# SYMBIOSIS ACROSS DIET-INDUCED PHENOTYPES OF LARVAL SEA URCHINS

by

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A dissertation submitted to the faculty of
The University of North Carolina at Charlotte
in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in
Biology

Charlotte

2020

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# ABSTRACT

TYLER JOSEPH CARRIER. Symbiosis across diet-induced phenotypes of larval sea urchins. (Under the direction of ADAM M. REITZEL)

Larval sea urchins have served as a fundamental system for understanding development, phenotypic plasticity, and life-history evolution over much of the last century. In the recent decade, this understanding has begun to extend to the microbiota associated with larval sea urchins. This dissertation highlights the communities of bacterial symbionts that associate echinoid larvae and how they relate to the ecology and evolution of larval feeding. In particular, larvae acclimate to food-restricted environments by elongating their feeding apparatus to increase their feeding capacity. Chapter 1 of this dissertation shows that expression of this alternative phenotype is correlated with shifts in the bacterial community associated with larval sea urchins and that this response is induced by the environment. Chapter 2 then defines the temporal progression of the larval host and the associated microbiota towards a phenotype-specific bacterial community. Daily profiling of these responses shows that larval urchins follow a four-stage progression and that shifts in the bacterial taxa precede morphological plasticity, suggesting a temporal asynchrony in distinct acclimation responses by larval sea urchins. Chapter 3 then compares these responses between three sea urchin populations from different ocean gyres, showing that despite significant differences in bacterial community structure within each population based on food availability, development, phenotype, and time, variation the bacterial taxa correlated more strongly with geographic location. Lastly, Chapter 4 then shows that these coordinated shifts in larval phenotype and the bacterial symbionts are lost during the major life-history transition from planktotrophy

(feeding) to lecithotrophy (non-feeding). In this transition we also find that eggs from the lecithotrophy are dominated by a novel bacterial lineage with close relatives known to manipulate host reproduction. Collectively, this dissertation suggests that the bacterial communities associated with larval sea urchins are integral to their biology and ecology.

# ACKNOWLEDGEMENTS

The last four-plus years have been an incredibly rewarding yet uphill journey for me both scientifically and personally, and I have so many people to thank. First and foremost, thank you to my advisor Adam Reitzel for taking a chance on me, for providing me with the independence and intellectual freedom I wanted and needed, and for supporting me to be who I see myself becoming. Your selfless mentorship has allowed me to be a better scientist and, more importantly, a better and happier person. Thank you.

Whether beginning this scientific journey on the same day or joining in within the final strides, I owe a great deal to Whitney Leach, Remi Ketchum, Jason Macrander, Devin Clegg, Amy Klock, Edward Smith, and Shelcie Menard for the emotional and intellectual support, humor, and feedback during our time in the Reitzel Lab. I am also incredibly grateful for Jason Hodin and Billie Swalla (Friday Harbor Laboratories), Daniel Rittschof (Duke University), and Maria Byrne (University of Sydney) for hosting me in their laboratories and allowing me to culture larvae; for Sam Dupont (University of Gothenburg), Brittany Leigh and Seth Bordenstein (Vanderbilt University), and Dione Deaker (University of Sydney) for sharing your expertise and time in our collaborations; and all the faculty and staff of the Friday Harbor Laboratories, Darling Marine Center, Duke University Marine Lab, Sven Lovén Centre for Marine Sciences, and the Sydney Institute of Marine Science for the endless logistical support during my visits.

Lastly, conducting field work, culturing larvae, and next-generation sequencing does not come without a financial investment. I, therefore, have several funding bodies to thank. This dissertation would not have been possible without a Graduate Research

Fellowship and Graduate Research Opportunities World award from the National Science Foundation, a Charles Lambert Memorial Endowment fellowship from the Friday Harbor Laboratories, a Sigma Xi Grants-in-Aid of Research grant, a North Carolina Sea Grant award (2016-R/MG-1604), and a Human Frontier Science Program award (RGY0079/2016).

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# CHAPTER 1

# SYMBIOTIC LIFE OF ECHINODERM LARVAE

Citation

**Carrier TJ, Reitzel, AM**. 2020. Symbiotic life of echinoderm larvae. Frontiers in Ecology and Evolution **7:**509.

Abstract

Echinoderms larvae have served as a fundamental system for understanding development and life history evolution over much of the last century. In the last few decades, our understanding of echinoderm larvae has expanded to the microbiota that they associate with. These symbionts and the communities that they form in relation to echinoderm larval host is the focus of this review. Our synthesis of the literature suggests three primary themes. First, larval echinoderms associate with 'subcuticle bacteria' that appear to colonize select tissue types. Second, the bacterial communities associated with larval echinoderms exhibit compositional shifts that are correlated with several fundamental properties of larval biology (*e.g.*, development and morphological plasticity) and ecology (*e.g.*, feeding environment). Third, echinoderm larvae exhibit specific responses to pathogenic bacteria that may aid in maintaining the symbiont community and avoid dysbiosis. To our knowledge, no studies have focused on if climate-related stressors impact the composition of these symbiont communities or how changes in bacteria may modulate response by larvae to these environmental stressors. We conclude by outlining techniques that need to be established in echinoderm larvae to transition from correlations between larvae and their associated microbiota to the function of these symbionts.

## 1.1 Echinoderms and their larvae

The phylum Echinodermata is characterized by their pentaradial symmetry and global distribution in marine ecosystems. This group first appeared in the Cambrian and is composed of ~7,000 extant species (Appeltans et al., 2012) that are grouped into five classes: Crinoidea (feather stars and sea lilies), Holothuroidea (sea cucumbers), Asteroidea (sea stars), Ophiuroidea (brittle stars), and Echinoidea (sea urchins). Echinoderm life cycles are primarily bi-phasic, where the adults reside on the sea floor and the embryonic stages are suspended in the water column (Mileikovsky, 1971; Thorson, 1950; Young & Chia, 1987). This phylum is, perhaps, most recognized for its remarkable diversity of larval forms that have fascinated biologists for more than a century (Levin & Bridges, 1995).

Echinoderm development, in general, follows either a lecithotrophic (non-feeding) or planktotrophic (feeding) trajectory (Mileikovsky, 1971; R. R. Strathmann, 1985; Thorson, 1950). Lecithotrophs develop from relatively large and energy-rich eggs (~300 µm-1 mm in diameter, or where $s \geq 1$; McEdward & Miner, 2006; Vance, 1973) with sufficient maternal investment to complete development and undergo metamorphosis. The developmental period for lecithotrophs typically lasts a few days and, due to a shorter pelagic larval duration, can result in marginal dispersal between populations (Mileikovsky, 1971; R. R. Strathmann, 1985; Thorson, 1950). Planktotrophs, on the other hand, develop from more energy-poor eggs (~100 µm-~300 µm in diameter, or where $s<1$; McEdward & Miner, 2006; Vance, 1973) with sufficient maternal input to complete embryogenesis and develop into larvae with initial feeding structures. The remaining

energetic supply required for larval development and metamorphosis is acquired from exogenous resources (*e.g.*, phytoplankton, detritus, and other particles) that are concentrated by a cilia-lined feeding apparatus (Feehan, Grauman-Boss, Strathmann, Dethier, & Duggins, 2018; Strathmann, 1987). These particulates are often dilute, leading to a pelagic larval duration lasting weeks to months and, in some cases, years (Mileikovsky, 1971; Olson & Olson, 1989; Strathmann, 1985; Thorson, 1950).

Nearly four decades ago, Ribkin and colleagues (1986) recognized that the planktotrophic larvae of the asteroid *Porania antarctica* selectively interact with the environmental microbiota through bacterivory. It has since been observed that additional planktotrophic echinoderms exhibit bacterivory and that this feeding mode is believed to be important, but not essential, to the metabolic requirements of the larva (Ayukai, 1994; Douillet, 1993; Gosselin & Qian, 1997; Moal, Samain, Corre, Nicolas, & Glynn, 1996; Pearse, Bosch, Pearse, & Basch, 1991). Based on maximum clearance rates and particle abundance, it is estimated that echinoderm larvae interact with ~20 million bacteria each day by feeding alone (Carrier, Macrander, & Reitzel, 2018). It is, however, unknown which bacterial (or other microbial) groups that echinoderm larvae may target and whether these microbes are selected strictly for bacterivory or as a symbiont that may be acquired by horizontal transmission.

Just prior to recognizing that echinoderm larvae were bacterivorous, Cameron and Holland (1983) observed that bacteria were living inside the tissues of healthy larvae. In this review we synthesize the properties of these bacteria and how they relate to the biology and ecology of the echinoderm larval host. In the first section, we provide an overview of our understanding of echinoderm larvae and their bacterial symbionts through

the lens of microscopy and next-generation sequencing. In the second section, we summarize how larval-associated microbiota may be relevant in coping with anthropogenic stressors and outline the techniques needed to transition towards understanding the function of these symbionts.

## 1.2 Bacterial symbionts of echinoderm larvae

Over the last few decades our understanding of echinoderm larvae and their microbes has gone through two primary phases. The first phase uses microscopy and focuses on ‘subcuticle bacteria’ (or, due to their location within larval tissues, could be also be characterized as endosymbionts but this has not been explicitly tested). The second phase has developed in recent years and uses next-generation sequencing and other molecular tools to characterize larval-associated bacterial communities. This phase may be further divided into two focal points: the dynamics of these bacterial communities under different ecological conditions and the immune responses of the larval host when faced with pathogenic bacteria.

### 1.2.1 Subcuticle bacteria

While determining how to preserve the thin cuticle overlying the epidermis for transmission and scanning electron microscopy, Holland and Nealson (1978) observed that adults for each of the five echinoderm classes had a high abundance of what they called ‘subcuticle bacteria.’ Holland and Nealson (1978) did not test whether embryonic or larval stages also contained subcuticle bacteria. They did, however, speculate on the nature of transmission for echinoderms, stating that because the eggs are “unattached to

any follicle cells, and no bacteria have never been observed on or in echinoderm eggs" that "it is probable that each new generation of such echinoderms acquires its subcuticular bacteria from the surrounding sea water." Moreover, Holland and Nealson (1978) suggested that, if acquired during the embryonic or larval stages, that the echinoderm must select for these symbiotic bacteria from a diverse community of environmental microbiota.

Shortly thereafter and on multiple occasions since these original observations, studies have identified subcuticle bacteria in the developmental stages of echinoderms. These symbionts, thus far, have been found in three asteroids (Bosch, 1992; Cameron & Holland, 1983; Cerra, Byrne, & Hoegh-Guldberg, 1997), one ophiuroid (Walker & Lesser, 1989), and one echinoid (Heyland, Schuh, & Rast, 2018; Schuh et al., 2019) (Figure 1.1; Table 1). In these five species, subcuticle bacteria have been observed within the mouth and gut lumen, out-pockets of the extracellular matrix that surrounds the larval body, embedded in the inner layer of the secondary cuticle of the rudiment epidermis, and are engulfed and, in some cases, digested by epidermal cells (Bosch, 1992; Cameron & Holland, 1983; Cerra et al., 1997; Heyland et al., 2018; Schuh et al., 2019; Walker & Lesser, 1989).

