

Phenotypic Plasticity of Feeding Structures in Marine Invertebrate Larvae

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Researchers have worked over the last thirty years to examine and understand phenotypic plasticity in larvae of marine invertebrates. These studies range from documenting the association between algal food availability and larval morphology in different species to examining the ecological and evolutionary implications of the plastic response. However, investigators have used disparate systems (genera and species of echinoid echinoderms mostly, but also other taxa), employed various rearing and measuring techniques and levels of genetic replication, and relied on different statistical methodologies for data analysis. They have also obtained results ranging from non-plastic to plastic, and reached different conclusions about the functional, ecological, and evolutionary roles of phenotypic plasticity in marine larvae. Our goals with this chapter are twofold: to review the literature in order to help researchers more quickly understand the extent and breadth of this field of research, and to identify gaps in our understanding in order to provide guidance for future research. We begin with a more general introduction of phenotypic plasticity, and then focus on feeding-structure plasticity in larvae of marine invertebrates.

8.1 Phenotypic Plasticity

Research on the expression and evolution of phenotypic plasticity is a rich field, and readers will find valuable entries into the literature through the books of West-Eberhard (2003) and DeWitt and

Scheiner (2004). Here we summarize some of the main concepts in order to facilitate discussion of plasticity in marine invertebrate larvae later in the chapter. Phenotypic plasticity is defined as the ability of a genotype to produce different phenotypes (developmental, physiological, morphological, behavioral, or life history traits) in response to different biotic or abiotic environmental factors. Plasticity can be adaptive, maladaptive, or effectively neutral. When an organism possesses plasticity that confers a fitness advantage, then plasticity is considered adaptive. Researchers have focused on examples in which there is evidence that plasticity is adaptive, likely because of the evolutionary and ecological consequences of phenotypes that improve an organism's fitness. However, phenotypic plasticity can be non-adaptive and still have important ecological consequences (Miner et al., 2005). It is often difficult to experimentally test whether plasticity is adaptive because phenotype and environment are typically confounded, and evidence often comes from both experiments and functional arguments. In addition, adaptation that leads to a fitness advantage (enhanced reproductive success of phenotypes across generations) is difficult to demonstrate for larval traits of organisms because of the time required to rear organisms to the adult stage. Plastic phenotypes that are inducible are also classified broadly as either defensive or offensive (Miner et al., 2005). Plastic phenotypes that protect an organism when at risk of death or injury are inducible defenses, whereas those that help an organism gain resources

when resources are limiting are inducible offenses. In some cases, inducible defenses and offenses both occur and are integrated in an organism.

There are several general categories of phenotypic plasticity—developmental, morphological, physiological, behavioral, or life historical. Responses assigned to the same category often have similarities in the timing and reversibility of the response. Morphological changes typically require relatively more time to produce than physiological and behavioral changes. Physiological, behavioral, and morphological changes are often, although not always, reversible when the stimulating environment is allayed. Changes to an organism's developmental trajectory or timing of transitions between life-history stages can be difficult or impossible to reverse. Though specifically defined responses are relatively easy to fit into one of these categories, organisms generally respond to their environment with an integrated response of many traits that are defined by more than one category. For example, some species of plant elongate their stem and grow taller when shaded by other plants (West-Eberhard, 2003). The response is morphological but is the direct result of underlying physiological and developmental plasticities. In addition to cause and effect relationships among plastic responses, energy can link different plasticity responses in an organism. Responses that require more energy can cause changes in development or life history transitions, such as metamorphosis from larva to juvenile (West-Eberhard, 2003; Gilbert and Epel, 2015). Often, delays in life history transitions result from an initial energetically expensive morphological or physiological plastic response.

In order for plasticity to evolve, organisms must detect reliable cues of environmental change (chemical, tactile, etc.) and respond at an appropriate temporal scale. Thus, plasticity must occur after these cues are received and processed. In order for the organism to properly match phenotype to environment, these cues must be reliable, providing information of environmental variability (e.g., magnitude and frequency). In addition, an induced phenotype must be well timed with environmental change. To ensure that the induced plastic response is effective, the period of time (i.e., lag-time) between sensation of the cue and

production of the phenotype must be appropriate to the scale of environmental change (Padilla and Adolf, 1996).

The degree of plasticity for a given genotype is often measured as either the difference in the magnitude of phenotypes expressed between two environments or the slope of the reaction norm across environments. The reaction norm for a given genotype depicts graphically the relationship between phenotype and a specific environmental factor. The phenotype is plastic when the slope of the relationship deviates from zero, and non-plastic (or constant) when equal to zero. The degree of phenotypic plasticity can vary among genotypes within a population. Natural selection can act on this variation, and thus the degree of phenotypic plasticity for a given trait is considered a trait in and of itself, with the potential to evolve. Numerous studies have documented the expression of phenotypic plasticity by organisms in general, but fewer have demonstrated variation for plasticity among genotypes both within and among populations, and fewer still have demonstrated that phenotypic plasticity has evolved in response to environmental changes and results in higher fitness. Research on phenotypic plasticity has a long history among terrestrial plants and insects, and a relatively recent history among marine invertebrates, particularly in early life stages (e.g., larvae).

Marine invertebrates and their larvae face selective pressures in their environments that are similar in some ways to those faced by terrestrial plants and the freshwater juvenile stages of some terrestrial animals (e.g., predation, competition, and dispersal), yet are uniquely different in others (e.g., salinity and CO₂-induced ocean acidification). Also, most freshwater invertebrate species are taxonomically different, developing directly and not through larval stages like marine invertebrates, although a few exceptions exist. Thus marine invertebrates and their larvae constitute a set of research systems that provide a counterpoint to terrestrial and freshwater organisms for testing our ideas about the biology of phenotypic plasticity. We believe that food-induced phenotypic plasticity exhibited by the planktotrophic (i.e., feeding) larvae of many

marine invertebrates provides one of the most comprehensive examples of phenotypic plasticity among marine organisms.

8.2 Feeding Larvae of Marine Invertebrates

The life histories of most species of marine invertebrates involve transitions through a series of successive developmental and ontogenetic stages (see Nielsen, this volume). Larvae are typically classified by whether they can acquire exogenous food and complete larval development using endogenous reserves in an egg. Feeding planktotrophic larvae must feed to gain nutrition from the planktonic environment, as they do not possess enough energy in an egg to complete development and metamorphose into a juvenile, whereas lecithotrophic larvae utilize primarily egg energy and might have the ability to feed, though feeding is not required. The combination of these two classification schemes results in four possible types of larvae, although only three are evolutionarily viable: feeding planktotrophic larvae, feeding lecithotrophic larvae, and nonfeeding lecithotrophic larvae. The fourth possibility, nonfeeding planktotrophic larvae, are not viable because there is no way for larvae to gain the energy needed to metamorphose. The vast majority of species with larval forms are either feeding planktotrophic, typically referred to as just “planktotrophic” larvae, or nonfeeding lecithotrophic larvae, typically referred to as just “lecithotrophic” larvae (Thorson, 1950; McEdward and Miner, 2001; Marshall and Keough, 2008). Less common are species with feeding lecithotrophic larvae, typically referred to as “facultative planktotrophic” larvae (Allen and Pernet, 2007). In this chapter, we focus on plasticity of the feeding structures of feeding larvae, however, plasticity of other life history characters are likely to exist and play important roles for larvae of all developmental modes. For example, adults might preferentially, and plastically, provision eggs with different types and amounts of biochemical constituents, and thus energy, by consuming different food resources or altering patterns (e.g., rate and timing) of oogenesis. While this type of plasticity will impact larval development

and growth, the consequences of plasticity of egg organic and energy composition also affect adult fecundity and lie outside the scope of this review.

