14 studies) concerned vertebrates, and two concerned insects. For the remaining studies on marine invertebrates, 15 concerned latent effects resulting from delayed metamorphosis, ten concerned the post-metamorphic effects of reduced food levels experienced during larval development, three concerned the effects of pollutants, and one paper each concerned the effects of reduced salinity and differences in food quality. The present review includes studies on the effects of several newly tested stressors, including ocean acidification, hypoxia, and dietary quality (Table 14.1). I also now include several recent studies on the latent

Table 14.1 Summary of Recent Research on Latent Effects in Marine Invertebrates.

Taxon	Species	Stress	Parameter measured	Latent effect	Reference
Cnidaria	*Porites astreoides*	H_2O_2 oxidative stress, 24 h	Survival in field	No	Ross et al., 2012
		Thermal stress: 3°C, 24 h		Yes	
	Montastraea faveolata	Low salinity stress	Juvenile survival	Yes	Vermeij et al., 2006
	Acropora tenuis	Delayed metamorphosis: 2, 4, 6 wk	Colony development, survival	No	Graham et al., 2013
Gastropoda	*Crepidula fornicata*	Phytoplankton species	Juvenile growth	Sometimes	Pechenik and Tyrell, 2015
		Food level	Juvenile growth	Yes	
	Crepidula fornicata	Low salinity: 12, 24, 48 h	Juvenile growth	No	Diederich et al., 2011
	Crepidula fornicata	Low salinity and thermal stress	Juvenile growth	Sometimes	Bashevkin and Pechenik, 2015
	Crepidula onyx	Low salinity: 12, 24, 48 h	Juvenile growth	No	Diederich et al., 2011
		Food level	Juvenile growth	Yes	Chiu et al., 2007
			Juvenile survival	Sometimes	
			Juvenile filtration rates	Yes	

(continued)

Table 14.1 (*Continued*)

Taxon	Species	Stress	Parameter measured	Latent effect	Reference
			Juvenile carbon assimilation	No	
		Short-term starvation	Juvenile survival	No	Chiu et al., 2008
			Juvenile growth	Yes	
		Hypoxia at 2 food levels, 8–10 d	Juvenile growth, filtration rates	Yes (low food)	Li and Chiu, 2013
	*Crepipatella dilatata**	Hypoxia: 24, 48, 72 h	Juvenile survival	Yes	Segura et al., 2014
			Juvenile growth	Yes	
	Crepipatella peruviana	Low salinity: 12, 24, 48 h		No	
	Crepipatella peruviana	Low salinity: 6 h	Size at metamorphosis	Yes	Montory et al., 2016
			Juvenile growth	No	
			Feeding, respiration rates	No	
Bivalvia	*Haliotis diversicolor*	Delayed metamorphosis, 3–11 d	Juvenile growth, survival	Yes	Onitsuka et al., 2010
Polychaeta	*Capitella teleta**	Hypoxia: 24, 36, 48, 72, or 96 h	Juvenile survival	No, negatively	Pechenik et al., 2016
			Juvenile growth	Possible positive	
		Salinity: 24, 48, or 96 h	Juvenile survival	Sometimes	
	Hydroides dianthus	Food level, 10 d	Juvenile mortality	Yes	Allen and Marshall, 2010
			Juvenile growth	Yes	
Crustacea	*Balanus amphitrite*	Delayed metamorphosis, 0–3 d	Juvenile growth	Yes	Thiyagarajan et al., 2007
		Low salinity, 0–3 d		Yes	
	Balanus glandula	Food level	Juvenile growth	Some	Emlet and Sadro, 2006
				Some	
	Carcinus maenas	Differences in presumed food levels experienced in the field	Juvenile survival, growth	Yes	Rey et al., 2016
Echinodermata	*Echinometra* (4 spp.)	Delayed metamorphosis, 5 months	Juvenile growth, survival	Yes	Rahman et al., 2014
Urochordata	*Ciona intestinalis*	Delayed metamorphosis, 3 d	Post-metamorphic growth	No	Jacobs et al., 2008
		Delayed metamorphosis, 7 d		Yes	
	Molgula socialis	Delayed metamorphosis, 2 d	Post-metamorphic growth	Yes (increased)	Jacobs et al., 2008
	Ascidiella aspersa	Delayed metamorphosis, 3–4 d	Post-metamorphic growth	No	Jacobs et al., 2008

*indicates studies in which brooded embryos, rather than larvae, were stressed

impact of stresses experienced by brooded embryos (Table 14.1). Interest in latent effects has been growing. Perhaps even more importantly, some marine invertebrates may make especially good models for understanding the mechanisms through which latent effects are mediated.

14.2 Latent Effects of Exposure to Toxicants

Only two papers concerning latent effects resulting from pollution stress were cited in my previous review, and surprisingly little additional work

has been published for marine invertebrates on that topic in the intervening ten years. Exposing young larvae of the oyster *Crassostrea gigas* to the widespread aquatic pollutant nonylphenol at the initial low concentrations of 1 µg l^{-1} and 100 µg l^{-1} impacted adult reproductive biology many months later, and in unexpected ways (Nice et al., 2003). Seven-day-old larvae were exposed to the pollutant for 48 h and then returned to control conditions for the rest of their larval development. The spat were then reared to adulthood under control conditions and examined ten months later, after which eggs and sperm were mixed to obtain a next generation of larvae. Although there was no measureable impact of previous larval exposure at either concentration on juvenile growth rates over the 10-month rearing period, there was a marked increase in the proportion of hermaphrodites in the population, and a significant and dramatic reduction in larval survival in the next generation 48 h after successful fertilization, even when one of the parents included in a cross was a control individual. On the other hand, exposing larvae of the Mediterranean sponges *Crambe crambe* and *Scopalina lophyropoda* to cadmium (5 µg l^{-1}) or copper (30 µg l^{-1}) for up to seven days had no effect on juvenile survival over the following six months (Cebrian and Uriz, 2007).