The function of these subcuticle bacteria remains essentially unknown. Two cases, however, may suggest that these symbionts interact with and functionally benefit the larval host. First, *A. squamata* has a "vestigial" pluteus (*i.e.*, greatly reduced larval arms and lacking a ciliated mouth) that is brooded within their central plate. Using transmission and scanning electron microscopy, Walker & Lesser (1989) found that a rod-shaped bacterium from the *Octadecabacter* was abundant and actively dividing within the tissues

of this "vestigial" pluteus (Morrow et al., 2018). This *Octadecabacter*, which strictly associates with these larvae and is not found in the environment, can uptake dissolved free amino acids that are then incorporated into bacterial proteins and increase the total amino acid uptake for *A. squamata* (Lesser & Blakemore, 1990; Lesser & Walker, 1992; Morrow, Tedford, Pankey, & Lesser, 2018; Walker & Lesser, 1989). Second, clonal larvae of the sea star *Luidia* collected from the Gulf Stream had one to three rod-shaped morphotypes of subcuticle bacteria (Bosch, 1992). For *Luidia* as well as *Acanthaster*, some symbionts were located in the gut and auto-fluoresced (Bosch, 1992; Carrier et al., 2018; Galac, Bosch, & Janies, 2016), suggesting the potential ability to be phototrophic. Collectively, these examples suggest, but do not show explicitly, that bacterial symbionts may have metabolic functions that could potentially benefit the larval host.

### 1.2.2 Bacterial communities

The subcuticle bacteria discussed above are a portion of the collection of bacteria associated with echinoderm larvae. Using next-generation sequencing, larval-associated bacterial communities have been reported for six species of echinoderm larvae: two asteroids (Carrier et al., 2018; Galac et al., 2016) and four echinoids (Carrier, Dupont, & Reitzel, 2019; Carrier & Reitzel, 2018; Carrier & Reitzel, 2019, 2019; Schuh et al., 2019) (Figure 1.1; Table 1). In general, these bacterial communities are composed of a couple hundred bacterial species (*i.e.*, Operational Taxonomic Units or OTUs, as defined by ≥97% similarity of the phylogenetically-conserved 16S rRNA gene) (Carrier et al., 2019; Carrier & Reitzel, 2018; Carrier & Reitzel, 2019; Carrier et al., 2018; Galac et al., 2016). The predominant bacterial families encompassing these diverse communities are the α-

and γ-Proteobacteria (Proteobacteria) and the Flavobacteriaceae (Bacteroidetes) (Carrier et al., 2019; Carrier & Reitzel, 2019, 2019; Carrier et al., 2018; Galac et al., 2016).

Like many other studies of animal and plant microbes, the bacterial communities associated with echinoderm larvae are species-specific and taxonomically distinct from the environmental microbiota (Carrier & Reitzel, 2018; Carrier et al., 2018; Galac et al., 2016), suggesting that these communities are, at least in part, selected by the host. This host-specificity, however, appears to be lost when larvae are cultured under traditional laboratory settings for rearing the developmental stages of marine invertebrates (*i.e.*, fine-filtered or artificial saltwater) (Schuh et al., 2019). Moreover, *Strongylocentrotus purpuratus* larvae cultured under traditional laboratory settings associate with bacterial communities that are less diverse in total taxa and the phylogenetic breadth of those taxa, and retain ~40% of the OTUs harbored by 'wild-type' counterparts (Schuh et al., 2019). This implies that studying larval-associated bacterial communities is most accurately performed at near-natural conditions, such as by filtering ambient seawater to 5-μm to remove most debris and planktonic predators while retaining the environmental microbiota (Carrier & Reitzel, 2018; Hodin et al., 2019).

Larval-associated bacterial communities are variable in community membership and composition but exhibit non-random shifts that correlate with multiple components of larval biology and ecology. These communities, for example, are established on unfertilized eggs but not the sperm of sea urchins (Carrier & Reitzel, 2019; Schuh et al., 2019). When cultured using coarsely (5-μm) filtered seawater, echinoderm larvae exhibit a development-based succession in symbiont composition and, using fluorescent *in situ* hybridization, these bacteria appear to be localized to the mouth and gut lumen (Carrier

& Reitzel, 2019; Schuh et al., 2019). Following fertilization, the diversity of these communities increases by ~20% during the early embryonic stages and decreased by nearly ~85% following hatching and through metamorphosis (Carrier & Reitzel, 2019). From egg to hatching, the early embryonic stages appear to converge taxonomically with the environmental microbiota but then exhibit a host-mediated selection by diverging from this community following the onset of feeding (Carrier & Reitzel, 2019). In cases where embryonic development includes asexual reproduction (*e.g.*, cloning), the larval clones deviate little from the parent larva by maintaining a high proportion of particular bacteria, including phototropic species (Carrier et al., 2018; Galac et al., 2016).

The six species of echinoderm larvae with profiled bacterial communities are planktotrophs and, by definition, are required to feed. Five of these species (echinoids: *Strongylocentrotus purpuratus*, *S. droebachiensis*, *Mesocentrotus franciscanus*, and *Lytechinus variegatus*; asteroid: *Acanthaster* sp.) have been differentially fed to test whether community composition varies with food quantity. In each case, bacterial communities are diet-specific with well-fed larvae distinguished from diet-restricted treatments (Carrier & Reitzel, 2018; Carrier & Reitzel, 2019; Carrier et al., 2018). When diet-restriction is prolonged, larval sea urchins compensate by elongating their feeding arms to increase water filtration capacity (Hart & Strathmann, 1994; McAlister & Miner, 2018; Miner, 2004). This change in morphology is correlated with a shift in the composition of the bacterial symbionts, such that larval urchins associate with phenotype-specific microbiota (Carrier & Reitzel, 2018; Carrier & Reitzel, 2019; Carrier et al., 2018).

Establishment of a phenotype-specific bacterial community for larvae of the sea urchin *L. variegatus* follows a four-stage succession (Carrier & Reitzel, 2019). First,

larvae across diets associated with bacterial communities similar in both composition and structure. Second, different food environments (*i.e.*, algal concentrations) induced diet-specific bacterial communities in both membership and composition. Third, the bacterial communities of diet-restricted larvae associated with similar bacterial communities that are also distinct from that of well-fed larvae, with the latter coinciding with a reduction in community diversity. Lastly, composition and structure are maintained from the prior successional stage and now correlate with short- and long-arm phenotypes (Carrier & Reitzel, 2019). This suggests that changes in the larval-associated bacterial community shifts prior to the expression of the environmentally elicited morphological phenotypes, and that microbial communities may respond to environmental variation more quickly than morphological changes.

Recent research also suggests that microbial communities differ not only between species but also between populations (*e.g.*, ascidians: Dishaw et al., 2014; sponges: Marino, Pawlik, López-Legentil, & Erwin, 2017; fish: Llewellyn et al., 2016; seaweed: Marzinelli et al., 2015). Differential feeding of larvae of the echinoid *S. droebachiensis* from three populations in different ocean basins showed parallel responses that result in diet-specific bacterial communities (Carrier et al., 2019). Despite each population associating with a diet-specific bacterial community, variation in OTU membership and community composition correlated more strongly with geographic location (Carrier et al., 2019). Moreover, when comparing the taxonomic membership between populations, 20-30% of bacterial taxa were specific to a single location while ~10% were shared between all three locations (Carrier et al., 2019). Collectively, these data suggest that larvae for a given species associates with a population-specific bacterial community. It is, however,

worth noting that this comparison was not performed using common garden culturing, and that it would be expected that these communities are more taxonomically similar, but still population-specific, when cultured using identical seawater.

### 1.2.3 Pathogenic bacteria

Biological responses to foreign 'particles' by echinoderm larvae were first recognized in the late 19th century (Metchnikoff, 1891; Tauber, 2003). More recently, echinoderm larvae have been used as a comparative system to define the cellular and molecular mechanisms of immunity when combating pathogenic bacteria. Due to the availability of a genome (Sodergren et al., 2006), the majority of this work has used the echinoid *Strongylocentrotus purpuratus*. The immune response by *S. purpuratus* to pathogenic bacteria was recently reviewed by (Buckley & Rast, 2017) and (Heyland et al., 2018). We refer the reader to these in-depth reviews for the molecular underpinnings of larval immunity, as this section will focus on the ecological components.

From the amoebic disease (*Paramoeba invadens*) of *S. droebachiensis* in Nova Scotia (Feehan, Johnson-Mackinnon, Scheibling, Lauzon-Guay, & Simpson, 2013; Scheibling & Stephenson, 1984) to the major epizootic that decimated *Diadema antillarum* throughout the Caribbean (Lessios, 2016; Lessios, Robertson, & Cubit, 1984) or, more recently, the densovirus-associated wasting disease of asteroids (Harvell et al., 2019; Hewson et al., 2014), the ecological impacts of disease on echinoderms has been well-documented over the last few decades (Feehan & Scheibling, 2014). Less of this work, however, has focused on embryonic and larval echinoderms. Echinoderm larvae potentially interact with ~20 million bacteria each day by feeding alone (Carrier et al.,

2018), and a portion of these bacteria may be consumed through bacterivory, be symbionts acquired by horizontal transmission, or pathogenic bacteria that require an immune response.

When faced with variation in food quantity, larval echinoids exhibit a trade-off in the expression of immune and metabolic genes (Carrier, King, & Coffman, 2015; Carrier et al., 2018), such that well-fed larvae upregulate metabolism and suppress immunity. High food availability is also suitable environmental conditions for pathogens or for bacteria to express pathogenic characteristics. If faced with a pathogen in these feeding conditions, food-induced suppression of the immune system may result in a suboptimal physiological response and larvae may be less able to regulate the associated microbiota. Echinoid larvae may then be at risk of pathogen-induced disease or exhibiting dysbiosis, both of which are hypothesized to be precursors to larval mortality (Carrier et al., 2018).