The duration of larval growth for feeding larvae is influenced by temperature and the availability of energetic resources (see Pernet, this volume; Jaekle, this volume). In general, higher temperatures increase metabolic and growth rates, shortening the amount of time individuals spend as larvae (O'Connor et al., 2007). Most feeding larvae actively acquire and assimilate energy and needed resources by capturing and consuming unicellular algae using elaborate ciliated feeding structures, which vary greatly among taxonomic groups (Strathmann, 1987). Exogenous energetic materials can also be acquired passively through the uptake of dissolved organic materials (Manahan et al., 1983). The degree to which feeding larvae must acquire exogenous food and resources is correlated with egg size (Herrera et al., 1996; Miner et al., 2005; McAlister and Moran, 2013). Some species require no or very little additional energy or resources beyond what is present in the egg (Allen and Pernet, 2007). Other species cannot survive beyond the stage when they gain the ability to feed. Small eggs contain less energy than larger eggs (Jaekle, 1995; McEdward and Morgan, 2001), and presumably larvae that develop from relatively smaller eggs likely require more food for metabolism and morphogenesis than larvae that develop from larger eggs. Feeding larvae of some echinoid species also require the hormone thyroxine to metamorphose, which they gain from their diet (Heyland and Hodin, 2004; Heyland et al., 2004). The possibility exists that small eggs might also not contain enough of these non-energetic compounds needed for metamorphosis, although this remains to be more critically tested.

In preparation for the transition between larval and juvenile life, and to ensure successful metamorphosis, feeding larvae from various taxa begin constructing juvenile structures by metamorphosis within the larval body. Taxonomic groups of marine species vary in the timing and duration of this transition, as well as in the mechanisms underlying this process. In many species larvae can metamorphose rapidly and transition quickly between pelagic and benthic environments (e.g., echinoderms), in other species the transition is gradual (e.g., molluscs).

However, in species in which larvae and juveniles live in different environments (e.g., the plankton and benthos), the ecological transition is almost always quick, regardless duration of the morphological transition; thus metamorphosis represents a critical developmental milestone for larvae. With metamorphosis serving as a definitive end-point for larvae, natural selection should favor mechanisms that decrease the duration of the larval period—for example, plasticity of structures associated with food capture or processing or via increased maternal provisioning, both of which ameliorate the effects of oligotrophic conditions (see following and Marshall et al., this volume).

8.3 Plasticity of Feeding Structures in Planktotrophic Larvae

8.3.1 Food Limitation, Resource Acquisition, and Energetic Trade-offs

Larvae in the sea are presumably food limited because the unicellular algae they eat are dilute and patchily distributed in time and space (Conover, 1968; Paulay et al., 1985; Fenaux et al., 1988, 1994), and larvae have limited ability to track patchy food. Thus, natural selection has likely favored strategies that improve the acquisition of scarce resources and reduce the amount of energy and materials that need to be obtained from the environment. Researchers in the late 1980s discovered an example of the first strategy; morphological phenotypic plasticity in response to the amount of food was demonstrated in feeding larvae from echinoid echinoderm species (Boidron-Metairon, 1988; Fenaux et al., 1988; Hart and Scheibling, 1988). Larvae fed a dilute concentration of food respond by growing longer skeletal arms, which increases the length of the ciliated band used for food collection (Strathmann, 1971; McEdward, 1986b; see Figure 8.1), thereby allowing larvae to capture more phytoplankton food particles from a greater volume of water (Hart, 1991). Furthermore, Hart and Strathmann (1994) demonstrated that plasticity of ciliated band length was correlated with plasticity of arm length. Subsequent work has documented similar feeding-structure plasticity in larvae of other echinoid species, ophiuroid,

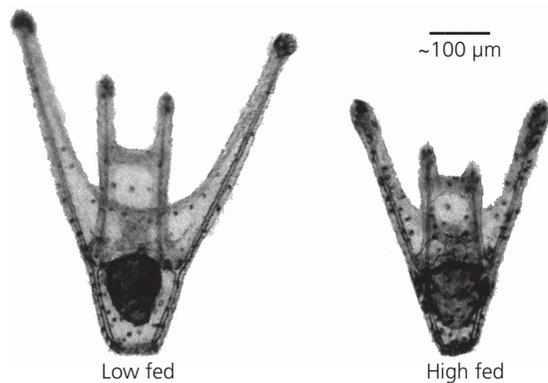


Figure 8.1 Low-fed (left) and high-fed (right) full-sib larvae of *Lytechinus variegatus* at four days post-fertilization. Note longer larval arms, both absolutely and relative to body length, of low-fed larva.

and holothuroid echinoderms, as well as in molluscan veligers (Table 8.1), although there are many phyla and larval types for which feeding-structure plasticity is undemonstrated or undocumented (Table 8.2). By increasing the length of the ciliated band and supporting body form, the larval surface-to-volume ratio increases. This could also increase the intake of dissolved organic matter (Manahan et al., 1983), albeit also increasing the surface area over which dissolved organics can leak, and thus the net benefit is not clear.

Investing energy to lengthen larval feeding structures in low food conditions might result, however, in decreased energetic investment in larval food-processing structures (e.g., the larval stomach; Miner, 2005) or juvenile structures required for metamorphosis (e.g., the juvenile rudiment; Boidron-Metairon, 1988; Strathmann et al., 1992; Bertram et al., 2009; see also Adams et al., 2011). In addition to increasing ciliary band length, larvae might also adjust capacity for capturing food relative to demand for energy and materials for growth of post-larval structures. For example, another response to starvation might be to retain a given ciliary band length but to reduce parts of the larval body not essential for larval life (e.g., the rudiment or epaulets). This reduction of unessential larval tissues presumably reduces demand for energy and materials for maintenance, and uses the resorbed tissues as a source of both energy and materials, as demonstrated in annelid trochophores (Pawlik and Mense,

Table 8.1 Marine Invertebrate Species Tested for Larval Feeding-Structure Plasticity.