More studies seem warranted, using a greater variety of toxicants and a greater range of species and taking into consideration the impact of other stressors experienced during and after larval development (Byrne and Przeslawski, 2013). Future studies should also investigate the potential for latent effects caused by the ingestion of microplastics during larval development (Galloway and Lewis, 2016). In addition, not all toxicants are produced or released by humans; the frequency of harmful algal blooms seems to be increasing in many parts of the world (Gilbert et al., 2014). Future studies could examine the potential impact of exposing larvae to sublethal concentrations of algal toxins on post-metamorphic fitness.

14.3 Latent Effects following Hypoxia

As hypoxic conditions become increasingly widespread in coastal marine environments around the world, due to climate change and the increasing output of organic wastes and agricultural nutrients (Wu, 2002; Zhang et al., 2013), interest in the potential consequences of spreading hypoxic stress continues to grow. For the calyptraeid gastropod *Crepidula onyx*, exposing well-fed (2×10^5 cells ml^{-1}) larvae to oxygen levels as low as 2 mg O_2 l^{-1} for eight to ten days had no significant impact on the growth rates of field-transplanted juveniles (Li and Chiu, 2013). However, when larvae of this species were exposed to the same level of low-oxygen stress but at reduced food levels (1×10^5 cells ml^{-1}), subsequent juvenile growth rates and filtration rates were significantly reduced compared with those exhibited by control individuals, even though there were no significant differences in the size or lipid content of juveniles from the different treatment groups at metamorphosis (Li and Chiu, 2013). Thus latent effects were observed following the exposure of larvae to the combined stresses of low food and reduced oxygen, but were not observed if the larvae had been well-fed when exposed to the same levels of hypoxic stress.

Although most studies documenting effects that cross the metamorphic boundary concern stresses experienced during larval life, several researchers have recently begun examining the post-metamorphic impact of environmental stresses experienced by encapsulated or brooded embryos (e.g., hypoxia and increased ammonia accumulation in brood chambers). Although brooding is generally viewed as protective (Gillespie and McClintock, 2007; reviewed by Pechenik, 1979; Noisette et al., 2014), brooding can, in some situations, expose developing embryos to stresses they would otherwise avoid by being free-living, something that has only recently been considered for marine invertebrates (Chaparro et al., 2009; 2014; Segura et al., 2014).

Calyptraeid gastropods, for example, brood their encapsulated embryos within the female mantle cavity for at least several weeks before releasing larvae or metamorphosed juveniles, depending on the species, into the surrounding seawater (Collin, 2004). Normally the females bring seawater into the mantle cavity and flush out older seawater and wastes through the actions of gill cilia (reviewed by Chaparro et al., 2002; Shumway et al., 2014). However, in the Quempillén River estuary in southern Chile, ambient salinity frequently drops below a level (22 psu) at which

females completely seal themselves off from the ambient environment (Chaparro et al., 2009); such isolation from the surroundings occurs frequently during peak reproductive activity at this site, and can last for at least three days at a time (Chaparro et al., 2009). Although such isolation by the mother protects developing embryos from exposure to severe salinity stress (Chaparro et al., 2009), it soon exposes them to greatly reduced oxygen levels. Indeed, with only one to two milliliters of fluid in the adult mantle cavity, oxygen levels typically fall below 1 mg O_2 l^{-1} within a few hours of isolation from the external environment (Chaparro et al., 2014). When brooded embryos of the direct-developing gastropod *Crepipatella dilatata* were exposed to oxygen levels <1 mg O_2 l^{-1} for 24 to 72 h, there was no effect on the average size at which juveniles emerged from the female, but there was a pronounced decline in subsequent juvenile growth rates under control conditions over the subsequent four-week monitoring period, regardless of how advanced the embryos had been when exposed to the stress; indeed, final mean juvenile shell length was approximately 20% less than that of control individuals for those exposed to severe hypoxia for even just 24 h as brooded embryos, and more than 50% less for those that had been exposed to hypoxia for 72 h (Segura et al., 2014). A single 24 h exposure of juveniles to hypoxic stress was also enough to significantly reduce juvenile survival over the first 30 days after their release from the mothers.

In contrast, exposing brooding females of the deposit-feeding polychaete *Capitella teleta* to oxygen concentrations <1 mg O_2 l^{-1} for up to 96 h (the maximum period tested) had no effect on mean numbers of larvae emerging from brood tubes, mean time to emergence, mean juvenile survival or growth rates, or on subsequent fecundity or time to reproductive activity in the next generation (Pechenik et al., 2016). Whether these results reflect the benefits of brood protection or simply a high embryonic tolerance to these stresses is an open question.

14.4 Latent Effects from Food and Nutrient Limitation

A number of papers have considered how food availability in the larval stage might impact post-metamorphic performance. For the calyptraeid gastropod *Crepidula onyx*, for example, starving larvae of certain ages for two days or rearing the larvae to metamorphic competence at a low phytoplankton concentration (1×10^4 cells ml^{-1} instead of 20×10^4 cells ml^{-1}) both resulted in significantly reduced juvenile growth: final juvenile shell sizes were up to 54% smaller than those of control individuals that had been reared as larvae at the higher food concentration, for both laboratory-reared and field-transplanted juveniles (Chiu et al., 2007; 2008). In neither study did larval nutritional experience influence subsequent juvenile survival in the laboratory, but feeding the larvae at the lower food concentration did result in higher mortality for field-transported juveniles (Chiu et al., 2007).