When faced with pathogenic bacteria, echinoid larvae exhibit immune responses, for example, by expressing genes in the interleukin 17 (IL17) complex (Buckley et al., 2017; Buckley & Rast, 2017; Ho et al., 2016). IL17s are known to both serve as a primary barrier to foreign bodies and to regulate the composition of larval-associated microbiota (Buckley & Rast, 2017). For *S. purpuratus*, exposure to the pathogen *V. diazotrophicus* coincides with successive expression of gut epithelial-specific IL17 subtypes to prevent progression of *V. diazotrophicus* and maintain the gut microbiota (Buckley et al., 2017; Buckley & Rast, 2017). When *S. purpuratus* larvae are made nearly germ-free, they become more susceptible to *Vibrio*-induced infections and mortality than counterparts with their native bacteria (Schuh et al., 2019). Pathogens that elicit a response appear to be lineage-specific, as not all *Vibrio* species or strains induce the expression of the IL17s

(Buckley et al., 2017; Buckley & Rast, 2017). Such responses by the larval host may contribute to maintaining homeostatic symbioses (*e.g.*, Mortzfeld & Bosch, 2017), but the functional underpinnings for larval echinoderms has yet to be determined (but see Buckley et al., 2017; Buckley & Rast, 2017; Ho et al., 2016).

### 1.3 Larval echinoderms in a changing environment

Our understanding of echinoderm larvae and their relationship with microbial symbionts has been studied at ambient conditions. Marine invertebrates and their life history stages are, however, encountering a suite of anthropogenic stressors (Byrne, Ross, Dworjanyn, & Parker, 2018) that may disrupt homeostatic symbioses (*e.g.*, Rosenberg, Koren, Reshef, Efrony, & Zilber-Rosenberg, 2007). To our knowledge, no studies have focused on how climate-related stressors (*e.g.*, temperature and pH) affect the associated microbiota of echinoderm larvae. Similar to the cnidarian planula (Mortzfeld et al., 2015) and sponge amphiblastula (Webster, Botte, Soo, & Whalan, 2011), we hypothesize that an acclimation response to abiotic or biotic stressors would include shifts in symbiont composition. This, in particular, provides larvae with an opportunity to acquire bacterial symbionts with genes that are novel to the larval hologenome and that may aid in ameliorating physiological stress. Whether at ambient conditions or facing climate-related stressors, the function of larval-associated symbionts remains uncertain and if the host benefits from these partnerships.

Determining if and how microbial symbionts contribute to the larval holobiont requires a transition from 16S rRNA-based profiling to function-based studies (Williams & Carrier, 2018). The functional potential and expression profiles of microbial symbionts

may be assessed using metagenomics (*e.g.*, Slaby, Hackl, Horn, Bayer, & Hentschel, 2017) and metatranscriptomics (*e.g.*, Moitinho-Silva et al., 2014). The impact that particular taxa or the symbiont community have on the larval host may then be assessed by generating microbe-free larvae through an antibiotic treatment or gnotobiotic chambers (Bates et al., 2006; Leigh, Liberti, & Dishaw, 2016; Rawls, Samuel, & Gordon, 2004; Schuh et al., 2019; Smith, McCoy, & Macpherson, 2007) and adding back single or a mix of culturable taxa (Domin et al., 2018). Such techniques can be coupled with established visualization approaches (electron microscopy: Cerra et al., 1997; fluorescent *in situ* hybridization: Schuh et al., 2019) to define the spatial distribution of these symbiont and which tissues they colonize. Lastly, bacteria are not the sole member of host-associated microbial communities, as viruses, archaea, and fungi are common co-inhabitants (e.g., Webster & Thomas, 2016). Similar molecular and sequencing approaches can be used to characterize if and how they interact with the larval host.

## 1.4 Conclusion

Our understanding of symbioses between larval echinoderms and microbes has primarily developed in the last few years; yet, in this time we suggest that three primary themes have materialized. First, larval echinoderms associate with subcuticle bacteria that appear to colonize select tissues. Second, the bacterial communities associated with larval echinoderms exhibit compositional shifts that are correlated with several fundamental properties of larval biology and ecology. Third, the echinoderm larval host exhibits strict responses to pathogenic microbiota that may aid in maintaining the symbiont community to avoid dysbiosis.

Echinoderms larvae continue to serve as a fundamental system for understanding development and life history evolution (Love & Strathmann, 2018). The diversity in form and function may act as a strong foundation to understand how and to what extent bacteria and other microbes influence the many dimensions of larval biology (Hammer, Sanders, & Fierer, 2019). In particular, these diverse developmental approaches enable the fields of animal-microbe symbiosis and larval biology to uniquely test for functional links between host and symbiont during, for example, morphological plasticity and life history transitions. Because after all, echinoderm larvae have always and will continue to live and evolve in a sea of microbes (Bordenstein & Theis, 2015; McFall-Ngai et al., 2013; Zilber-Rosenberg & Rosenberg, 2008).

Acknowledgements

A special thanks to the editors of *Symbiosis in a Changing Environment* for the invitation and the reviewers to providing critical feedback to an earlier version of this manuscript. T.J.C. was supported by an NSF Graduate Research Fellowship and A.M.R. was supported by Human Frontier Science Program Award RGY0079/2016.

References

Appeltans, W., Ahyong, S. T., Anderson, G., Angel, M. V., Artois, T., Bailly, N., . . . Costello, M. J. (2012). The magnitude of global marine species diversity. *Current Biology, 22*, 2189-2202.

Ayukai, T. (1994). Ingestion of lltraplankton by the planktonic larvae of the crown-of-thorns starfish, *Acanthaster planci*. *Biological Bulletin, 186*, 90-100.

Bates, J. M., Mittge, E., Kuhlman, J., Baden, K. N., Cheesman, S. E., & Guillemin, K. (2006). Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. *Developmental Biology, 297*, 374-386.

Bordenstein, S. R., & Theis, K. R. (2015). Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biology, 13*, e1002226.

Bosch, I. (1992). Symbiosis between bacteria and oceanic clonal sea star larvae in the western North Atlantic Ocean. *Marine Biology, 114*, 495-502.

Buckley, K. M., Ho, E. C. H., Hibino, T., Schrankel, C. S., Schuh, N. W., Wang, G. Z., & Rast, J. P. (2017). IL17 factors are early regulators in the gut epithelium during inflammatory response to *Vibrio* in the sea urchin larva. *eLife, 6*, e23481.

Buckley, K. M., & Rast, J. P. (2017). An organismal model for gene expression networks in the gut-associated immune response. *Frontiers in Immunology, 8*, 1297.

Byrne, M., Ross, P. M., Dworjanyn, S. A., & Parker, L. (2018). Larval ecology in the face of changing climate—impacts of ocean warming and ocean acidification. In T. J. Carrier, A. M. Reitzel, & A. Heyland (Eds.), *Evolutionary Ecology of Marine Invertebrate Larvae*. Oxford, UK: Oxford University Press.

Cameron, R. A., & Holland, N. D. (1983). Electron microscopy of extracellular materials during the development of a seastar, *Patiria miniata* (Echinoderm: Asteroidea). *Cell Tissue Research, 243*, 193-200.

Carrier, T. J., Dupont, S., & Reitzel, A. M. (2019). Geographic location and food availability offer differing levels of influence on the bacterial communities associated with larval sea urchins. *Fems Microbiology Ecology, 95*, fiz103.

Carrier, T. J., King, B. L., & Coffman, J. A. (2015). Gene expression changes associated with the developmental plasticity of sea urchin larvae in response to food availability. *Biological Bulletin, 228*, 171-180.

Carrier, T. J., Macrander, J., & Reitzel, A. M. (2018). A microbial perspective on the life-history evolution of marine invertebrate larvae: if, where, and when to feed. *Marine Ecology, 39*, e12490.

Carrier, T. J., & Reitzel, A. M. (2018). Convergent shifts in host-associated microbial communities across environmentally elicited phenotypes. *Nature Communications, 9*, 952.

Carrier, T. J., & Reitzel, A. M. (2019). Bacterial community dynamics during embryonic and larval development of three confamilial echinoids. *Marine Ecology Progress Series, 611*, 179-188.

Carrier, T. J., & Reitzel, A. M. (2019). Shift in bacterial taxa precedes morphological plasticity in a larval echinoid 166: 164.

Carrier, T. J., Wolfe, K., Lopez, K., Gall, M., Janies, D. A., Byrne, M., & A.M., R. (2018). Diet-induced shifts in the crown-of-thorns (*Acanthaster* sp.) larval microbiome. *Marine Biology, 165*, 157.

Cerra, A., Byrne, M., & Hoegh-Guldberg, O. (1997). Developments of the hyaline layer around the planktonic embryos and larvae of the asteroid *Patiriella calcar* and the presence of associated bacteria. *Invertebrate Reproduction and Development, 31*, 337-343.

Dishaw, L. J., Flores-Torres, J., Lax, S., Gemayel, K., Leigh, B., Melillo, D., . . . Gilbert, J. A. (2014). The gut of geographically disparate *Ciona intestinalis* harbors a core microbiota. *PLoS ONE, 9*, e93386.

Domin, H., Zurita-Gutiérrez, Y. H., Scotti, M., Buttlar, J., Hentschel, U., & Fraune, S. (2018). Predicted bacterial interactions affect *in vivo* microbial colonization dynamics in *Nematostella*. *Frontiers in Microbiology, 9*, 728.

Douillet, P. (1993). Bacterivory in Pacific oyster *Crassostrea gigas* larvae. *Marine Ecology Progress Series, 98*, 123-134.

Feehan, C. J., Grauman-Boss, B. C., Strathmann, R. R., Dethier, M. N., & Duggins, D. O. (2018). Kelp detritus provides high-quality food for sea urchin larvae. *Limnology and Oceanograhpy, 63*, 5299-5306.

Feehan, C. J., Johnson-Mackinnon, J., Scheibling, R. E., Lauzon-Guay, J.-S., & Simpson, A. B. G. (2013). Validating the identity of *Paramoeba invadens*, the causative agent of recurrent mass mortality of sea urchins in Nova Scotia, Canada. *Diseases of Aquatic Organisms, 103*, 209-227.

Feehan, C. J., & Scheibling, R. E. (2014). Effects of sea urchin disease on coastal marine ecosystems. *Marine Biology, 161*, 1467-1485.

Galac, M. R., Bosch, I., & Janies, D. A. (2016). Bacterial communities of oceanic sea star (Asteroidea: Echinodermata) larvae. *Marine Biology, 163*, 162.

Gosselin, L. A., & Qian, P.-Y. (1997). Can bacterivory alone sustain larval development in the polychaete Hydroides elegans and the barnacle Balanus amphitrite? *Marine Ecology Progress Series, 161*, 93-101.

Hammer, T. J., Sanders, J. G., & Fierer, N. (2019). Not all animals need a microbiome. *Fems Microbiology Letters, 336*, fnz117.

Hart, M. W., & Strathmann, R. R. (1994). Functional consequences of phenotypic plasticity in echinoid larvae. *Biological Bulletin, 186*(3), 291-299.