Phylum Class Species	Plasticity? Measure Type	Statistical Methodology	Genetic Replication	Reference
Echinodermata				
Asteroidea				
<i>Acanthaster planci</i>	Yes: 1, 2	ANOVA, PCA	2F, 1M	Wolfe et al. 2015
<i>Luidia foliolata</i>	Yes: 0	ANOVA	1F, 1M for 1 full-sib family	George 1994
<i>Pisaster ochraceus</i>	Yes: 1, 2	ANOVA, Canonical Discriminant Analysis	Unspecified; multiple F and M	George 1999
<i>Sclerasterias mollis</i>	Yes: 1	PCA, Wilcox 3-way	4 parents (presume 2F, 2M) per population	Poorbagher et al. 2010a
Echinoidea				
<i>Centrostephanus rodgersii</i>	No: 1, 2	ANOVA, ANCOVA, PCA	1F, 1M each for 3 full-sib families	Soars et al. 2009
<i>Clypeaster subdepressus</i>	Yes: 2	ANOVA	1F, 1M for 1 full-sib cross	Reitzel and Heyland 2007
<i>Dendraster excentricus</i>	Yes: 0	ANOVA	Unspecified # of parents	Boidron-Metairon 1988
	Yes: 2, 3	ANCOVA	1F, 1M each for 2 full-sib families	Hart and Strathmann 1994
	Yes: 1	ANCOVA	1F, 1M each for 2 full-sib families	Miner 2007
<i>Diadema antillarum</i>	No: 1	Repeated measures ANCOVA	1F, 1M for 1 full-sib family	McAlister 2008
<i>Diadema mexicanum</i>	No: 1	Repeated measures ANCOVA	1F, 1M each for 4 full-sib families	McAlister 2008
<i>Echinometra lucunter</i>	No: 1	Repeated measures ANCOVA	1F, 1M each for 3 full-sib families	McAlister 2008
<i>Echinometra vanbrunti</i>	No: 1	Repeated measures ANCOVA	1F, 1M each for 3 full-sib families	McAlister 2008
<i>Echinometra viridis</i>	No: 1	Repeated measures ANCOVA	1F, 1M each for 3 full-sib families	McAlister 2008
<i>Encope michelini</i>	No: 0	ANOVA, MANOVA	Unspecified # of parents	Eckert 1995
<i>Eucidaris tribuloides</i>	No: 1	Repeated measures ANCOVA	1F, 1M each for 3 full-sib families	McAlister 2008
<i>Eucidaris thoursii</i>	No: 1	Repeated measures ANCOVA	1F, 1M for 1 full-sib family	McAlister 2008
<i>Evechinus chloroticus</i>	Yes: 1	ANOVA, MANOVA, PCA	1F, 1M for 1 full-sib family	Sewell et al. 2004
<i>Heliocidaris tuberculata</i>	Yes: 1	ANOVA, ANCOVA, PCA	1F, 1M for 1 full-sib family	Soars et al. 2009
<i>Leodia sexiesperforata</i>	No: 2	ANOVA	1F, 1M for 1 full-sib family	Reitzel and Heyland 2007
<i>Lytechinus variegatus</i>	Yes: 0	ANOVA	Unspecified # of parents	Boidron-Metairon 1988 Section 1.03
	Yes: 1	ANOVA	Unspecified # of parents	McEdward and Herrera 1999
	Yes: 1	Profile Analysis (Repeated measures ANOVA and MANOVA)	1F, 1M for 1 full-sib family	Miner and Vonesh 2004
	Yes: 1	ANCOVA	18F, 6M breeding design for 29 full-sib, half-sib families	McAlister 2007a
<i>Melitta tenuis</i>	Yes: 2	ANOVA	1F, 1M for 1 full-sib family	Reitzel and Heyland 2007
<i>Paracentrotus lividus</i>	Yes: 0	Not reported	Unspecified # of parents	Fenaux et al. 1988
	Yes: 1, 2, 3	ANOVA	1F, 1M each for 3 full-sib families	Strathmann et al. 1992
	Yes: 3	ANOVA	Full-sibling cultures, Unspecified # of parents	Fenaux et al. 1994
<i>Pseudochinus huttoni</i>	Yes: 1, 2, 3	ANOVA, PCA	9–10F per parental diet treatment, 1M	Poorbagher et al. 2010b

(continued)

Table 8.1 (Continued)

Phylum Class <i>Species</i>	Plasticity? Measure Type	Statistical Methodology	Genetic Replication	Reference
<i>Strongylocentrotus droeachiensis</i>	Yes: 1	PCA	4F, 4M pooled gametes	Hart and Scheibling 1988
	Yes: 1, 2, 3	ANCOVA	8F (4F, 2 locations), 1M for 8 half-sib families	Bertram and Strathmann 1998
	No: 1	ANOVA, PCA	3F, 3M pooled gametes	Meidel et al. 1999
	N/A (examined gene expression)	N/A (examined gene expression)	Unspecified # of parents	Carrier et al. 2015
<i>Strongylocentrotus franciscanus</i>	Yes: 1, 2	ANCOVA	3F, 1M pooled gametes	Miner 2005
	Yes: 1	ANCOVA	2F, 5M pooled gametes	McAlister 2007b
<i>Strongylocentrotus purpuratus</i>	Yes: 1, 2	ANCOVA	3F, 1M pooled gametes	Miner 2005
	Yes: 1	ANCOVA	1F, 1M each for 2 full-sib families	Miner 2007
	Yes: 1	ANCOVA	2F, 7M pooled gametes	McAlister 2007b
	Yes: 0	ANOVA	Unspecified # of parents	Adams et al. 2011
<i>Tripneustes gratilla</i>	Yes: 1, 2	ANOVA, PCA	1F, 1M for 1 full-sib family	Byrne et al. 2008
Holothuroidea				
<i>Australostichopus mollis</i>	Yes: 2, 3	ANOVA, PCA	Unspecified # of parents	Morgan 2008
<i>Apostichopus japonicus</i>	Yes: 2	PCA	1 F, 1M for 1 full-sib family	Sun and Li 2013
Ophiuroidea				
<i>Macrophiothrix koehleri</i>	Yes: 1, 2	ANCOVA, Linear Mixed Model, Cubic spline	6 separate experiments, Unspecified # of parents	Podolsky and McAlister 2005
<i>Macrophiothrix longipeda</i>	Yes: 1, 2	ANCOVA, Linear Mixed Model, Cubic spline	2 separate experiments, Unspecified # of parents	Podolsky and McAlister 2005
<i>Macrophiothrix lorioli</i>	No: 1, 2	ANCOVA, Linear Mixed Model, Cubic spline	5 separate experiments, Unspecified # of parents	Podolsky and McAlister 2005
<i>Macrophiothrix rhabdoti</i>	No: 1, 2	ANCOVA, Linear Mixed Model, Cubic spline	1 experiment, Unspecified # of parents	Podolsky and McAlister 2005
Mollusca				
Bivalvia				
<i>Crassostrea gigas</i>	Yes: 3	ANCOVA	9F, 2M pooled gametes	Strathmann et al. 1993
Gastropoda				
<i>Crepidula fornicata</i>	Yes: 3	ANCOVA	1F, unknown M	Estrella Klinzing and Pechenik 2000
Annelida*				
Polychaeta				
<i>Phragmatopoma lapidosa californica</i>	Yes: N/A	Not reported	Unspecified # of parents	Pawlik and Mense 1994
<i>Hydroides dianthus</i>	Yes: N/A	ANOVA	15–25F, 1M pooled gametes	Toonen and Pawlik 2001

(continued)

Table 8.1 (Continued)

Phylum Class Species	Plasticity? Measure Type	Statistical Methodology	Genetic Replication	Reference
Bryozoa*				
Gymnolaemata				
<i>Membranipora membranacea</i>	Yes: 3	ANOVA	Unknown. Wild caught larvae	Strathmann et al. 2008

Note. "Measure type" refers to measure of relative feeding-structure length to (1) midline body length or other structure functional only in larva, (2) stomach (functional in both larva and juvenile), or (3) rudiments of juvenile structures (not functional in larva). A measure type of (0) indicates that data from absolute feeding-structure length was used to assess plasticity, although data from body length, stomach length, or juvenile rudiment length may have also been reported. ANOVA = analysis of variance; ANCOVA = analysis of covariance, MANOVA = multivariate analysis of variance, PCA = principle components analysis, F = female, M = male, N/A = not applicable. Phyla in which plasticity of other larval structures, settlement and/or metamorphic competency (in association with feeding, starvation and subsequent re-feeding) has been demonstrated, albeit not plasticity of feeding structures per se, are indicated by *.