Similarly, rearing nauplius larvae of the barnacle *Balanus glandula* on a low concentration of the diatom *Skeletonema costatum* decreased mean juvenile growth rates and also decreased the survival of juveniles that had been transplanted to field sites for at least the first two to six days after metamorphosis, when most of the mortality occurred (Emlet and Sadro, 2006). Field-transplanted juveniles of the tube-dwelling polychaete *Hydroides diramphus* also showed reduced survival if the larvae had been reared at lower food concentrations (Allen and Marshall, 2010).

When larvae of the gastropod *Crepidula fornicata* were reared at a low concentration of the flagellate *Isochrysis galbana* (clone T-ISO, 1×10^4 cells ml^{-1}) for two or four days early in development, no latent effects on subsequent post-metamorphic growth rates were detected (Pechenik et al., 2002). However, when larvae of this species were reared at this same low food concentration for their entire larval development, juveniles fed ad libitum as their reward for metamorphosing grew about 37% more slowly than control juveniles for the first three days after metamorphosis, and nearly 45% more slowly for the first six days after metamorphosis (Pechenik and Tyrell, 2015).

The post-metamorphic impact of differences in nutritional quality during larval development has recently been explored for marine invertebrates, apparently for the first time. In contrast to either starving larvae for a period of time or rearing them at a greatly reduced food concentration, Pechenik and Tyrell (2015) reared the larvae of *Crepidula*

fornicata at high concentrations of three different algal diets that generally supported good survival but at different rates of growth: *Isochrysis galbana*, (clone T-ISO; the diet producing the fastest larval growth), *Dunaliella tertiolecta* (clone DUN), and *Pavlova lutheri* (clone MONO). Following metamorphosis, all juveniles were reared on the diet producing the fastest growth, T-ISO. In two experiments, larvae reared on one of the nutritionally poorer diets resulted in substantially reduced mean post-metamorphic growth rates. However, in other experiments larval diet had no effect on mean juvenile growth rates despite substantial differences in the mean growth rates of larvae that had been reared on the different diets. Implications of these studies seem especially intriguing, as climate change and ocean acidification appear to be shifting both phytoplankton species ranges and the nutritional composition of individual phytoplankton species (Hinga, 2002; Tortell et al., 2002; Hayes et al., 2005; Rossoll et al., 2012; Hettinger et al., 2013b; Leu et al., 2013; Wynne-Edwards et al., 2014).

It should be noted that even though some researchers have transplanted juveniles to field sites for further monitoring once metamorphosis has occurred (Emlet and Sadro, 2006; Thiyagarajan et al., 2007; Allen and Marshall, 2010; Diederich et al., 2011; Graham et al., 2013; Hettinger et al., 2013a), the larvae have always been stressed in the laboratory. Typically, feeding larvae are reared on one or several different phytoplankton species. Even high food concentrations of phytoplankton species producing the best growth in the laboratory may be imposing stress on larvae. In the field, larvae will probably have access to many dozens of phytoplankton species at any one time. Thus, what appears to be an ideal diet in the laboratory may in fact be suboptimal, compared to what larvae are ingesting in nature. Indeed, larvae of *Crepidula fornicata* collected from the field typically grew much faster in the laboratory over the next several days than larvae that had hatched in the laboratory and were then reared on the same unigal diet (*Isochrysis galbana*, clone T-ISO) (Pechenik and Levine, 2007), suggesting that the field-collected larvae were somehow healthier than those obtained and reared in the laboratory. Hatchery operators might try rearing juveniles obtained from field-collected larvae. It would also be interesting to examine the consequences of nutritional stress for larvae reared under field conditions; this might be feasible for larvae that are large at hatching, such as those of *Crepidula fornicata*, which typically hatch at shell lengths between about 400 to 450 µm (Pechenik, 1984; Pechenik et al., 1996a; b; Henry et al., 2010; Pechenik and Tyrell, 2015). Along these lines, larvae of the green crab *Carcinus maenas* were collected in the field during upwelling events of different magnitudes, allowed to metamorphose in the laboratory, and were then reared in the laboratory through the fifth juvenile instar (Rey et al., 2016). Larvae that seem to have experienced better food conditions in the field showed higher juvenile survival and better juvenile growth in the lab than those that had been collected during other larval supply events and reared under the same conditions (Rey et al., 2016).

14.5 Latent Impact of Salinity Stress

In a study by Thiyagarajan et al. (2007), cyprids of the barnacle *Balanus amphitrite* were exposed to the low salinity of ten psu for 24 h before the larvae were allowed to metamorphose. Even though there was no measureable impact of the treatment on cyprid lipid reserves, subsequent mean juvenile growth rates were up to 70% lower than those of control animals for at least the next five days, in both the laboratory and the field. Exposing larvae to reduced salinities also impacted post-metamorphic development for the South American suspension-feeding gastropod *Crepipatella peruviana* (Montory et al., 2016). Veligers of that species were exposed in the laboratory to salinities as low as 15 psu for only six hours, matching the salinity reductions recorded from local tide pools during a heavy rain during a single low tide. Clearance rates were reduced for the first five days after metamorphosis for individuals exposed to 15 psu as larvae, and mean juvenile shell sizes were still significantly smaller than those of control individuals ten days later. Those individuals that had been exposed to a salinity of 15 or 20 psu as larvae also showed an approximately 20% decrease in subsequent juvenile survival.