Harvell, C. D., Montecino-Latorre, D., Caldwell, J. M., Burt, J. M., Bosley, K., Keller, A., . . . Gaydos, J. K. (2019). Disease epidemic and a marine heat wave are associated with the continental-scale collapse of a pivotal predator (*Pycnopodia helianthoides*). *Science Advances, 5*, eaau7042.

Hewson, I., Button, J. B., Gudenkauf, B. M., Miner, B., Newton, A. L., Gaydos, J. K., . . . Harvell, C. D. (2014). Densovirus associated with sea-star wasting disease and mass mortality. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 17278-17283.

Heyland, A., Schuh, N. W., & Rast, J. P. (2018). Sea urchin larvae as a model for postembryonic development. In M. Kloc & J. Z. Kubiak (Eds.), *Marine Organisms as Model Systems in Biology and Medicine* (pp. 137-161): Springer.

Ho, E., Buckley, K. M., Schrankel, C. S., Schuh, N. W., Hibino, T., Solek, C. M., . . . Rast, J. P. (2016). Perturbation of gut bacteria induces a coordinated cellular immune response in the purple sea urchin larva. *Immunology and Cell Biology, 94*, 861–874.

Hodin, J., Heyland, A., Mercier, A., Pernet, B., Cohen, D. L., Hamel, J.-F., . . . George, S. B. (2019). *Culturing echinoderm larvae through metamorphosis* (Vol. 150).

Holland, N. D., & Nealson, K. H. (1978). The fine structure pf the echinoderm cuticle and subcuticular bacteria of echinoderms. *Acta Zoologica, 59*, 169-185.

Leigh, B. A., Liberti, A., & Dishaw, L. J. (2016). Generation of germ-free *Ciona intestinalis* for studies of gut-microbe interactions. *Frontiers in Microbiology, 7*, 2092.

Lesser, M. P., & Blakemore, R. P. (1990). Description of a novel symbiotic bacterium from the brittle star, Amphipholis squamata. *Applied and Environmental Microbiology, 56*, 2436-2440.

Lesser, M. P., & Walker, C. W. (1992). Comparative study of the uptake of dissolved amino acids in sympatic brittlestars with and without endosymbiotic bacteria. *Comparative Biochemistry and Physiology, 101*, 217-223.

Lessios, H. A. (2016). The great *Diadema antillarum* die-off: 30 years later. *Annual Review of Marine Science, 8*, 267-283.

Lessios, H. A., Robertson, D. R., & Cubit, J. D. (1984). Spread of *Diadema* mass mortality through the Caribbean. *Science, 226*, 335-337.

Levin, L. A., & Bridges, T. S. (1995). Pattern and diversity in reproduction and development. In L. R. McEdward (Ed.), *Ecology of Marine Invertebrate Larvae* (pp. 1-48): CRC Press.

Llewellyn, M. S., McGinnity, P., Dionne, M., Letourneau, J., Thonier, F., Carvalho, G., . . . Derome, N. (2016). The biogeography of the atlantic salmon (*Salmo salar*) gut microbiome. *ISME Journal, 10*, 1280-1284.

Love, A. C., & Strathmann, R. R. (2018). Marine invertebrate larvae: model life histories for development, ecology, and evolution. In T. J. Carrier, A. M. Reitzel, & A. Heyland (Eds.), *Evolutionary Ecology of Marine Invertebrate Larvae*. Oxford, United Kingdom: Oxford University Press.

Marino, C. M., Pawlik, J. R., López-Legentil, S., & Erwin, P. M. (2017). Latitudinal variation in the microbiome of the sponge *Ircinia campana* correlates with host haplotype but not anti-predatory chemical defense. *Marine Ecology Progress Series, 565*, 53-66.

Marzinelli, E. M., Campbell, A. H., Valdes, E. Z., Vergés, A., S., N., Wernberg, T., . . . Steinberg, P. D. (2015). Continental-scale variation in seaweed host-associated bacterial communities is a function of host condition, not geography. *Environmental Microbiology, 17*, 4078-4088.

McAlister, J. S., & Miner, B. G. (2018). Phenotypic plasticity of feeding structures in marine invertebrate larvae. In T. J. Carrier, A. M. Reitzel, & A. Heyland (Eds.), *Evolutionary Ecology of Marine Invertebrate Larvae*. Oxford, UK: Oxford University Press.

McEdward, L. R., & Miner, B. (2006). Estimation and interpretation of egg provisioning in marine invertebrates. *Integrative and Comparative Biology, 46*, 224-232.

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C. G., Carey, H. V., Domazet-Loso, T., Douglas, A. E., . . . Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences of the United States of America, 110*(9), 3229-3236.

Metchnikoff, E. (1891). *Lectures on the Comparative Pathology of Inflammation: Delivered at the Pasteur Institute in 1891*. London, UK: Kegan Paul, Trench, Trubner and Company.

Mileikovsky, S. A. (1971). Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evalution. *Marine Biology, 10*, 193-213.

Miner, B. G. (2004). Evolution of feeding structure plasticity in marine invertebrate larvae: a possible trade-off between arm length and stomach size. *Journal of Experimental Marine Biology and Ecology, 315*, 117-125.

Moal, J., Samain, J. F., Corre, S., Nicolas, J. L., & Glynn, A. (1996). Bacterial nutrition of great scallop larvae. *Aquaculture International, 4*, 215–-223.

Moitinho-Silva, L., Seridi, L., Ryu, T., Voolstra, C. R., Ravasi, T., & Hentschel, U. (2014). Revealing microbial functional activities in the Red Sea sponge *Stylissa carteri* by metatranscriptomics. *Environmental Microbiology, 16*, 3683-3698.

Morrow, K. M., Tedford, A. R., Pankey, M. S., & Lesser, M. P. (2018). A member of the Roseobacter clade, *Octadecabacter* sp., is the dominant symbiont in the brittle star *Amphipholis squamata*. *FEMS Microbiology Ecology, 94*, fiy030.

Mortzfeld, B. M., & Bosch, T. C. G. (2017). Eco-Aging: stem cells and microbes are controlled by aging antagonist FoxO. *Current Opinion in Microbiology, 38*, 181-187.

Mortzfeld, B. M., Urbanski, S., Reitzel, A. M., Kunzel, S., Technau, U., & Fraune, S. (2015). Response of bacterial colonization in *Nematostella vectensis* to

development, environment and biogeography. *Environmental Microbiology, 18*(6), 1764-1781.

Olson, R. R., & Olson, M. H. (1989). Food limitation of planktotrophic marine invertebrate larvae: does it control recruitment success? *Annual Review of Ecology and Systematics, 20*, 225-247.

Pearse, J. S., Bosch, I., Pearse, V. B., & Basch, L. V. (1991). Bacterivory by bipinnarias: in the Antartic but not in California *American Zoologist, 31*, 6A.

Rawls, J. F., Samuel, B. S., & Gordon, J. I. (2004). Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 4596-4601.

Rivkin, R. B., Bosch, I., Pearse, J. S., & Lessard, E. J. (1986). Bacterivory: a novel feeding mode for asteroid larvae. *Science, 233*, 1311-1314.

Rosenberg, E., Koren, O., Reshef, L., Efrony, R., & Zilber-Rosenberg, I. (2007). The role of microorganisms in coral health, disease, and evolution. *Nature Reviews Microbiology, 5*(5), 355-362. doi:10.1038/nrmicro1635

Scheibling, R. E., & Stephenson, R. L. (1984). Mass mortality of *Strongylocentrotus droebachiensis* (Echinodermata: Echinoidea) off Nova Scotia, Canada. *Marine Biology, 78*, 153-164.

Schuh, N. W., Carrier, T. J., Schrankel, C. S., Reitzel, A. M., Heyland, A., & Rast, J. P. (2019). Bacterial exposure mediates developmental plasticity and resistance of lethal *Vibrio lentus* infection in purple sea urchin (*Strongylocentrotus purpuratus*) larvae. *Frontiers in Immunology*.

Slaby, B. M., Hackl, T., Horn, H., Bayer, K., & Hentschel, U. (2017). Metagenomic binning of a marine sponge microbiome reveals unity in defense but metabolic specialization. *The ISME Journal, 11*, 2465-2478.

Smith, K., McCoy, K. D., & Macpherson, A. J. (2007). Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Seminars in Immunology, 19*, 59-69.

Sodergren, E., Weinstock, G. M., Davidson, E. H., Cameron, R. A., Gibbs, R. A., Weinstock, G. M., . . . Consortium, S. U. G. S. (2006). The genome of the sea urchin *Strongylocentrotus purpuratus*. *Science, 314*, 941-952.

Strathmann, R. R. (1985). Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *Annual Review of Ecology and Systematics, 16*, 339-361.

Strathmann, R. R. (1987). Larval feeding. In A. C. Giese, J. S. Pearse, & V. B. Pearse (Eds.), *Reproduction of Marine Invertebrates: General aspects, seeking unity in diversity*: Blackwell Scientific Publications.

Tauber, A. L. (2003). Metchnikoff and the phagocytosis theory. *Nature Reviews Molecular Cell Biology, 4*, 897-901.

Thorson, G. (1950). Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews, 25*, 1-45.

Vance, R. R. (1973). On reproductive strategies in marine benthic invertebrates. *The American Naturalist, 107*, 339-352.

Walker, C. W., & Lesser, M. P. (1989). Nutrition and development of brooded embryos in the brittlestar *Amphipholis squamata*: do endosymbiotic bacteria play a role? *Marine Biology, 103*, 519-530.

Webster, N. S., Botte, E. S., Soo, R. M., & Whalan, S. (2011). The larval sponge holobiont exhibits high thermal tolerance. *Environmental Microbiology Reports, 3*, 756-762.

Webster, N. S., & Thomas, T. (2016). The sponge hologenome. *mBio, 7*(2), e00135-00116.

Williams, E. A., & Carrier, T. J. (2018). An -omics perspective on marine invertebrate larvae. In T. J. Carrier, A. M. Reitzel, & A. Heyland (Eds.), *Evolutionary Ecology of Marine Invertebrate Larvae* (pp. 288-304). Oxford, UK: Oxford University Press.

Young, C. M., & Chia, F.-S. (1987). Abundance and distribution of pelagic larvae as influenced by predation, behavior, and hydrographic factors. In A. C. Giese, J. S. Pearse, & V. B. Pearse (Eds.), *Reproduction of Marine Invertebrates IX. General aspects: seeking unity in diveristy*. Palo Alto, California: Blackwell Scienfic Publications.

Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *Fems Microbiology Reviews, 32*, 723-735.