Table 8.2 Phyla with Feeding Larvae for Which We Do Not Know (or Negative Data Has Not Been Reported) Whether Larvae Possess Feeding-Structure Plasticity

Phylum	Feeding larva
Cnidaria	planula
Platyhelminthes	müller's larva
Annelida	trochophore*
Mollusca	trochophore
Sipuncula	trochophore
Bryozoa	cyphonaute*
Phoronida	actinotroph
Arthropoda	nauplius and zoea
Hemichordata	tornaria

*Indicates larval types in which plasticity of other larval structures, settlement and/or metamorphic competency (in association with feeding, starvation, and subsequent re-feeding) has been demonstrated, albeit not plasticity of feeding structures per se.

1994; Toonen and Pawlik, 2001) and bryozoan cyphonautes (Strathmann et al., 2008) (see Table 8.1; Table 8.2).

As a consequence of these types of plasticity, food-limited larvae exhibit delayed time to metamorphosis, a potentially dangerous prospect for planktonic-feeding organisms (Thorson, 1950; Rumrill, 1990; Morgan, 1995). Strathmann et al. (1992) suggested that plasticity in larval arm length provides information about larval feeding history in the field, although this idea has not been

rigorously tested in a field setting. Thus there exist several different, yet intricately connected examples of phenotypic plasticity in response to food availability in this system: morphological plasticities of feeding-structure size (larval arms and ciliated band lengths), food-processing structure size (stomach length or volume), and developmental plasticities of the time to initial formation of the juvenile rudiment and duration of the larval period (time to metamorphosis). For the latter, the time period is presumably shorter in low-fed larvae that exhibit morphological plasticity of feeding structures as compared to low-fed larvae that do not exhibit this form of plasticity. Difficulties in experimentally preventing the expression of morphological plasticity by larvae in low food conditions have prohibited a direct test of this hypothesis. Studies that employ targeted phenotypic engineering (e.g., through developmental manipulation or genetic modification, to "trick" larvae into expressing a short-arm phenotype in a low food environment) could be used to test this assumption, and thus the adaptive significance of plasticity in this system. A study by Adams et al. (2011) suggests that this type of manipulation or modification is feasible (see later).

With respect to the second strategy, larger egg size typically reflects an increase in maternally provisioned energetic materials, which are used to build larger larvae and to fuel more rapid larval development (McAlister and Moran, 2012; 2013; see Marshall et al., 2008, and Moran and McAlister,

2009, for reviews). Egg size has been linked to several life history traits or events, including larval form (McEdward, 1986a), developmental mode (Strathmann, 1985), and the length of larval development (Thorson, 1950; Vance, 1973; Strathmann, 1985). Herrera et al. (1996) demonstrated variation in feeding period with egg size; development time to metamorphosis is inversely related to egg size among various echinoid species. Poor larval feeding environments might have selected for the evolution of large eggs to minimize high planktonic mortality (Rumrill, 1990). Alternatively, conditions of sperm limitation might have selected for large eggs to increase fertilization success (Levitán, 1993; Podolsky and Strathmann, 1996), indirectly affecting larval traits. Several studies indicate that arm-length plasticity is primarily expressed (or at least is detectable) during early larval development (Boidron-Metairon, 1988; Hart and Scheibling, 1988; Eckert, 1995; Miner, 2007; Adams et al., 2011; though see Hart and Strathmann, 1994; George, 1999) and that the capacity for plasticity of arm length early in development is associated with the amount of maternally provisioned energetic reserves, and thus with egg size (Bertram and Strathmann, 1998; McAlister, 2007b; Reitzel and Heyland, 2007; Bertram et al., 2009; Poorbagher et al., 2010a; 2010b). These results suggest that larvae utilize endogenous resources for the initial production of food-collecting structures, and then exploit exogenous resources for the development of other, later-appearing structures.

Given that feeding-structure plasticity is common in planktotrophic species of echinoids, coupled with the general interest in egg size and evolution of marine invertebrates, it is not surprising that researchers have tested whether egg size is associated with feeding-structure plasticity in larvae. Indeed, egg size varies greatly (>80-fold difference in volume as calculated from values below) among species of echinoids with planktotrophic larvae. In some species mothers provision their offspring with a small portion of the energy needed to complete larval development and metamorphose into a juvenile (e.g., *Arbacia stellata*, egg volume 0.13 nl, egg energy 1.0 mJ; Moran et al., 2011); whereas in other species, mothers provide all of the energy needed to complete larval development and metamorphosis (e.g., the facultative

planktotroph *Clypeaster rosaceus*, egg volume 10.77 nl, egg energy 110 mJ; Miner et al., 2002).

There are two general hypotheses for the relationship between egg size and degree of morphological plasticity. Embryos that develop from larger eggs have access to greater endogenous materials and energy and thus have the propensity to display a greater degree of morphological plasticity than larvae that develop from smaller eggs (Herrera et al., 1996; McAlister, 2007b). Alternatively, selection might have favored a greater capacity for plasticity in embryos that develop from smaller eggs to more effectively utilize exogenous resources (McAlister, 2007b). The results from other studies support both of these hypotheses. Comparative measurements between closely related species and physical manipulations of egg size in a single species demonstrate that large egg size is associated with a greater degree of plasticity (McAlister, 2007b). Alternatively, comparative studies among multiple species of ophiuroids (Podolsky and McAlister, 2005) and echinoids (Reitzel and Heyland, 2007) have supported the alternate hypothesis. Egg size is likely a coarse measure of egg energetic content, however, it provides no information about egg biochemical composition (Moran and McAlister, 2009; Moran et al., 2011). Increases in egg size can be obtained through increased maternal provisioning or through simple hydration (Podolsky and Strathmann, 1996; McAlister and Moran, 2012). Thus, outside of a controlled phylogenetic context, using egg size alone to make assumptions about egg composition and energetic content and their association with other life history characters might be problematic (McAlister and Moran, 2012). We suspect that the relationship between plasticity and egg size is a combination of both hypotheses, and reflects a nonlinear negative relationship between these variables for feeding larvae (Figure 8.2). For larvae that develop from relatively small eggs (i.e., egg energy runs out shortly after larvae gain the ability to feed), low levels of endogenous energy likely limit the production of long arms when food is scarce. At the other extreme, larvae that develop from relatively large eggs (i.e., egg energy fuels nearly all of larval development) are not energy limited and thus plasticity of arm length does not improve fitness if the main role of arms is feeding. Studies that manipulate the timing of exposure to exogenous food can elucidate



Figure 8.2 Degree of phenotypic plasticity of feeding-structure length relative to egg size. Feeding larvae that develop from small or large eggs exhibit lower degrees of plasticity (less difference between phenotype values of high- and low-fed larvae) than larvae that develop from intermediate size eggs.

whether the degree of the plastic response changes during the course of larval development and, further, if the response is confined to specific developmental windows or time periods.