In contrast, Diederich et al. (2011) exposed larvae of three calyptraeid gastropods (*Crepidula fornicata, Crepidula onyx, and Crepipatella peruviana* [formally,

Crepipatella fecunda]) to salinities of 30 (control), 20, 15, or 12 psu, for 24 or 48 hours, and found no effects on subsequent mean juvenile growth rates for any of the three species, even when larval growth rates failed to recover to control levels after the stress period had ended. There was no juvenile mortality for any of the three species tested in that study. Longer exposures of larvae to reduced salinity did produce significant latent effects on juvenile growth in *Crepidula fornicata*, however, in four of the six experiments conducted by Bashevkin and Pechenik (2015), and the effects were generally negative. In one of their experiments, however, the effect was positive: juveniles grew faster than controls at reduced salinity (20 psu) if the larvae had been reared at that same reduced salinity. Again, larval and juvenile mortality were both very low in those studies.

When brooding females of the deposit-feeding polychaete *Capitella teleta* were exposed to salinities as low as ten psu for 24, 48, or 96 h, dramatically fewer larvae subsequently emerged from the brood tubes over the following weeks, suggesting substantial pre-hatching mortality resulting from exposures as short as 24 h at salinities as high as 20 psu (Pechenik et al., 2016). Surprisingly, however, all but the longest duration of low-salinity exposure (and, thus, the most intense level of salinity stress) imposed on brooding females had no subsequent detrimental effects on juvenile survival or juvenile growth, for those larvae that did survive to metamorphose. Indeed, exposing brooding females to a salinity of 25 psu for 96 hours significantly increased post-metamorphic survival of the larvae that were released. In contrast, stressing the larvae of *C. teleta* at low salinities for as little as 24 h significantly reduced post-settlement survival and juvenile growth rates in a previous study (Pechenik et al., 2001).

Exposing brooding females of the direct-developing gastropod *Crepipatella dilatata* to a reduced ambient salinity of ten psu for 72 h resulted in reduced juvenile oxygen consumption rates, clearance rates, and growth rates that were still apparent four weeks after metamorphosis (Chaparro et al., 2014). However, these latent effects were most likely due to the impact of mothers sealing their mantle cavities off from the surrounding seawater, with consequent changes in mantle cavity oxygen levels, ammonia concentrations, and pH, rather than to a direct effect of reduced salinity.

14.6 Latent Effects of Delayed Metamorphosis

Planktonic marine invertebrate larvae must typically develop for a time, usually ranging from hours to weeks, before becoming competent to undergo metamorphosis (reviewed by Pechenik, 1990). Once competent, metamorphosis can be delayed for hours, days, weeks, or months in different species in the absence of appropriate environmental cues, or in the presence of inhibitory factors (Pechenik, 1990; Marshall et al., 2003). Nearly half the studies concerning latent effects that were reviewed previously (Pechenik, 2006) dealt with effects of delayed metamorphosis. The following five more recent papers add to the previous theme that delayed metamorphosis produces substantial latent effects in some species but not others, and that effects are sometimes seen among the progeny of some parents but not among the progeny of other parents within the same species.

Delaying metamorphosis of the abalone *Haliotis diversicolor* (Onitsuka et al., 2010) for up to 12 or 17 days and of four sea urchin species in the genus *Echinometra* (Rahman et al., 2014) from one to five months decreased both juvenile survival and juvenile growth rates significantly. Larvae of *Haliotis diversicolor* are lecithotrophic; in contrast, those of *Echinometra* are planktotrophs, and were fed the diatom *Chaetoceros gracilis* during the delay period. Similarly, delaying cyprid metamorphosis for the barnacle *Balanus amphitrite* by just three to four days resulted in reduced growth rates of juveniles that were transplanted to two different field sites (Thiyagarajan et al., 2007). In contrast, whether the nonfeeding larvae of the coral *Acropora tenuis* were allowed to metamorphose two, four, or six weeks after fertilization had no significant detrimental impact on post-settlement survival or time to initiate colony formation in field-transplanted individuals (Graham et al., 2013); indeed, budding began sooner in the four-week-old cohorts than in those that had been induced to metamorphose two weeks earlier. Finally, delaying metamorphosis for seven days

reduced mean juvenile growth rates for the solitary seasquirt *Ciona intestinalis* in studies by Jacobs et al. (2008), but had no effect on the two other ascidian species tested in the same study.

14.7 Latent Effects of Ocean Acidification

The data indicating declines of approximately 30% in pH in the world's oceans during the past several hundred years have become increasingly convincing, and alarming (Doney et al., 2009; Byrne, 2011; Barton et al., 2012; Kroeker et al., 2013), and the cause seems unassailable: excessive release of CO_2 into the atmosphere from a range of human activities including power production; manufacturing; fuel-consuming air, sea, and land transportation; cement production; and deforestation (Doney et al., 2009; Friedlingstein et al., 2014). Approximately one-third of that excess CO_2 has been absorbed by the world's oceans, resulting in a marked increase in seawater acidity despite the presence of a bicarbonate buffering system (Pörtner, 2008; Doney et al., 2009). By the year 2100, the average pH in the world's oceans is expected to drop from 8.1 to about 7.7–7.8 (Doney et al., 2009; IPCC, 2013).