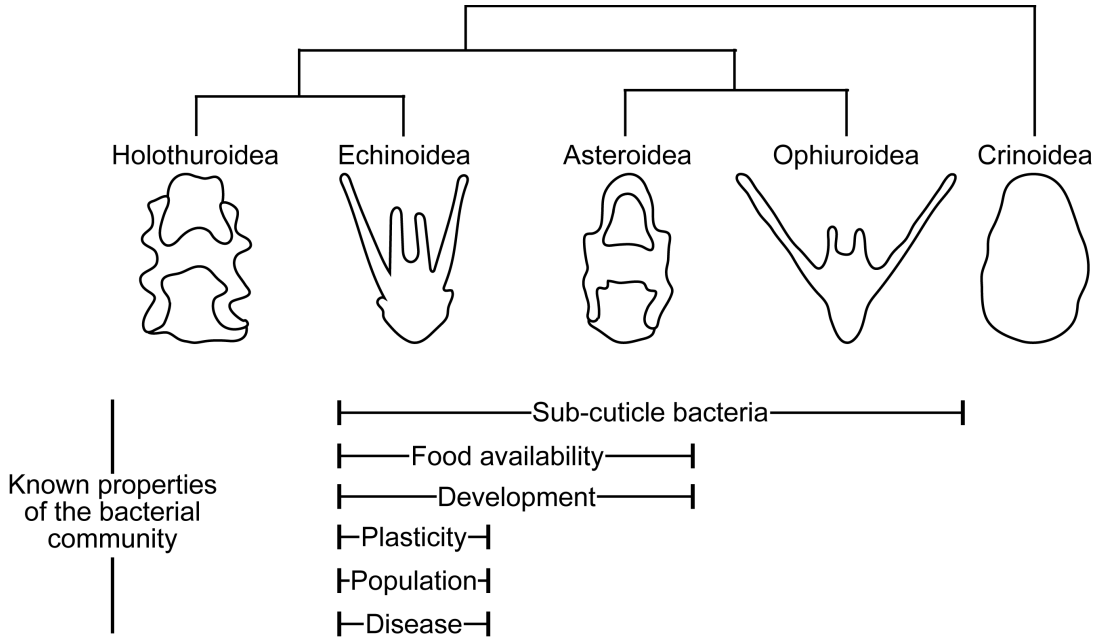


Figure 1.1. Echinoderm larvae and their symbionts. Properties of echinoderm larval biology and ecology with known correlated shifts in the associated bacterial communities.

| | Species | Type | Reference |
|---|---|---|---|
| Asteroids | *Acanthaster* sp. | Bacterial community by 16S rRNA profiling | Carrier *et al.,* 2018 |
| | *Luidia* sp. | Sub-cuticle bacteria by electron microscopy | Bosch, 1992 |
| | *Patiria miniata* | Sub-cuticle bacteria by electron microscopy | Cameron and Holland, 1983 |
| | *Patiriella calcar* | Sub-cuticle bacteria by electron microscopy | Cerra *et al.*, 1997 |
| Crinoidea | N/A | N/A | N/A |
| Echinoids | *Lytechinus variegatus* | Bacterial community by 16S rRNA profiling | Carrier and Reitzel, 2019 |
| | *Mesocentrotus franciscanus* | Bacterial community by 16S rRNA profiling | Carrier and Reitzel, 2018; Carrier and Reitzel, 2019 |
| | *Strongylocentrotus purpuratus* | Bacterial community by 16S rRNA profiling | Carrier and Reitzel, 2018; Carrier and Reitzel, 2019 |
| | | Sub-cuticle bacteria by *in situ* hybridization | Schuh *et al.*, 2019 |
| | *Strongylocentrotus droebachiensis* | Bacterial community by 16S rRNA profiling | Carrier and Reitzel, 2018; Carrier and Reitzel, 2019; Carrier *et al.* 2019 |
| Holothuroidea | N/A | N/A | N/A |
| Ophiuroidea | *Amphipholis squamata* | Sub-cuticle bacteria by electron microscopy | Walker and Lesser, 1989 |

Table 1.1. List of echinoderm species noted to associate with bacterial symbionts.

CHAPTER 2

# CONVERGENT SHIFTS IN HOST-ASSOCIATED MICROBIAL COMMUNITIES ACROSS ENVIRONMENTALLY ELICITED PHENOTYPES

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## Citation

**Carrier TJ, Reitzel, AM.** 2018. Convergent shifts in host-associated microbial communities across environmentally elicited phenotypes. Nature Communications **9:**952.

## Abstract

Morphological plasticity is a genotype-by-environment interaction that enables organisms to increase fitness across varying environments. Symbioses with diverse microbiota may aid in acclimating to this variation, but whether the associated bacteria community is phenotype-specific remains understudied. Here we induce morphological plasticity in three species of sea urchin larvae and measure changes in the associated bacterial community. While each host species has unique bacterial communities, the expression of plasticity results in the convergence on a phenotype-specific microbiome that is, in part, driven by differential association with α- and γ-proteobacteria. Furthermore, these results suggest that phenotype-specific signatures are the product of the environment and are correlated with ingestive and digestive structures. By manipulating diet quantity over time, we also show that differentially associating with microbiota along a phenotypic continuum is bidirectional. Taken together, our data support the idea of a phenotype-specific microbial community and that phenotypic plasticity extends beyond a genotype-by-environment interaction.

## 2.1 Introduction

Phenotypic plasticity, the ability of a single genotype to produce multiple distinct phenotypes, is a genotype-by-environment interaction that enables organisms to acclimate to environmental variation (DeWitt, Sih, & Wilson, 1998; Sterns, 1989; West-Eberhard, 2003). For many organisms, including plants, amphibians, and marine invertebrates, plasticity confers a fitness advantage (*i.e.*, is adaptive) when the phenotype matches the environment (Miner, Sultan, Morgan, Padilla, & Relyea, 2005). The context-dependent expression of alternate phenotypes is, therefore, presumed to be an evolvable trait influenced by natural selection (Miner, Sultan, Morgan, Padilla, & Relyea, 2005). To date, ecological and evolutionary theory, including that of phenotypic plasticity, is primarily viewed as a genotype-by-environment interaction (Agrawal, 2001; DeWitt et al., 1998;Miner et al., 2005; Sterns, 1989; Via & Lande, 1985). However, all eukaryotes, including plants and animals, are not strictly biological individuals (Gilbert, Sapp, & Tauber, 2012) but, instead, are holobionts that comprise a host and consortium of associated microbiota (Bordenstein & Theis, 2015; McFall-Ngai et al., 2013; Rosenberg, Sharon, & Zilber-Rosenberg, 2009; Theis et al., 2016; Zilber-Rosenberg & Rosenberg, 2008).

The hologenome theory of evolution proposes that multicellular eukaryotes establish partnerships with microbiota (*e.g.*, eukaryotes, bacteria, Archaea, fungi, and viruses) that are, in part, heritable and affect fitness (Bordenstein & Theis, 2015; Rosenberg et al., 2009; Theis et al., 2016; Zilber-Rosenberg & Rosenberg, 2008). Variation in these microbial communities may arise from differential associations

following changes in the environment or host and/or microbial genomes (Bordenstein & Theis, 2015). The hologenome of a holobiont is, therefore, a host genome-by-microbial metagenome-by-environment ($G_H$x$G_M$xE) interaction, whereby acclimation is the result of changes in both $G_M$ and $G_H$ (Bordenstein & Theis, 2015; Carrier & Reitzel, 2017). Environment-mediated shifts in the structure (*i.e.*, composition and abundance) of host-associated microbial communities often vary in response to biotic challenges, such as diet type (David et al., 2014; Ley et al., 2008) and starvation (Kohl, Amaya, Passement, Dearing, & McCue, 2014). Shifts in host-associated microbial communities may, therefore, co-occur with the expression of environmentally elicited and adaptive morphological characters.

A system to test the hypothesis that host-associated microbial communities are phenotype-specific are the feeding (planktotrophic) larvae of marine invertebrates. Planktotrophic larvae require exogenous nutrients to progress through development and undergo metamorphosis (Strathmann, 1985). The abundance and distribution of phytoplankton in coastal seas are spatially and temporally heterogeneous and often diluted in offshore waters (Olson & Olson, 1989). Several groups of planktotrophic larvae, including echinoids (phylum Echinodermata, class Echinoidea), respond to heterogeneous feeding environments by exhibiting morphological plasticity (McAlister & Miner, 2017). When experiencing starvation, larvae allocate energetic resources from development of the larval body towards the structures for ingestion (*i.e.*, post-oral arms) while absorbing stomach tissues, enabling larvae to increase their feeding capacity in low food environments (Adams, Sewell, Angerer, & Angerer, 2011; Boidron-Metairon, 1988; Byrne, Sewell, & Prowse, 2008; Carrier, King, & Coffman, 2015; Miner, 2004; Soars,

Prowse, & Byrne, 2009). The role of and responses by the associated microbial community along this morphological continuum remains unknown, even though echinoderm larvae associate with diverse microbial communities (Galac, Bosch, & Janies, 2016) and encounter tremendous numbers of environmental microbiota (Azam & Malfatti, 2007).

While within-species comparisons may discern the potential values of phenotypic plasticity, comparisons of conserved responses between closely related species to common environmental variation provides a broader inference for characterizing shared and species-specific adaptive responses. Here, we use larvae of three confamilial echinoid species (*Strongylocentrotus purpuratus*, *Mesocentrotus franciscanus*, and *S. droebachiensis*; Supplementary Fig. 2.1A-C; Lee, 2003) that differ in their expression of plasticity (Moran & McAlister, 2009), in order to test the hypothesis that the associated microbial community co-varies with expression and magnitude of morphological plasticity. Through a series of differential feeding experiments paired with sequence-based analysis of the associated bacterial community, we provide evidence that the microbiome shifts following the expression of phenotypic plasticity and the magnitude to which this character is expressed.

## 2.2 Materials and Methods

### 2.2.1 Adult urchin collection and larval rearing

Adult urchins were collected from populations throughout the Salish Sea in April 2016. Specifically, individual *S. purpuratus* were hand-collected at low tide at Slip Point, Clallam Bay, WA (48°15'39" N, 124°15'03" W) and transferred overnight to the Friday

Harbor Laboratories (FHL; University of Washington; Friday Harbor, WA, USA). Similarly, *S. droebachiensis* were hand-collected at low tide, except at Cattle Point, San Juan Island, WA (48°27'00" N, 122°57'43" W), and were transferred to FHL within the hour. *M. franciscanus*, on the other hand, were collected by SCUBA off Bell Island, WA (48°35'49" N, 122°58'55" W) and transferred to FHL within two hours. Collected urchins were suspended in sub-tidal cages off the dock at FHL and fed *Nereocystis* spp. (sugar kelp) *ad libitum* until spawning two weeks later.