8.3.2 Patterns of Expression and Environmental Cues

Although several studies have focused on understanding the energetic trade-offs discussed previously, more research has involved functional

demonstrations and documentation of the expression of feeding-structure plasticity. These studies have included assessments of the presence, magnitude, variability, and timing of the response within specific taxa (Table 8.1). Other studies have investigated the environmental cues that induce the feeding-structure response, indicating that in addition to low algal food availability, algal exudates (Miner, 2007) and thyroxin compounds (Heyland and Hodin, 2004; Heyland et al., 2004) can also play a role.

Many researchers measure plasticity early in larval development and have demonstrated that for the majority of species, larvae fed scarce amounts of food after several days during the first week or two of development exhibit longer larval arms than larvae fed abundant food (see Table 8.1). Across studies, starved larvae exhibit on the order of an 8–30% increase in arm length over their well-fed counterparts. Although the percentage difference in arm length between well- and under-fed larvae can be relatively small, because the relationship between absolute arm length and food availability over time is concave (both decreasing and decelerating; Miner and Vonesh, 2004), morphological changes made early in development will have a greater influence on an organism's overarching ontogenetic trajectory than will changes made later in development (Figure 8.3). Additionally, small differences made early in development, coupled with adjustments of metabolic rate, might also be very important if they increase the threshold at which larvae starve. It is important to note, however, that not all species may

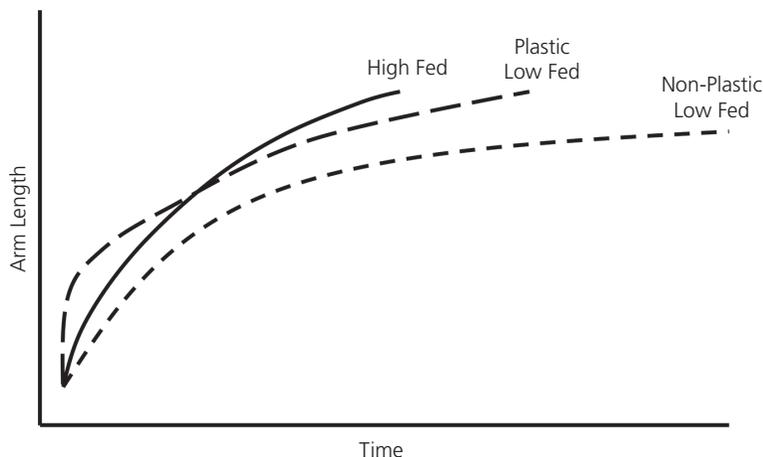


Figure 8.3 Absolute arm length relative to time for high-fed larvae (solid line), low-fed larvae exhibiting plasticity (long-dash line), and low-fed larvae not exhibiting plasticity (short-dash line). Phenotypically plastic low-fed larvae exhibit longer arms early in development and theoretically enjoy a fitness advantage of reaching metamorphosis sooner than non-plastic low-fed larvae.

be phenotypically plastic (Eckert, 1995; McAlister, 2008; Soars et al., 2009; see caveat later), which suggests that the functional arguments for plasticity, its magnitude, and the cost-benefit relationship for maintaining the ability to express plasticity, are needed for each case.

Another important metric for feeding-structure plasticity is the relative relationship between feeding-structure length and a measure of body size, such as midline body length. In a system in which food is the inducing environmental variable, as well as the resource necessary for growth, measurements of feeding-structure length (e.g., arm length) relative to body length can indicate whether low-fed larvae are expressing phenotypic plasticity throughout the duration of larval ontogeny. In this scenario, graphical plots of arm length vs. body length will exhibit a more steeply sloped relationship for poorly fed than for well-fed larvae (Figure 8.4). Slopes calculated for individuals fed different food levels can then be used to assess the degree of plasticity expressed by a given genotype, by plotting and calculating the slope of the reaction norm between these values.

Different genotypes are expected to vary in their degree of plasticity, though differences in the degree of plasticity among genotypes in marine invertebrates remain largely unexplored (but see McAlister, 2007a).

This might be due in part to the logistical complications of simultaneously rearing multiple, replicated-treatment cultures of larvae and to the large numbers of genotypes that must be examined for valid statistical comparisons. Because phenotypic variation among genotypes provides an opportunity for evolution by natural selection, studies that examine the genotypic-level variation in phenotypic plasticity are needed. Data describing the across-environment pattern of phenotypic expression (e.g., mean and variance) among genotypes in a population could provide important clues about the evolutionary potential of the population. Examinations of this type could be extended to an inter-population level of analysis. Although two studies have investigated differences in phenotypic plasticity between populations (Bertram and Strathmann, 1998; Poorbagher et al., 2010b), these studies were concerned more with the effects of nutrition on the expression of plasticity than investigating differences in the degree of inter- and intra-population variation for plasticity. Researchers have proposed that phenotypic plasticity varies by latitude: species, populations, and genotypes from higher latitudes will express a greater degree of phenotypic plasticity than those from lower latitudes (McAlister, 2008; Soars et al., 2009). This idea is untested, but could be examined using multi-genotype, common-garden analyses.

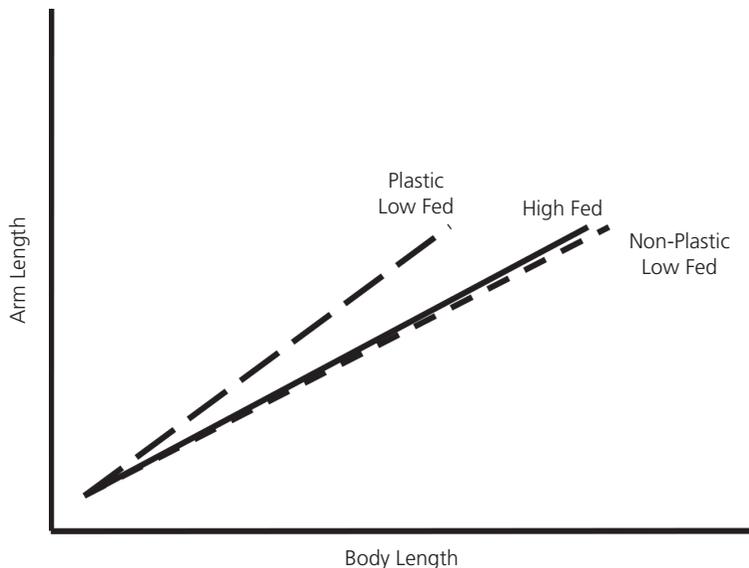


Figure 8.4 Arm length relative to body length. Phenotypically plastic low-fed larvae (long-dash line) exhibit a more steeply sloped relationship between these structures than high-fed (solid line) or non-plastic low-fed (short-dash line) larvae. High-fed larvae reach metamorphosis sooner than plastic and non-plastic low-fed larvae.