Although a good number of studies have examined the effects of ocean acidification (OA) on aspects of larval growth and development (reviewed by Byrne and Przeslawski, 2013; Hettinger et al., 2013b; see also Byrne et al., this volume), and a number of studies have considered the effects of maternal exposure to reduced pH on offspring responses (e.g., Parker et al., 2012; Pansch et al., 2014; reviewed by Ross et al., 2016), few studies have so far considered how experiencing OA stress early in development might influence post-metamorphic fitness. In one study of such latent effects, larvae of the sea urchin *Strongylocentrotus droebachiensis* were reared to metamorphosis at pH 7.7 (control = 8.1) (Dupont et al., 2013). The resulting juveniles grew more quickly over the next three months when reared at the reduced pH than when reared at the control pH (~ 0.29 mm $month^{-1}$ vs. ~0.16 mm $month^{-1}$). Post-metamorphic mortality was especially high (about 95%) for animals in that treatment, however, so it's not clear whether larval experience really promoted a greater tolerance of reduced pH after metamorphosis or whether the results reflect the preferential survival of faster-growing juveniles. Similar results were obtained for the bay scallop *Argopecten irradians*, but again, the higher rates of subsequent juvenile growth following larval exposure to the stress may reflect differential survival of particular genotypes rather than a true latent effect (Gobler and Talmage, 2013).

In contrast, clear detrimental latent effects resulting from larval exposure to acidification stress were reported by Hettinger et al. (2012; 2013a) for the Olympic oyster *Ostrea lurida*. When larvae were reared to metamorphosis at pH 7.8, juvenile growth rates in the laboratory for the first week after metamorphosis were about 26% lower than those of control juveniles that had been reared as larvae at pH 8.1, regardless of whether juveniles were reared under the control or reduced pH conditions (Hettinger et al., 2012). Similar effects of larval pH experience were subsequently documented in juveniles that had been transplanted to field situations following metamorphosis (Hettinger et al., 2013a). The impact of being reared at reduced pH during larval life persisted for at least four months after metamorphosis in that latter study, with no indication of compensatory growth by individuals that had experienced the stressful pH as larvae (Hettinger et al., 2013a). Intriguingly, the effects on juvenile growth were not mitigated by transferring individuals to more benign field conditions following metamorphosis. Additional studies investigating the potential for latent effects following larval exposure to ocean acidification seem warranted.

14.8 Latent Impact of Thermal Stress

Few studies have examined the effects of early thermal stress on the post-metamorphic development of marine invertebrates. Ross et al. (2013) exposed larvae of the coral *Porites astreoides* to elevated temperature (elevated by 3 °C, to 30 °C) for 24 hours, and later transplanted the metamorphosed spat to a field site. Temperature stress experienced during the larval stage significantly increased post-metamorphic mortality ($p = 0.044$) over the following 24 days, although mortality of control individuals was also quite high (90%).

14.9 Caution in Interpreting Latent Effects

Interpreting data from latent effects studies can be complicated by developmental mortality, as alluded to earlier and as noted by Marshall and Morgan (2011). If larval or post-metamorphic mortality is substantial, then what appear to be latent effects on juvenile growth, adult fecundity, or other measures could reflect differential survival of certain genotypes rather than a shift in individual morphological development or physiology. Mortality of laboratory-reared larvae and juveniles can be quite high, even under what appear to be ideal rearing conditions (Yund and McCartney, 2016), and if that mortality is not random then results may be difficult to interpret. Indeed, as mentioned in a previous section, the favorable effects of rearing larvae at reduced pH on the growth of juveniles reared at the same low pH reported for the sea urchin *Strongylocentrotus droebachiensis* (Dupont et al., 2013) and the bivalve *Argopecten irradians* (Gobler and Talmage, 2013) could have been mediated by genotype-specific differential juvenile mortality before growth measurements were made; juvenile mortalities for individuals in this treatment were extremely high (up to 95%). Thus, the observed more rapid growth of juveniles that had been stressed in that treatment as larvae might reflect the differential survival of the most rapidly growing juveniles, which is certainly an intriguing possibility.

However, latent effects have also been documented in cases where there was negligible larval or juvenile mortality, in which case the effects must be due to something more interesting. Such species may be especially useful models for studying the underlying basis for latent effects. The larvae and juveniles of *Crepidula fornicata*, for example, can be routinely reared in the laboratory with 0 to 7% mortality (Pechenik and Lima, 1984; Pechenik and Tyrell, 2015; Bashevkin and Pechenik, 2015); the larvae are large at hatching (typically 400–450 µm in shell length) and grow quickly; can be easily induced to metamorphose when competent (Pechenik and Gee, 1993); and grow rapidly following metamorphosis (up to about 200 µm per day for the first week or so after metamorphosis) with high survival (Pechenik, 1984; Pechenik et al., 1996a; Bashevkin and Pechenik, 2015; Pechenik and Tyrell, 2015). This is not the first time that *C. fornicata* has been suggested as a promising research model (Henry et al., 2010).

Juvenile mortality for the Olympia oyster *Ostrea lurida* was also low in the laboratory experiments of Hettinger et al. (2012), again implying that the decreased mean post-metamorphic growth rates observed for individuals that had experienced reduced pH stress as larvae have a basis other than differential juvenile mortality, although it does not rule out the possibility of selective mortality during larval development. In a subsequent study (Hettinger et al., 2013a), the researchers found that the pH experienced as larvae significantly reduced juvenile growth rates in field-transplanted individuals even four months post-settlement, again without the larval treatment having had a significant differential impact on juvenile mortality.