Adult urchins were spawned with a one- to two-mL intracoelomic injection of 0.50 M KCl. For each species, gametes from up to three males and three females were separately pooled. Fertilization of eggs and larval rearing followed Strathmann (1987), except, to include the environmental microbiota, embryos and larvae were reared using 5.0-µm filtered seawater (instead of traditional filtration at 0.22-µm). Briefly, embryos were incubated in one-liter of filtered seawater (FSW) at ambient temperature and salinity (Supplementary Fig. 2.24), and two hours post-fertilization were transferred to three-L of FSW, divided into triplicates, and larval density was fixed to 2 larvae•mL$^{-1}$, with subsequent dilutions with development. Larval cultures were given 90 to 95% water changes every other day.

Monocultures of *R. lens* were grown at room temperature with f/2 media and a combination of ambient and artificial lighting (Guillard, 1975).

### 2.2.2 Experimental feeding and larval morphometrics

At 48 hours post-fertilization, prism-stage larvae were divided into three replicate jars for each of the four experimental feeding treatments varying in *R. lens* quantity:

10,000, 1,000, 100, or 0 cells•mL$^{-1}$. For each species, larvae fed 10,000 cells•mL$^{-1}$ were reared through metamorphosis while starved larvae were diet-restricted until developmental stasis was reached. Larvae (n=100) of each species from all treatments and replicates were sampled weekly, preserved in RNAlater, and stored at -20 °C until extractions of nucleic acids were performed. We also tested for how diet shifts influence development and associated microbes in *S. droebachiensis* by starving larvae for three weeks and then switching them to 10,000 cells•mL$^{-1}$ through metamorphosis. Larvae from this experiment were preserved and the nucleic acids were extracted in an identical manner.

In addition to sampling larvae to assay the associated microbial communities, twenty larvae from a single replicate for each dietary treatment were sampled for morphometric analysis. Larvae were imaged using a compound microscope (Nikon Eclipse E600; camera: QImaging MicroPublisher 5.0 RTV) and morphometrics (length of larval body, post-oral arms, and stomach area; Supplementary Fig. Fig. 2.1D) were measured using ImageJ (NIH software, ver. 1.9.2; Schneider, Rasband, & Eliceiri, 2012). We tested for whether larval morphology and stomach volume were influenced by differential feeding over time using a two-way ANOVA (JMP Pro v. 13). Where statistical differences were observed ($p<0.05$), we used a post-hoc test to determine the affect at each time point and for each diet.

### 2.2.3 Assaying microbial communities

We extracted total DNA from larval samples using the GeneJet Genomic DNA Purification Kit (Thermo Scientific). For filtered seawater samples, we extracted eDNA

using the FastDNA Spin Kit for Soil (MP Biomedical). DNA was then quantified using the NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific) and diluted to 5 $ng \bullet \mu L^{-1}$ using RNase/DNase-free water.

Bacterial sequences were amplified using universal primers for the V3/V4 regions of the 16S rRNA gene (Forward: 5′ CTACGGGNGGCWGCAG, Reverse: 5′ GACTACHVGGGTATCTAATCC; Klindworth et al., 2013). Products were purified using the Axygen AxyPrep Mag PCR Clean-up Kit (Axygen Scientific), indexed via PCR using the Nextera XT Index Kit V2 (Illumina Inc.), and then purified again. At each of these three clean up states, fluorometric quantitation was performed using a Qubit (Life Technologies) and libraries were validated using a Bioanalyzer High Sensitivity DNA chip (Agilent Technologies). Illumina MiSeq sequencing was performed at the University of North Carolina at Charlotte.

Forward and reverse sequences were paired and trimmed using PEAR (Zhang, Kobert, Flouri, & Stamatakis, 2014) and Trimmomatic (Bolger, Lohse, & Usadel, 2014), respectively, converted from fastq to fasta using custom script, and, prior to analysis of bacterial 16S rRNA sequences, chimeric sequences were detected using USEARCH (Edgar, Haas, Clemente, Quince, & Knight, 2011) and removed using filter_fasta.py. Using QIIME 1.9.1 (Caporaso et al., 2010), bacterial 16S rRNA sequences were analyzed and grouped into operational taxonomic units (OTUs) based on a minimum 97% similarity. The biom table generated by the pick_open_reference_otus.py script was filtered of OTUs with less than ten reads as well as sequences matching chloroplast for cryptophytes (*i.e.*, *R. lens*; Supplementary Data 1-3).

Using the filtered biom table and "biom summarize-table" function to count total sequences per sample, the rarefaction depth of 18,225 was determined and applied to all subsequent analyses (Supplementary Fig. 2.23). Beta diversity was calculated using the weighted UniFrac (Lozupone & Knight, 2005), and principal coordinate analyses (PCoA) were visualized in EMPeror (Vazquez-Baeza, Pirrung, Gonzalez, & Knight, 2013) and stylized in Adobe Illustrator CS6. Community composition was generated using summarize_taxa_through_plots.py script and visualized using Prism 7 (GraphPad Software). Community similarity across phenotypes, dietary states, developmental stages, and their decoupling were compared statistically using an ANOSIM as part of the compare_categories.py script.

A step-by-step listing of QIIME scripts used to convert raw reads to OTUs for visualization of the data is located in Supplementary Note 1.

## 2.3 Results

### 2.3.1 Larval morphometrics

Morphological plasticity in echinoid larvae is induced following exposure to a low phytoplankton environment. To induce plasticity in *S. purpuratus*, *M. franciscanus*, and *S. droebachiensis*, larvae were fed either 10,000, 1,000, 100, or 0 cells•mL$^{-1}$ of the cryptophyte *Rhodomonas lens*. As predicted, each species exhibited a significant morphological change upon four weeks of differential feeding (Fig. 2.1; Supplementary Fig. 2.2; Supplementary Tables 1-3; ANOVA, $p<0.001$). Plasticity of the feeding structures was observed for *S. purpuratus*, *M. franciscanus*, and *S. droebachiensis*, where larvae at the same developmental stage fed the same diet exhibited a higher post-oral arm

to mid-body line ratio with time (Fig. 2.1; Supplementary Fig. 2.2; Supplementary Tables 2-3; ANOVA, $p<0.001$). For the time points where plasticity was expressed, the magnitude of morphological change was inversely correlated with the degree of maternal investment (Fig. 2.1; Supplementary Fig. 2.3).

For *S. purpuratus*, larvae fed 100 cells•mL$^{-1}$ exhibited morphological plasticity following two versus three or four weeks of diet-restriction (Fig. 2.1A; Supplementary Fig. 2.2A), where the ratio between post-oral arms and larval body increased, on average, by 10.9% (±0.8%). For *M. franciscanus*, plasticity was observed when comparing larvae fed 100 cells•mL$^{-1}$ following one versus two or three weeks of diet-restriction (Fig. 2.1B; Supplementary Fig. 2.2B), where the ratio between post-oral arms and larval body increased, on average, by 9.1% (±1.4%). Lastly, for *S. droebachiensis*, larvae fed 1,000 cells•mL$^{-1}$ expressed plasticity following two versus three or four weeks of diet-restriction (Fig. 2.1C; Supplementary Fig. 2.2C), where the ratio between post-oral arms and larval body increased, on average, by 4.5% (±2.1%).

### 2.3.2 Microbiome across morphological plasticity states

We used our morphological plasticity data as reference points to compare the structure of the microbiome along this phenotypic transition. The composition of the associated microbial community for larvae of each species was distinct between phenotypes when developmental stage and diet were identical (ANOSIM, *S. purpuratus*: $p<0.004$, *M. franciscanus*: $p<0.006$, *S. droebachiensis*: $p=0.046$; Fig. 2.2A-C; Supplementary Fig. 2.4-2.6).

Next, we tested whether the magnitude of morphological change was correlated with the magnitude to which the associated bacterial community was restructured. We determined that the number of differentially associated OTUs from pre- to post-expression of plasticity was directionally proportional to the magnitude of morphological change ($R^2$=0.938; Fig. 2.3A) and inversely proportional to egg size (Supplementary Fig. 2.7A). Specifically, *S. purpuratus*, *M. franciscanus*, and *S. droebachiensis* differentially associated with 446, 302, and 152 OTUs (Supplementary Fig. 2.7), respectively. Furthermore, the ratio between over- to under-represented OTUs was directionally proportional to the magnitude of morphological change ($R^2$=0.880; Fig. 3B; Supplementary Fig. 2.7C) and inversely proportional to egg size (Supplementary Fig. 2.7B).

Recruitment and expulsion of bacteria and/or a shuffling of relative proportion of the resident communities are not mutually exclusive mechanisms for differentially associating with microbial taxa (Bordenstein & Theis, 2015). By comparing the relative abundance of bacteria at higher taxonomic levels along this phenotypic transition, we observed that larvae trade-off in associating with α- and γ-proteobacteria. Larvae from each species associated with relatively more γ- and less α-proteobacteria following the expression of phenotypic plasticity (Fig. 2.2D-F). Furthermore, we observed a similar phenotype-specific trade-off at both the family and genus level for each species of larvae. Specifically, the γ-proteobacteria Colwelliaceae, *Oleispira*, and *Pseudomonas* for *S. purpuratus*, Colwelliaceae for *M. franciscanus*, and Flavobacteriaceae (*e.g.*, *Polaribacter*) for *S. droebachiensis* represent a greater portion of the associated microbial communities of larvae having exhibited phenotypic plasticity (Supplementary Fig. 2.8; Supplementary

Table 4-5; ANOVA, $p<0.008$). On the other hand, the α-proteobacteria Bradyrhizobiaceae for *S. purpuratus*, *Sphingomonas* for *M. franciscanus*, and Bradyrhizobiaceae for *S. droebachiensis* represent a reduced portion of the associated microbial communities of larvae having exhibited phenotypic plasticity (Supplementary Fig. 2.8; Supplementary Table 4-5; ANOVA, $p<0.008$).

### 2.3.3 Diet- and development-based shifts in the microbiome

Nutritional developmental plasticity in echinoid larvae is induced when shifted from a well-fed to diet-restricted feeding regime (Carrier et al., 2015; McAlister & Miner, 2017; B.G. Miner, 2004; Soars et al., 2009). To test if differences in the structure of the associated microbial community were a product of the feeding environment (*i.e.*, quantity of phytoplankton), we compared community similarity across dietary states following one week (*i.e.*, pre-expression of plasticity) of differential feeding and at a later time point (*i.e.*, post-expression of plasticity).

For *S. purpuratus* and *M. franciscanus*, but not *S. droebachiensis*, the structure of the microbiome was similar across food treatments following one week of differential feeding (ANOSIM, *S. purpuratus*: $p=0.325$, *M. franciscanus*: $p=0.808$, *S. droebachiensis*: $p<0.002$; Fig. 2.4A-C; Supplementary Fig. 2.9A-C, 2.10-2.12). At later time points following the expression of phenotypic plasticity, the structure of the microbiome was distinct across food treatments for all species (ANOSIM, *S. purpuratus*: $p<0.002$, *M. franciscanus*: $p<0.003$, *S. droebachiensis*: $p<0.002$; Fig. 2.4D-F; Supplementary Fig. 2.9D-F, 2.13-2.15). This difference between weeks pre- and post-expression of plasticity

supports that larval phenotype and the associated microbiota was likely the product of differential feeding.