Hart and Strathmann (1994) showed that poorly fed larvae produced longer arms and ciliated bands and exhibited approximately a 20% increase in maximum clearance rates over well-fed larvae, providing evidence that arm length plasticity is functionally significant. Strathmann et al. (1992) demonstrated that full-sibling larvae exhibit heterochronic shifts in the relative timing of the development of food-collecting arms and of the juvenile rudiment. However, production of larger food-collecting structures occurs at the expense of food processing (stomach) or postlarval (rudiment) structures (Strathmann et al., 1992; Miner, 2005). Whether the delay in the development of postlarval structures is due to larvae preferentially allocating energy, materials, or both to feeding structures or a consequence of larvae having fewer resources because they are fed less is currently unclear (but see Adams et al., 2011, for some evidence for preferential allocation of energetic reserves). These results suggest that feeding-structure plasticity is evolutionarily adaptive: larvae that alter feeding-structure length to match environmental conditions via plasticity will enjoy a fitness advantage relative to those that do not. Strathmann et al. (1992) hypothesize that plasticity in allocation toward the growth and developmental timing of body parts is associated with the evolution of lecithotrophy from planktotrophy. Herrera et al. (1996) demonstrated that species whose larvae develop from larger eggs, and thus possess greater endogenous energy stores, attain later developmental stages (characterized by the growth of successive pairs of larval arms to a typical maximum of four pairs and lastly the appearance of a juvenile rudiment adjacent to the larval stomach) than species from smaller eggs. Thus the effects of maternal nutrition on larval development and morphogenesis might mimic the effects of greater endogenous food supplies.

Strathmann et al. (1993) suggest that larval feeding-structure plasticity has evolved at least twice in marine invertebrates. Larvae of both echinoderms and molluscs display feeding-structure plasticity, but are distantly related and have very different types of larvae with different feeding mechanisms. Echinoderms are deuterostomes and the juvenile develops as a separate structure within the larval body. Feeding larvae of echinoderms

capture particles of food by reversing a short section of their ciliary band, which is composed of simple cilia. Molluscs are protostomes and many structures are unchanged during metamorphosis from larva to juvenile, but the larval feeding structure is resorbed or shed at metamorphosis. Feeding larvae of molluscs capture particles with an opposed band feeding mechanism and compound cilia. For these reasons, larvae of echinoderms and molluscs are thought to have evolved independently. Because feeding-structure plasticity has evolved in both groups, the implication is that plasticity is either ancestral to both groups, or is a coevolved, homoplastic trait (Podolsky and McAlister, 2005).

Among echinoderms, phenotypic plasticity of larval feeding structures has been examined in approximately 22 echinoid, four asteroid, two holothuroid, and four ophiuroid species (Table 8.1). The majority of species that exhibit no measured plasticity in response to food-limiting conditions are tropical (Eckert, 1995; McAlister, 2008; Soars et al., 2009), which suggests there are general differences in the expression and evolution of plasticity between species in temperate vs. tropical ecosystems. There also might be differences in plasticity on smaller geographic scales, such as between more closely located populations of a given species. For example, two populations of the subtropical subspecies *Lytechinus variegatus carolinus*, located approximately 185 km apart (collection sites near Beaufort and Wilmington, North Carolina) differ (McAlister, unpublished data), with larvae from one population demonstrating arm-length plasticity and the other seemingly not.

To date, studies examining feeding-structure plasticity have utilized measures of absolute feeding-structure length as well as three different types of relative measure: (1) to body length or some other measure restricted to ephemeral larval structures, (2) to stomach, wherein the stomach is a structure that is functional in both the larva and postlarval juvenile, and (3) to rudiments of juvenile structures that are not functional until after the larval stage (see Section 8.3.4 for statistical considerations). While feeding-structure plasticity may potentially be demonstrated as present through any of these metrics, the conclusion that plasticity is absent in some species (Eckert, 1995; McAlister, 2008; Soars et al., 2009) or populations may be premature unless a study

specifically includes measures of feeding-structure length relative to rudiments of juvenile structures (see Table 8.1). For example, if there was no change in arm or ciliary band length in response to low food, but development of the juvenile rudiment was delayed until the larva had attained a stage with longer arms or ciliary band, then that developmental delay would confer some of the hypothesized benefits of producing absolutely longer arms or ciliary band earlier in the larval period. Demand for energy and materials for growth would thus be postponed until the capacity to capture food had been increased. Although studies collecting absolute or relative measures similar to (1) or (2) are certainly valuable, and may be logistically more feasible, future studies should strive to include measures of both larval structures and the rudiments of juvenile structures to definitively confirm presence or absence of plasticity.

Furthermore, from the population and geographical patterns presented earlier, many new questions arise: What environmental factors or organismal functions drive or constrain the expression of feeding-structure plasticity between taxa? Are there measurable environmental differences between sites or do differing results reflect some type of seasonal or individual sampling bias? Are differences in expression proximally tied to ecological differences between ecosystems or locales, or have ultimate evolutionary responses occurred in lineages? Has there been a loss of chemosensory structures in non-plastic species? Is it difficult to regain these structures once lost? Does absence of plasticity merely reflect absence of measures that would detect it? Answers to these questions will require researchers to continue empirical analysis within a comparative framework while collecting all relevant metrics. At the same time, our understanding of the expression and evolution of plasticity will be deepened by delving into the developmental and physiological mechanisms that are involved during feeding-structure production.

8.3.3 Developmental Mechanisms of Feeding-Structure Plasticity

Although there is currently limited research on the developmental or physiological mechanisms that cause feeding-structure plasticity in marine larvae,

researchers are well poised to rapidly advance this area of research (reviewed by Miner, 2011; see also Adams et al., 2011; Carrier et al., 2015). First, sea urchin larvae have long been a model system for development, and the developmental mechanisms for some of the structures involved in feeding-structure plasticity in echinoid larvae are well understood (e.g., formation of the larval skeleton). Second, recent advances in molecular techniques allow researchers to measure changes in the timing and quantities of gene expression in larvae exposed to different concentrations of food. Last, the interest in ecological development is rapidly growing and as new techniques emerge, feeding-structure plasticity in echinoids is very well suited to advance this field (Hofmann et al., 2005; Adams et al., 2011; Carrier et al., 2015; Gilbert and Epel, 2015).

Here we focus on four general questions that require answers to understand how larvae adjust their morphology in response to algal concentration: (1) How do larvae detect algal concentration? (2) How do larvae adjust the size of the ciliary band, skeleton (in echinoids and ophiuroids), and stomach? (3) How do larvae regulate the timing of production of rudimentary juvenile structures? (4) How do larvae coordinate these different responses? We focus on several studies in which researchers have direct answers about the developmental mechanisms of feeding-structure plasticity in larvae, and draw a few examples from a large body of research on the development of echinoderm larvae to highlight some genes and pathways that are likely involved in feeding-structure plasticity.

There is indirect evidence about the type and location of receptors that larvae use to detect algal concentrations. Echinoid larvae can respond to algal concentrations before they can even ingest algae (Miner, 2005; Adams et al., 2011). These results suggest that larvae use receptors expressed in epithelial cells to detect algal concentrations (Shilling, 1995; Miner, 2007). At least in some echinoid species, these receptors are unlikely mechanoreceptors because larvae do not alter their morphology in response to plastic beads that are similar in size and concentration to algae (Miner, 2007). More likely the receptors are chemoreceptors because larvae reduce the size of their feeding structures in response to algal exudates (Miner, 2007) and amino acids

(Shilling, 1995) in these species. There is some evidence that thyroxine is a signal molecule between algae and larvae (Heyland and Hodin 2004), though researchers have not identified a chemoreceptor or specific chemical signal. In other echinoid species, larvae might use both mechanoreceptors and chemoreceptors in tandem to detect algal concentrations. Larvae of a sand dollar only produce smaller feeding structures in response to intact algae and not algal exudates or plastic beads (Miner, 2007). It is possible that larvae use the same receptors to detect and capture algae and to alter the size of their feeding structures. There is also evidence that suggests larvae are more sensitive to environmental cues when starved. Starved larvae up-regulate cAMP response element-binding protein (CREB), ETS domain-containing protein (Elk), and an excitatory amino acid transporter (EAAT), which in other organisms are known to increase the sensitivity to environmental stimuli (Carrier et al., 2015). Interestingly, thyroxine receptor and dopamine receptor (see later) activity can cross-regulate CREB (Méndez-Pertuz, 2003; Neve et al., 2004).