14.10 Consequences of Larval Stress are Not Always Negative

Most of the latent effects of larval experience that have been reported to date have been negative, with juvenile or adult survival or performance being reduced in some way. The previously mentioned study with corals (Graham et al., 2013), in which budding began sooner in a cohort from larvae that had had their metamorphosis delayed for two weeks longer than those from another cohort, is one exception. Similarly, in one experiment with the polychaete *Capitella teleta* (Pechenik et al., 2016), brooded embryos that had been exposed to hypoxic stress (1 ml O_2 l^{-1}) for 36 hours grew more quickly following metamorphosis than those that had experienced shorter periods of stress during brooding. A similarly intriguing result has been reported for male zebra finches, the first result of its kind for any vertebrate: male birds that were stressed as nestlings were later found to have a higher reproductive fitness than those that were not stressed during early development (Crino et al., 2014).

In some other experiments, the outcome of being stressed in the larval stage depended on whether juveniles were also reared under stressful conditions. For example, in one experiment with *Crepidula fornicata*, larvae that had been reared at the

reduced salinity of 20 psu showed significantly faster growth as juveniles when maintained at the same low salinity after metamorphosis, compared with the growth of juveniles that had been reared as larvae at 30 psu and then transferred to the lower salinity after metamorphosing (Bashevkin and Pechenik, 2015); it appears that rearing larvae at that low salinity pre-acclimated individuals for successful development at that salinity. As mentioned earlier (see Section 14.7), similar results were obtained for the sea urchin *Strongylocentrotus droebachiensis* when larvae were reared at the low pH of 7.7 and juveniles were reared under the same conditions (Dupont et al., 2013), and for the scallop *Argopecten irradians* (Gobler and Talmage, 2013), although the extent to which the results of those two studies reflect selective post-metamorphic mortality rather than true latent effects is not clear. A number of studies with insects and vertebrates have also found that stresses experienced during early development can induce adult phenotypes to tolerate similar environmental stresses better (reviewed by Wang et al., 2016b). Future studies with marine invertebrates might focus specifically on the extent to which stressful larval experiences pre-adapt juveniles or adults for stressful conditions after metamorphosis. The likelihood of such findings would seem to be greater the more that larval environmental conditions anticipate those likely to be experienced by juveniles or adults.

14.11 Mechanisms Accounting for Latent Effects

The mechanisms underlying the latent effects that have now been documented for various marine invertebrates have not been well explored and are poorly understood (Williams and Degnan, 2009). There are two major suggestions in the marine invertebrate literature: (1) an effect on energy availability or allocation following metamorphosis, and (2) epigenetic shifts in gene expression patterns. The two may not be mutually exclusive.

A number of researchers have suggested or implied a prominent role for depleted larval nutrient reserves in producing latent effects (Emlet and Sadro, 2006; Vermeij et al., 2006; Chiu et al., 2007; 2008; Johnson and Wendt, 2007; Thiyagarajan et al., 2007; Hettinger et al., 2012; Li and Chiu, 2013; Rahman et al., 2014). For example, in a study referred to earlier (Emlet and Sadro, 2006) in which rearing nauplius larvae of the barnacle *Balanus glandula* at a low food concentration led to reduced post-metamorphic survival and growth, those reductions were correlated with reduced lipid and protein content of the pre-metamorphosed cyprids (Emlet and Sadro, 2006), supporting a simple energetics-limitation hypothesis for the observed reduction in juvenile growth rates. Similar results were reported for the gastropod *Crepidula onyx* when larvae were starved for portions of larval development (Chiu et al., 2007). Vermeij et al. (2006) noted increased swimming activity of nonfeeding coral larvae (*Montastraea faveolata)* under conditions of decreased salinity, and hypothesized that increased rates of energy expenditure might have accounted for the observed decrease in post-metamorphic survival.

Along these same lines, the study by Li and Chiu (2013) that was referred to earlier showed that exposing the larvae of *Crepidula onyx* to hypoxic stress reduced post-metamorphic growth and filtration rates, but only when the stressed larvae had also been reared at a reduced food concentration; larvae exposed to hypoxic stress under high food conditions showed normal post-metamorphic growth, suggesting that having abundant nutrients prior to metamorphosis ameliorated the latent impact of hypoxic stress. Perhaps abundant embryonic nutrient stores also account for the remarkable lack of latent effects seen when brooded embryos of the polychaete *Capitella teleta* were exposed to severe hypoxia for up to 96 hours (Pechenik et al., 2016). Nutrient limitation during larval development may alter energy allocation and thus impede the normal development of feeding structures (Chiu et al., 2007) or digestive machinery. Even so, what might cause such specific shifts in morphological development, and are they indeed specific to feeding-related morphology or physiology?

Some of the best direct evidence for a role of nutritional limitation in generating reduced post-metamorphic growth rates comes from laboratory studies with the nonfeeding larvae of the bryozoan *Bugula neritina* (Wendt and Johnson, 2006; Johnson and Wendt, 2007). Larvae whose settlement was

delayed by 24 h in a medium with depleted dissolved organic matter (DOM) showed a reduced ability to metamorphose successfully, and those that did metamorphose had smaller than normal feeding lophophores; those effects of delayed metamorphosis were substantially (but not completely) offset for a parallel group of larvae in seawater enriched with DOM (Johnson and Wendt, 2007).

On the other hand, post-metamorphic growth rates at normal salinity (34 psu) were reduced for the barnacle *Balanus amphitrite* after cyprids were exposed for 24 hours to a salinity of only 10 psu, despite the fact that there was no measureable effect on cyprid lipid content prior to metamorphosis (Thiyagarajan et al., 2007). Does this argue against a direct nutritional mechanism for the observed latent effects, or were those latent effects caused by the reduced availability of carbohydrates, proteins, or some other non-lipid nutrient or micronutrient? Deterring metamorphosis of this species for three days also resulted in reduced post-metamorphic growth for field-transplanted individuals, and allowing the newly metamorphosed, field-transplanted individuals to grow in an area with higher food availability "did not fully compensate for the negative effects of delayed metamorphosis" (Thiyagarajan et al., 2007, p. 183); indeed, mean juvenile feeding rates were significantly higher for individuals whose metamorphosis had been delayed, again suggesting that something more than a deficiency in juvenile feeding ability was at play in reducing the juvenile growth rates of these individuals (Thiyagarajan et al., 2007).