A confounding factor specific to *S. droebachiensis* following one week of differential feeding was that developmental stage was variable across diets, where higher concentrations resulted in advanced stages (Supplementary Table 1). To test whether echinoid larvae associated a developmental stage-specific microbial community (as defined by the number of larval arms), we compared community similarity of 4-, 6-, and 8-arm *S. purpuratus* larvae reared on the same diet and exhibiting a similar plasticity state. Like to other taxa (McFall-Ngai & Ruby, 2000; McFall-Ngai, 2002), *S. purpuratus* associated with a developmental stage-specific microbial community (ANOSIM, $p<0.005$; Supplementary Fig. 2.16). We hypothesize that the difference in associated microbial community observed in *S. droebachiensis* one week post differential feeding was, in part, due to a mixed population of 4- and 6-arm larvae across diets (Supplementary Table 1).

### 2.3.4 De-coupling phenotypic plasticity

Each component of nutritional developmental plasticity for echinoid larvae (*i.e.*, diet, development, and phenotype) has specific microbial communities (Fig. 2.2, 2.4; Supplementary Fig. 2.16). These components, however, are biologically linked and thus our results may, in part, be explained by co-variation between these factors. To de-couple diet, development, phenotype, and time (*i.e.*, ecological drift), we compared the microbial communities of 4-, 6-, and 8-arm larvae of both *S. purpuratus* and *M. franciscanus* fed 100, 1,000, and 10,000 cells•$mL^{-1}$, respectively, to 4-arm larvae pre- and post-expression

of phenotypic plasticity (*i.e.*, larvae from Fig. 2.2A-B). For both species, we observe a diet-development coupling (*i.e.*, PC1) distinct from phenotype (*i.e.*, PC2) and time (*i.e.*, PC3) (Fig. 2.5; ANOSIM, *S. purpuratus*: $p<0.001$, *M. franciscanus*: $p<0.001$), further supporting that echinoid larvae associate with phenotype-specific microbial communities.

Differences in the associated microbial communities of the echinoid larval host across plasticity states may also have been the result of differences in the microbial communities prior to feeding. To test this, we compared the host-associated microbiota of pre-feeding larvae, finding that each biological replicate varied slightly but were more similar to each other than to other species of pre-feeding larvae (*i.e.*, species-specificity; $p<0.004$; Supplementary Fig. 2.17-2.20). This result mirrors a phylosymbiotic pattern (Brooks, Kohl, Brucker, van Opstal, & Bordenstein, 2016) (data not shown), although the number of echinoid species is insufficient for a robust comparison. Thus, we observed no support that differences in microbial signatures across phenotypes, dietary states, and developmental stages were due to pre-treatment differences.

Alternatively, these differences may have been the product of temporal shifts in the environmental microbiota during the course of the experiment. When comparing larval-associated and environmental microbiota from pre-feeding and late larval development, we observed each species of pre- (ANOSIM, $p<0.001$) and post-feeding (ANOSIM, $p<0.001$) larva were distinct from the environmental microbiota (Supplementary Fig. 2.21), suggesting that plasticity- and diet-specific microbial associates were unlikely to be the product of differential exposure to environmental microbiota. These results suggest that plasticity- and diet-specific microbial associates were unlikely to be the product of differential exposure to environmental microbiota.

### 2.3.5 Bidirectional shifts in the associated microbial community

Expression of nutritional plasticity can be reversible with a change in the feeding regime. To test whether the plasticity-associated microbial signature is reversible, *S. droebachiensis* larvae were starved (0 cells•mL$^{-1}$) for three weeks then switched to an *ad libitum* diet (10,000 cells•mL$^{-1}$) for three weeks (*i.e.*, until metamorphosis).

The microbiome of larvae fed *ad libitum* followed a development-specific trajectory while starved larvae, as before, remained distinct from well-fed siblings and was similar to a starvation-specific microbial community (Fig. 2.4 and 2.6). The structure of the microbial community associated with early-stage larvae, as discussed above, were more similar to each other (weeks 1 and 2) than to late-stage larvae, independent of diet. Within the later larval stages, a division in community similarity was observed between starved larvae (weeks 3, 4, and 6) and well-fed larvae (Supplementary Fig. 2.22). Furthermore, when starved larvae were switched to a well-fed diet, their associated microbial communities became more similar to larvae fed *ad libitum* with time (Fig. 2.6; Supplementary Fig. 2.22), a trajectory congruent with developmental morphology (Fig. 2.1C).

## 2.4 Discussion

Evolutionary and ecological theory predicts that variation in host-associated microbial communities corresponds with host phenotype (Bordenstein & Theis, 2015; Gilbert, 2016; Scott F. Gilbert, Bosch, & Ledon-Rettig, 2015). If the impact of associated microbes was sufficient to contribute to fitness of the holobiont, we would predict that the

host should be under selection to regulate what microbial species they associate in different environments (Bordenstein & Theis, 2015; Carrier & Reitzel, 2017; Kohl & Carey, 2016; Theis et al., 2016). Examples include, but are not limited to, aphids and *Buchnera* (Douglas, 1998), the bobtail squid *Euprymna* and *Vibrio fischeri* (Nyholm & Mcfall-Ngai, 2004), and the parasitic wasp *Nasonia* and *Wolbachia* (Bordenstein, O'Hara, & Werren, 2001), and on a community level, the gut and root microbiome of many animals (Ley et al., 2008) and plants (Berendsen, Pieterse, & Bakker, 2012). Previous studies, however, have not directly tested whether the microbiome correlates with environmentally-induced morphological plasticity in adaptive characters. Morphological plasticity is present in many species and is likely adaptive by facilitating a better matching phenotype for increased performance in the environment experienced by the holobiont (Bordenstein & Theis, 2015; DeWitt et al., 1998; Gilbert et al., 2015; McFall-Ngai et al., 2013; Miner et al., 2005). If the associated microbial community contributes to the relative fitness of the host experiencing a dynamic environment, we hypothesized that the community should shift with morphological plasticity.

Using larvae from three echinoid species ranging in their ability to express morphological plasticity, we observed that the microbiome predictably shifted for all three species of larvae. For each species, changes in the morphology of larvae experiencing food-restricted environments resulted in a corresponding shift in the microbial community. Interestingly, although similar patterns of differential association were observed between species of larvae, the microbial taxa were similar at higher taxonomic levels (*e.g.*, phylum and class) but not at the OTU level. Moreover, for the species of larvae experiencing a coarse environmental shift (*i.e.*, *S. droebachiensis* from unfed to

high food), a phenotype-specific microbial signature was reversible, implying that a microbiome-based means of acclimating to environmental variation is bidirectional and, perhaps, a fluid component of hologenomic acclimation.

Larvae of *S. purpuratus*, *M. franciscanus*, and *S. droebachiensis* partner with species-specific microbiota that exhibit similar patterns of differential association when exhibiting phenotypic plasticity. In light of species-specific patterning in larval-associated microbiota, we propose that the functional importance of the microbiome corresponding with phenotypic plasticity is similar between species while the bacterial taxa vary. Our hypothesis follows recent evidence that the microbial taxa associated with a host may not directly reflect the functional properties of that community (Shafquat, Joice, Simmons, & Huttenhower, 2014). Based on the degree to which larvae can exhibit morphological plasticity, we suggest that the particular functions of the microbial communities associated with *S. purpuratus* and *M. franciscanus* larvae expressing plasticity are more similar than that of *S. droebachiensis* (Brooks et al., 2016).

Corresponding with a predicted convergence in functional properties of the host-associated microbial communities across phenotypes, shifts in these communities may be mediated by differential gene expression of the larval host. Previous transcriptomic comparisons have shown that *S. droebachiensis* larvae exhibit a broad transcriptomic response to differential feeding (Carrier et al., 2015). Following the expression of phenotypic plasticity, *S. droebachiensis* larvae down-regulate genes associated with growth and metabolism while up-regulating genes involved with neurogenesis and environmental sensing, immunity and defense, and longevity (Carrier et al., 2015). Interestingly, the predicted function of up-regulated genes when larval echinoids undergo

phenotypic plasticity also correspond with well-known functional properties of microbes (Adams et al., 2011; Buckley et al., 2017; Buckley & Rast, 2017; Eisthen & Theis, 2016; Ho et al., 2017; Y. K. Lee & Mazmanian, 2010; Mortzfeld & Bosch, 2017; Round & Mazmanian, 2009; Sinclair, 2005; Sutherby et al., 2013). Therefore, a functional-based approach (Webster & Reusch, 2017) should be taken to determine whether the host gene expression or microbial interactions regulates, or perhaps directs, phenotypic plasticity.

The rate of environmental change and delay in the corresponding phenotypic response can limit the phenotype-environment match. Larvae of the sea urchin *Lytechinus variegatus* exposed to fine grain (two-day) variability in exogenous resources, for example, are unable to match phenotype with feeding regime because the delay required for phenotypic reconstruction exceeds the environmental variability (Miner & Vonesh, 2004). However, differentially associating with microbial communities when experiencing similar fine grain environmental variation, as similarly shown here with *S. droebachiensis* (10,000 versus 0 cells•$mL^{-1}$), may be quicker to modulate over short temporal oscillations than morphological changes, which are typically slow. Thus, when facing environments variability favoring the expression of alternate morphological traits, organisms may acclimate by differentially associating with microbial communities.

Phenotypic plasticity is common in animals and plants and, thus, our results of a phenotype-specific microbial community may be common when acclimating in variable environments. Polyphenism in anuran tadpoles, for example, is highly dependent on diet-type, such that the morphology of carnivore and omnivore morphs from the same clutch differs considerably (Pfennig, 1992a, 1992b). Namely, carnivorous tadpoles have a larger orbitohyoideus to snout length ratio, enabling more efficient predation on their preferred

dietary option (Pfennig, 1992a, 1992b). Terrestrial plants, on the other hand, are highly plastic with regards to resource acquisition. For example, low-nutrient soil environments mediate increased growth in the roots and harvestable areas for the rhizosphere, whereas low levels of light results in an increase in leaf area (Sultan, 2000). Anuran tadpoles and plants, therefore, may serve as comparative systems for studying the hologenomic evolution of phenotypic plasticity and whether additional environmental cues select for shared and unique mechanisms associated with acclimation.

Taken together, the data presented here support the hypothesis that sea urchin larvae have a phenotype-specific microbial community and that morphological change is correlated with restructuring the associated microbial community. Future research should determine whether the bacterial associates and other type of microbes, influence the expression of larval genes and what metabolites they contribute to the host will elucidate how these microbes may contribute to maximizing hologenomic fitness in a heterogeneous sea.