After larvae detect an external signal from algae, the nervous system and dopaminergic neurons are likely involved in the signaling pathway. Adams et al. (2011) demonstrated that dopamine is involved in the signaling pathway that induces smaller feeding structures, which occur when food is abundant. The ability to manipulate the phenotype of larvae through dopamine now allows researchers to decouple the effects of energy and materials from algae from the effects of the induced response (e.g., Adams et al., 2011). We look forward to future research on the role of dopamine, especially in other plastic species and related non-plastic species (see Table 8.1).

Compared to research on the developmental pathways involved in creating the larval skeleton in echinoids, there are fewer studies on the developmental pathways that larvae use to specify the ciliary band. However, our understanding is rapidly improving as researchers use new tools to improve the developmental map of echinoids (e.g., Li et al., 2014). In echinoids, the ciliary band is specified by interactions between the oral and aboral ectoderm (Davidson et al., 1998; Su et al., 2009), and the gene products of forkhead g (FoxG), hepatocyte nuclear factor 6 (Hnf6), and orthodenticle β 1/2 (Otx β) are a

few of the genes likely involved specifying to the ciliary band (Optim et al., 2004; Tu et al., 2006; Yankura et al., 2013). Of these genes, Hnf6 and Otx β are not regulated by genes that pattern the embryo, which might allow their expression patterns to be altered without disrupting general morphological development (Duboc et al., 2004; Su et al., 2009). The length of the ciliary band might be regulated by genes, like forkhead J1 (FoxJ1), which are associated with ciliogenesis. Starved plutei up-regulate FoxJ1 (Carrier et al., 2015), which is a transcriptional regulator associated with many aspects of cilia development in mouse (Choksi et al., 2014). Despite our improving understanding of the pathways that specify the ciliary band, understanding the mechanisms responsible for adjusting the size of the ciliary band is critical, and an area that we hope researchers investigate more in the future.

The pathways that control the length of the larval skeleton in echinoids are well studied in echinoids—though much less is known about ophiuroids, which also produce a larval skeleton (Dylus et al., 2016). Primary mesenchyme cells form the larval skeleton by producing an extracellular matrix and precipitating calcium carbonate onto this matrix (Killian and Wilt, 2008), and involve many hundreds of genes (Ettensohn 2009; Rafiq et al., 2014). The location and length of the skeleton are regulated by interactions between the primary mesenchyme cells and ectoderm (Hardin et al., 1992; Armstrong et al., 1993; Guss and Ettensohn, 1997). Vascular endothelial growth factor (VEGF), fibroblast growth factors (FGF), orthopedia (Otp), and tetraspanin are involved in the interaction between primary mesenchyme cells and ectoderm, and appear to affect size of the skeleton (DiBernardo et al., 1999, Cavalieri et al., 2007; Duloquin et al., 2007; Love et al., 2007; Röttinger et al., 2008)—the general shape of the skeleton appears fixed early in embryonic development (Armstrong and McClay, 1994). It is also possible that the transmembrane protein P16, which is necessary for skeletal spicules to elongate, is involved in the pathway (Cheers and Ettensohn, 2005). In addition, the proteins spicular matrix protein (SM 50), mesenchyme-specific protein (MSP 130), advillin, and carbonic anhydrase are associated with primary mesenchyme cells, and likely control, at least in part, the length of the

skeleton (Carson et al., 1985; Leaf et al., 1987; Peled-Kramar et al., 2002; Love et al., 2007). For example, advillin and carbonic anhydrase appear to promote lengthening the skeleton at the tips, whereas MSP 130 adds more calcium carbonate to the existing skeleton (Carson et al., 1985; Leaf et al., 1987; Peled-Kramar et al., 2002; Love et al., 2007). Similar to the pathways that specify the ciliary band, we hope researchers also focus on mechanisms that can regulate the length of the skeleton. It will be especially interesting to compare the pathways involved in skeletal plasticity between echinoids and ophiuroids because their skeletons are likely convergent.

Carrier et al. (2015) provide information about the pathways that might regulate the development of the rudiment. Plutei adjust a suite of genes associated with metabolism when exposed to different concentrations of unicellular algae. Starved plutei down-regulate genes associated with growth and mitochondrial activity and up-regulate genes associated with energy homeostasis. In particular, starved larvae up-regulate forkhead O (FoxO) and down-regulate target of rapamycin, which is associated with the formation of the rudiment. Given that both of these genes respond similarly in distantly related model species, it is possible that this pathway is conserved and involved in feeding-structure plasticity in all echinoderms (Carrier et al., 2015). Unlike adjusting of the length of the ciliary band and larval skeleton in certain regions in response to food, the response of the rudiment only requires a shift in the overall timing of when to produce the rudiment, which might indicate a simpler mechanism.

Electrical and chemical signals likely coordinate the morphological responses among structures (Adams et al., 2011; Miner, 2011). The nervous system is well developed in echinoderm larvae before larvae can feed (reviewed in Burke et al., 2006; also see Adams et al., 2011). Sensory neurons are located on the epithelium and nerves are closely associated with the ciliary band and stomach, especially the oral field and mouth. The close proximity between the skeletal rods, primary mesenchyme cells, and ectoderm makes it more likely that chemical signals coordinate responses in the ciliary band and skeleton. For example, dopamine is found in close association with ectoderm and primary mesenchyme cells, which produce the skeleton (Adams et al., 2011).

Partitioning-defective protein localizes in both primary mesenchyme and stomach cells, and might coordinate the response between these two structures (Shiomi and Yamaguchi, 2008).

We encourage research specifically on the developmental and physiological mechanisms of feeding-structure plasticity in larvae. We anticipate that the current knowledge will allow rapid progress for echinoids, and our understanding of echinoids will facilitate research on other groups. We especially encourage comparative studies that will permit tests of hypotheses about the evolution of larval feeding-structure plasticity among metazoans.

8.3.4 Experimental Designs and Analyses

Investigators studying feeding-structure plasticity of larvae are faced with a challenge because manipulating food concentrations alters rates of growth and development, as well as size of feeding structures (Hart and Scheibling, 1988). As a result, it is not possible to simply compare a metric of feeding-structure size between treatments at a given time. Fed larvae are typically much larger in general, including the size of feeding structures, and more developed than starved larvae at any given time. In what follows we discuss how investigators have tried to solve this challenge with the design of their experiments and the analysis of their data.