Although post-metamorphic feeding limitations may play a role in causing observed latent effects in at least some species, there is reason to believe that other, largely unexplored factors are probably also involved in at least some cases (reviewed by Pechenik, 2006; Chiu et al., 2007). Consider, for example, the impact of delayed metamorphosis on colony development of the seasquirt *Diplosoma listerianum*: branchial baskets were significantly smaller not just in the newly metamorphosed ancestrulae, but also in individuals subsequently produced asexually weeks later in field-transplanted colonies (Marshall et al., 2003). Similarly, exposing larvae of the bryozoan *Watersipora subtorquata* to sublethal levels of copper for only a few hours reduced colony survival months after colonies were transplanted to field sites (Ng and Keough, 2003), while forcing larvae of the bryozoan *Bugula neritina* to swim for an extra 23 to 24 hours significantly reduced colony growth rates and fecundity of field-transplanted colonies over the next two weeks, and delayed the onset of reproduction (Wendt, 1998). It is also worth remembering that effects on juvenile growth rates of the Olympia oyster *Ostrea lurida* also persisted for months after individuals were transplanted to field sites (Hettinger et al., 2013a). Such effects are unlikely to be caused simply by reductions in initial feeding ability following metamorphosis. In addition, some studies have failed to detect latent effects following stresses that have diminished larval energy reserves (e.g., delaying metamorphosis of the ascidian *Styela plicata*; Thiyagarajan and Qian, 2003) or which are likely to have diminished such reserves (e.g., salinity stress in three species of calyptraeid gastropod; Diederich et al., 2011).

What molecular mechanisms might account for the persistent latent effects that have been observed in so many studies? It is thought that some pollutants could be acting as potent endocrine disruptors (Nice et al., 2003), while the reduced juvenile growth rates documented for several suspension feeders following a variety of larval stresses are possibly due to reduced rates of functional gill development for at least the first few days or weeks after metamorphosis (Pechenik et al., 2002; Li and Chiu, 2013); to date nobody has determined exactly what about the gill is not working properly—is it simply a reduced size, or does the reduced functioning have a more interesting and subtle mechanical basis? But behind even these mechanisms there may often be a more fundamental cause of changes in juvenile and adult performance caused by events experienced much earlier in development. As suggested earlier (Pechenik, 2006), there is growing evidence that patterns of gene expression can be altered by the environment to modify phenotypes without making any changes in the actual gene sequences themselves. Indeed, Williams and Degnan (2009) have demonstrated distinct differences in patterns of gene expression, persisting for at least 40 hours after metamorphosis, for juveniles of the abalone *Haliotis asinine* that had

been triggered to metamorphose by contact with different coralline algal species. Such epigenetic effects have a number of different potential mechanisms (reviewed by Jablonka, 2013; Burggren, 2014; Skinner, 2015); to date the most attention has been focused on the selective methylation of DNA nucleotides (especially 5′ cytosine rings), namely, DNA methylation which then blocks the expression of the targeted genes (Suzuki and Bird, 2008; Williams and Degnan, 2009; Burggren and Crews, 2014), and histone modification (Gibson et al., 2012; Robichaud et al., 2012) which can either silence or activate gene expression. De-methylation can also be involved in regulating gene expression patterns (Kesäniemi et al., 2016). Few studies (e.g., Williams and Degnan, 2009) have so far sought to identify the molecular mechanisms that are responsible for the latent effects that have been documented in marine invertebrates, but the technology to do is becoming increasingly available (Williams and Degnan, 2009; Lyko et al., 2010; Robichaud et al., 2012; Flores et al., 2013; Gavery and Roberts, 2014), and interest in doing so is growing (Gibson et al., 2012; Robichaud et al., 2012).

Intriguingly, food levels and other nutritional deficiencies can be especially important causes of epigenetic shifts in patterns of gene expression in vertebrates (Mazzio and Soliman, 2014), as can temperature stress and exposure to toxicants (reviewed by Skinner, 2015). Understanding the mechanisms behind latent effects should enable us to explain some important questions about the organismal data collected to date. For example, why do we see clear latent effects in some experiments but not others using the same species, even when the larvae have experienced the same stress treatments at the same levels for the same amounts of time? If susceptibility is genetically determined in such cases, what makes the offspring of some parents more susceptible than those from other parents? Another especially intriguing question is why do we sometimes not see any latent effects following metamorphosis even when larval growth rates do not recover to control levels after the stress period has ended (Diederich et al., 2011)? Why do certain stresses produce clear latent effects in some species but not in other species? Also, to what extent is the variability we currently see in juvenile growth rates in the field due to direct genetic effects and to what extent to the effects of previous experience during embryonic or larval development?

Once the mechanisms lying behind the latent effects documented for marine invertebrates are understood, it should be possible to understand why the larvae of some species show these responses to certain stresses but not others, why the larvae of some species don't show latent effects in response to the same stresses, and why the larvae of some species may be more susceptible to stresses at certain stages of development.

Remarkably, there is increasing evidence, particularly from work with vertebrates, that environmentally induced epigenetic alterations in patterns of gene expression—particularly those involving consequences of nutritional stress—can at least sometimes be passed on to future generations; that is, environmentally induced alterations in patterns of gene expression involving nutritional stress can be transgenerational and play key roles in facilitating evolutionary change (Jablonka and Raz, 2009; Burggren, 2014; Burton and Metcalfe, 2014; Mendizabal et al., 2014; Skinner, 2015). Whether any of the latent effects discussed in this chapter are ever transmitted to future generations has not yet been assessed for any marine invertebrate (Marshall and Morgan, 2011; Gavery and Roberts, 2014), but is something that should be explored in future studies. Burggren (2014) has noted that what have been referred to as maternal or paternal "transgenerational effects" could in many cases be mediated by direct effects on gametes or early embryos in species with internal development, rather than being transmitted to the next generation from effects on adults. But such interpretive difficulties would not apply to the sorts of latent effects discussed in the present review.

14.12 Impact and Implications

Some of the immediate consequences of latent effects in the field are easy to imagine. Reduced growth rates, in particular, are likely to increase vulnerability to predation: to the extent that individuals become less vulnerable to predators as they grow (Gosselin and Qian, 1996; 1997; Hunt and Scheibling, 1997), slower growth should cause

longer periods of vulnerability. The population-level impact of latent effects may be especially great for organisms that are sessile after metamorphosis, as juveniles and adults of such species typically have fewer options for avoiding exposure to environmental stresses. Also, some stresses experienced early in development appear to increase juvenile mortality directly (e.g., in the gastropod *Crepipatella dilatata*: Chaparro et al., 2014). Similarly, latent effects that involve delaying time to reproductive maturity and reducing fecundity (e.g., Wendt, 1998) may well negatively impact local population dynamics, or at least the likelihood of successful perpetuation of the parental genotype.

On the other hand, to the extent that larval experiences of ocean acidification, nutritional limitation, hypoxia, or sublethal pollutant exposure can improve juvenile or adult function under similarly stressful conditions, latent effects could promote species persistence in a changing ocean.

The frequency with which the larvae of marine invertebrates experience delayed metamorphosis and other potentially stressful experiences prior to metamorphosis in the field, and the actual consequences of those experiences on juveniles in natural field situations, are unknown. Individual juveniles and adults in natural populations certainly differ greatly in behavior, growth rate, timing of sexual maturity, and fecundity for all organisms studied to date.

But the degree to which those differences reflect the impact of pre-metamorphic experience, rather than differences in underlying standard Mendelian genetics or local differences in physical conditions (e.g., Helmuth and Hofmann, 2001), is not clear. What we need to find is an internal black-box recorder of some sort that would provide a record of larval experience in field-collected juveniles or adults that exhibit differences in key measures of individual fitness. Something like that has been found for fish (otoliths) (McCormick, 1999), but we are still looking for something comparable for studies of marine invertebrates (Levin et al., 2015). Even if we find that a particular stressor causes detectable and reproducible changes in isotope ratios, trace element composition, or other components of molluscan larval shell composition in the laboratory (Levin et al., 2015), we also need to make sure that such changes are not also induced by any other factors.

To the extent that latent effects are indeed occurring commonly for marine invertebrates in the natural world, there is tremendous potential for climate change, pollution, hypoxia, and other environmental stresses to impact the geographic distributions of species and local community structure through those subtle effects. Thus, a widespread and increasing occurrence of latent effects in the field will make it even more difficult to predict the future impact of environmental change on future populations and communities. The potential for multiple environment-individual interactions adds further complexity: studies to date are limited to testing the effects of one to three multiple stressors at a time (e.g., ocean acidification, pollutants, temperature stress, hypoxia, salinity stress, or nutritional stress) (Table 14.1), whereas in the real world, embryos and larvae are probably subjected to many stresses simultaneously. The precise actual impact of human activity on marine populations defies prediction: there is probably only one way to conduct this particular experiment, and we are all participating.

14.13 Summary

1. A variety of stresses, including hypoxia, reduced pH, and food limitation, experienced during development can influence growth rates, survival, and other fitness characteristics following metamorphosis.
2. The brooding of embryos may protect against exposure to some environmental stresses during development, but can sometimes expose developing embryos to stresses they would otherwise have avoided by being free-living, resulting again in reduced fitness in later life.
3. The mechanisms through which such "latent effects" are mediated are unclear: energy-balance issues and epigenetic factors—in which gene expression patterns are altered without any changes in DNA sequences—seem to be involved.
4. The extent to which documented variability in factors such as growth, survival, and reproductive output in benthic field populations is explained by stresses experienced early in development remains to be determined.

The coming years will apparently bring increasing water temperatures; changing rainfall patterns and consequent shifts in coastal salinities and in the magnitudes of salinity fluctuation; increasing ocean acidity; increasing levels of pollution (including the spread of microplastics); increasing incidences of hypoxic events; and shifting patterns of phytoplankton abundance, species composition, and nutritional quality; all of which have the potential to increase physiological stresses on developmental stages and increase the energy costs of development through metamorphosis. This presents us with a good number of important questions: (1) To what extent do these stresses—alone and in combination—have latent impact on marine invertebrates, and what are those impacts? (2) Do they ever carry over to subsequent generations? (3) To what extent are some species more vulnerable to particular stresses than other species? (4) What accounts for differences in vulnerability? (5) To what extent does brooding protect offspring from exposure to environmental stress and from exhibiting latent effects later in development? (6) And what are the underlying mechanisms causing latent effects? It seems likely that many of these questions will be addressed, and possibly answered, over the next ten years.

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