Investigators often use a nested experimental design, where larvae are measured from a few replicate containers per treatment. The reasons for a nested design are primarily practical because rearing larvae in many replicate containers requires considerable time for maintenance, as well as specialized equipment to control temperature (e.g., incubators or shallow tanks of water to partially submerge containers)—containers typically only hold one or two liters and can fluctuate in temperature beyond the physiological limits of larvae if temperature is not rigorously controlled. Most studies have less than five replicates per treatment. The small number of replicates, however, means that statistical power of experiments is often low, which is of concern because differences between treatments range from 10% to 30%. To increase the statistical power and thus the ability to detect

plasticity, some investigators have analyzed data treating each individual larva as a replicate, after determining that containers were not significantly different within treatments (e.g., Strathmann et al., 1993; Hart and Strathmann, 1994), rather than using the mean for each container. This is a reasonable approach, but one with inherent problems. For example, if there are really differences among containers within treatments but only a few larvae are measured per container, then an investigator is much more likely to incorrectly determine that containers are not different (a Type II error). Differences among containers within treatments are likely common (Sewell et al., 2004), and therefore investigators should not use larvae within a container as independent replicates without a clear justification and power analysis of the test to determine whether containers within a treatment differ from one another. We recommend that investigators work to include more containers, which are true independent replicates, in their experiments.

Most investigators have tested two or three concentrations of algal food as experimental treatments and code algal concentration as a categorical variable. This approach allows researchers to determine whether there is an effect of food concentration, but provides little information about the relationship between feeding structures and algal concentrations—the shape of this relationship has important ecological and evolutionary consequences. An alternative design is to assign a different algal concentration to each container within some range of concentrations and code the algal concentration as a continuous variable (Miner and Vonesh, 2004). Both approaches allow for inferences about whether plasticity occurs, but the latter approach allows for stronger inferences about the shape of the reaction norm. Both linear and generalized linear models can be used with either approach, though generalized linear models provide more flexibility because the researcher can independently specify the relationship between the response and predictor variable and the error structure (Quinn and Keough, 2002)—however, they are more difficult to interpret with complex designs where multiple factors are manipulated.

Feeding interval can also affect the concentration of algae and the interpretation of results. Two processes can change the concentration of algae after

algae are added to a container: they can multiply, die or sink to the bottom and become unavailable. Algae are probably not very likely to divide much between feedings every one to three days because researchers rarely add nutrients to replicates or provide sufficient lighting for algal growth. However, if nutrients are added with algae and light is provided, then algal growth might be of concern. Larval grazing is likely a larger concern because larvae can greatly reduce algal concentrations. For example, feeding larvae of echinoids can clear 1 ml of water per day of food (Hart and Strathmann, 1994). If there are 500 larvae in a container with 2 L of seawater (a concentration of 0.25 larvae per ml), then the larvae will reduce the concentration of algae by 25% per day. There are two consequences that researchers should consider when algal densities change between feedings. First, the concentrations that are added to a replicate container do not reflect the actual concentrations that larvae experience, and researchers will incorrectly estimate the relationship between larval morphology and algal concentration. Second, investigators manipulate a variety of aspects about the food environment (e.g., mean, variance, maximum, and minimum of algal concentration). Currently it is unclear which parameters larvae use to adjust their morphology (Miner and Vonesh, 2004). Changes in algal concentrations among containers in a treatment might explain in part why in some studies larvae in different containers of a treatment differ in phenotype (Sewell et al., 2004).

Investigators have used either larval arm length or ciliary band length as a metric of feeding-structure size and have employed a variety of different statistical approaches to detect feeding-structure plasticity in larvae. Because food concentrations also affect growth, investigators often measure a covariate to account for larval size (e.g., midline body length, a skeletal component of the body, or shell length). Investigators often adjust for larval size, to compare larvae of an equivalent size, by dividing the response variable by the covariate and testing for differences with an analysis of variance (ANOVA; e.g., Sewell et al., 2004; Byrne et al., 2008), or an analysis of covariance (ANCOVA; e.g., Miner, 2005; McAlister, 2007b). An alternative approach used by some investigators is to measure a variety of metrics and use principle components analysis (PCA)

to reduce the number of variables before analyzing the principle components with ANOVA (Hart and Scheibling, 1988; Sewell et al., 2004; Miner et al., 2005; Soars et al., 2009). All approaches have potential problems that investigators should be aware of. Analyzing the ratio of the response variable to the covariate with ANOVA confounds which variable, the response or covariate, is causing differences. By contrast, ANCOVA explicitly tests whether larvae of a given size differ in the response variable and the interaction between the response and covariate. However, ANCOVA assumes there is no error in the covariate (Quinn and Keough, 2002), which is never true when measuring some aspect of larval size. It is likely that the covariate will have similar error as the response variable. PCA is likely the most problematic because transforming the original variables into composite variables mixes both among- and within-group variation, which often reduces the ability to detect differences among treatments (McCoy et al., 2006). McCoy et al. (2006) provide a more appropriate, albeit more complex, method (common principal components analysis combined with Burnaby's back projection method—CPCA/BBPM), and we recommend that investigators use this approach in the future.

To complicate the analysis even further, investigators often measure larvae from containers more than once during the experiment. Repeatedly sampling from containers presents the same challenges as measuring multiple larvae from a container—data collected at different times from a container are not independent. The common solution is to use repeated measures ANOVA or ANCOVA to test for differences among treatments. Recently, mixed effects models that utilize maximum likelihood are common in statistical packages and allow for random effects that deal with the non-independence of repeatedly sampling from containers and measuring more than one individual from a container at a given time.

Lastly, and with respect to genotypic variation and genetic diversity broadly, our knowledge of feeding-structure plasticity across taxa derives from data in which only a single female (and often only a single male for one full-sib family) was used to produce the larvae examined in a given study. This is the case for approximately 1/3 of the species (14 of 37) listed in Table 8.1. The collective

lack of genetic diversity across studies is striking and makes generalizing broad biological patterns, such as the temperate vs. tropical argument presented earlier, from single-family data difficult and potentially tenuous. Future studies should strive to maximize genetic diversity by incorporating as many full-sib families as is feasible, or alternatively by maximizing the number of gametes from a given sex, as appropriate for the hypotheses driving a particular study. For example, increasing the number of males in a given study will increase the amount of additive genetic variation among families, whereas increasing the number of females will diversify the effects toward growth of maternal provisions available from the egg. Researchers should incorporate and statistically account for these variables (numbers of males and females used and full- and half-sib families reared) to the degree possible given the space and time constraints associated with rearing multiple, replicate larval cultures.

8.4 Summary

1. There is compelling evidence that feeding-structure plasticity in marine invertebrates is adaptive, but we need a better understanding of the genetic variation of plasticity within and among populations. Researchers should strive to maximize genetic diversity in future studies.
2. There are important trade-offs among morphological structures and the timing of life history events that influence the evolution of feeding-structure plasticity.
3. Maternal provisions influence the evolution of feeding-structure plasticity; minimal provisioning limits the energy available to produce larger feeding structures, whereas maximal provisioning removes the need to produce larger feeding structures.
4. Feeding-structure plasticity has likely evolved at least twice among marine invertebrates.
5. Researchers are starting to understand the molecular mechanisms of feeding-structure plasticity in marine invertebrates. In light of our understanding of development in echinoids, this system is especially well suited for future research.

6. Researchers have used a variety of experimental designs and statistical analyses in studies of larval feeding-structure plasticity. Some of these designs are problematic and should be avoided in the future. In all cases, all metadata, including larval density, food concentration and feeding frequency, should be reported.

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