Acknowledgements

We thank Jason Hodin and Billie Swalla for providing laboratory space; Morgan Eisenlord, Gustav Pauley, Katie Dobkowski, and Joe Gaydos for collecting urchins; Colette Feehan and Richard Strathmann for larval rearing advise; Daniel Janies for sequencing resources; Karen Lopez for technical assistance with sequencing; and Kevin Lambirth, Jason Macrander, and Andrew Brooks for bioinformatics assistance. TJC was supported by an NSF Graduate Research Fellowship, Charles Lambert Memorial Endowment fellowship from the Friday Harbor Laboratories, and a Sigma Xi Grants-in-

Aid of Research grant; AMR was supported by NSF DEB1545539 and Human Frontier Science Program Award RGY0079/2016.

References

Adams, D. K., Sewell, M. A., Angerer, R. C., & Angerer, L. M. (2011). Rapid adaptation to food availability by a dopamine-mediated morphogenetic response. *Nature Communications, 2*, 592.

Agrawal, A. A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science, 294*, 321-326.

Azam, F., & Malfatti, F. (2007). Microbial structuring of marine ecosystems. *Nature Reviews Microbiology, 5*, 782-791.

Berendsen, R. L., Pieterse, C. M. J., & Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science, 17*, 478-486.

Boidron-Metairon, I. F. (1988). Morphological plasticity in laboratory-reared echinoplutei of *Dendraster excentricus* (Eschscholtz) and *Lytechinus variegatus* (Lamarck) in response to food conditions. *Journal of Experimental Marine Biology and Ecology, 119*, 31-41.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics, 30*(15), 2114-2120.

Bordenstein, S. R., O'Hara, F. P., & Werren, J. H. (2001). *Wolbachia*-induced incompatibility precedes other hybrid incompatibilities in *Nasonia*. *Nature, 409*, 707-710.

Bordenstein, S. R., & Theis, K. R. (2015). Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biology, 13*, e1002226.

Brooks, A. W., Kohl, K. D., Brucker, R. M., van Opstal, E. J., & Bordenstein, S. R. (2016). Phylosymbiosis: relationships and functional effects of microbial communities across host evolutionary history. *Plos Biology, 14*, e2000225.

Buckley, K. M., Ho, E. C. H., Hibino, T., Schrankel, C. S., Schuh, N. W., Wang, G. Z., & Rast, J. P. (2017). IL17 factors are early regulators in the gut epithelium during inflammatory response to *Vibrio* in the sea urchin larva. *Elife, 6*, e23481.

Buckley, K. M., & Rast, J. P. (2017). An organismal model for gene expression networks in the gut-associated immune response. *Frontiers in Immunology, 8*, 1297.

Byrne, M., Sewell, M. A., & Prowse, T. A. A. (2008). Nutritional ecology of sea urchin larvae: influence of endogenous and exogenous nutrition on echinopluteal growth and phenotypic plasticity in *Tripneustes gratilla*. *Functional Ecology, 22*, 643-648.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., . . . Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods, 7*(5), 335-336.

Carrier, T. J., King, B. L., & Coffman, J. A. (2015). Gene expression changes associated with the developmental plasticity of sea urchin larvae in response to food availability. *Biological Bulletin, 228*, 171-180.

Carrier, T. J., & Reitzel, A. M. (2017). The hologenome across environments and the implications of a host-associated microbial repertoire. *Frontiers in Microbiology, 8*, 802.

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., . . . Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature, 505*, 559-563.

DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution, 13*, 77-81.

Douglas, A. E. (1998). Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annual Review of Entomology, 43*, 17-37.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics, 27*(16), 2194-2200.

Eisthen, H. L., & Theis, K. R. (2016). Animal-microbe interactions and the evolution of nervous systems. *Philosophical Transactions of the Royal Society B Biological Sciences, 371*, 20150052.

Galac, M. R., Bosch, I., & Janies, D. A. (2016). Bacterial communities of oceanic sea star (Asteroidea: Echinodermata) larvae. *Marine Biology, 163*, 162.

Gilbert, S. F. (2016). Developmental plasticity and developmental symbiosis: the return of Evo-Devo. *Current Topics in Developmental Biology, 116*, 415-433.

Gilbert, S. F., Bosch, T. C. G., & Ledon-Rettig, C. (2015). Eco-Evo-Devo: developmental symbiosis and developmental plasticity as evolutionary agents. *Nature Reviews Genetics, 16*, 611-622.

Gilbert, S. F., Sapp, J., & Tauber, A. I. (2012). A symbiotic view of life: we have never been individuals. *Quarterly Review of Biology, 87*(4), 325-341.

Guillard, R. R. L. (Ed.) (1975). *Culture of phytoplankton for feeding marine invertebrates*. New York, USA: Plenum Press.

Ho, E., Buckley, K. M., Schrankel, C. S., Schuh, N. W., Hibino, T., Solek, C. M., . . . Rast, J. P. (2017). Perturbation of gut bacteria induces a coordinated cellular immune response in the purple sea urchin larva. *Immunology and Cell Biology, 94*, 861–874.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glockner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research, 41*(1), e1.

Kohl, K. D., Amaya, J., Passement, C. A., Dearing, M. D., & McCue, M. D. (2014). Unique and shared responses of the gut microbiota to prolonged fasting: a comparative study across five classes of vertebrate hosts. *FEMS Microbiology Ecology, 90*(3), 883-894.

Kohl, K. D., & Carey, H. V. (2016). A place for host-microbe symbiosis in the comparative physiologist's toolbox. *Journal of Experimental Biology, 219*, 3496-3504.

Lee, Y. H. (2003). Molecular phylogenies and divergence times of sea urchin species of Strongylocentrotidae, Echinoida. *Molecular Biology and Evolution, 20*, 1211-1221.

Lee, Y. K., & Mazmanian, S. K. (2010). Has the microbiota played a critical role in the evolution of the adaptive immune system? *Science, 330*, 1768-1773.

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., . . . Gordon, J. I. (2008). Evolution of mammals and their gut microbes. *Science, 320*, 1647-1651.

Lozupone, C., & Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology, 71*(12), 8228-8235.

McAlister, J. S., & Miner, B. G. (2017). Phenotypic plasticity of feeding structures in marine invertebrate larvae. In T. J. Carrier, A. M. Reitzel, & A. Heyland (Eds.), *Evolutionary Ecology of Marine Invertebrate Larvae*. Oxford, UK: Oxford University Press.

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C. G., Carey, H. V., Domazet-Loso, T., Douglas, A. E., . . . Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences of the United States of America, 110*(9), 3229-3236.

McFall-Ngai, M., & Ruby, E. G. (2000). Developmental biology and marine invertebrate symbioses. *Current Opinion in Microbiology, 3*, 603-607.

McFall-Ngai, M. J. (2002). Unseen forces: the influence of bacteria on animal development. *Developmental Biology, 242*, 1-14.

Miner, B. G. (2004). Evolution of feeding structure plasticity in marine invertebrate larvae: a possible trade-off between arm length and stomach size. *Journal of Experimental Marine Biology and Ecology, 315*, 117-125.

Miner, B. G., Sultan, S. E., Morgan, S. G., Padilla, D. K., & Relyea, R. A. (2005). Ecological consequences of phenotypic plasticity. *Trends in Ecology & Evolution, 20*, 685-692.

Miner, B. G., & Vonesh, J. R. (2004). Effects of fine grain environmental variability on morphological plasticity. *Ecology Letters, 7*, 794-801.

Moran, A. L., & McAlister, J. S. (2009). Egg size as a life history character of marine invertebrates: Is it all it's cracked up to be? *Biological Bulletin, 216*, 226-242.

Mortzfeld, B. M., & Bosch, T. C. G. (2017). Eco-Aging: stem cells and microbes are controlled by aging antagonist FoxO. *Current Opinion in Microbiology, 38*, 181-187.

Nyholm, S. V., & Mcfall-Ngai, M. J. (2004). The winnowing: Establishing the squid-*Vibrio* symbiosis. *Nature Reviews Microbiology, 2*(8), 632-642.

Olson, R. R., & Olson, M. H. (1989). Food limitation of planktotrophic marine invertebrate larvae: does it control recruitment success? *Annual Review of Ecology and Systematics, 20*, 225-247.

Pfennig, D. W. (1992a). Polyphenism in spadefoot toad tadpoles as a locally adjusted evolutionarily stable strategy. *Evolution, 46*, 1408-1420.

Pfennig, D. W. (1992b). Proximate and functional causes of polyphenism in an anuran tadpole. *Functional Ecology, 6*, 167-174.

Rosenberg, E., Sharon, G., & Zilber-Rosenberg, I. (2009). The hologenome theory of evolution contains Lamarckian aspects within a Darwinian framework. *Environmental Microbiology, 11*, 2959-2962.

Round, J. L., & Mazmanian, S. K. (2009). The gut microbiome shapes intestinal immune responses during health and disease. *Nature Reviews Immunology, 9*, 313-323.

Schneider, C., Rasband, W., & Eliceiri, K. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, 9*, 671-675.

Shafquat, A., Joice, R., Simmons, S. L., & Huttenhower, C. (2014). Functional and phylogenetic assembly of microbial communities in the human microbiome. *Trends in Microbiology, 22*(5), 261-266.

Sinclair, D. A. (2005). Toward a unified theory of caloric restriction and longevity regulation. *Mechanisms of Ageing and Development, 126*, 987-1002.

Soars, N. A., Prowse, T. A. A., & Byrne, M. (2009). Overview of phenotypic plasticity in echinoid larvae, 'Echinopluteus transversus' type vs. typical echinoplutei. *Marine Ecology Progress Series, 383*, 113-125.

Sterns, S. (1989). The evolutionary significance of phenotypic plasticity. *BioScience, 39*(7), 436-445.

Strathmann, M. F. (1987). *Reproduction and development of marine invertebrates of the northern Pacific coast: data and methods for the study of eggs, embryos, and larvae*: University of Washington Press.

Strathmann, R. R. (1985). Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *Annual Review of Ecology and Systematics, 16*, 339-361.

Sultan, S. E. (2000). Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science, 5*, 537-542.

Sutherby, J., Giardini, J. L., Nguyen, J., Wessel, G., Leguia, M., & Heyland, A. (2013). Histamine is a modulator of metamorphic competence in *Strongylocentrotus purpuratus* (Echinodermata: Echinoidea). *BMC Developmental Biology, 12*, 14.

Theis, K. R., Dheilly, N. M., Klassen, J. L., Brucker, R. M., Baines, J. F., Bosch, T. C. G., . . . Bordenstein, S. R. (2016). Getting the hologenome concept right: An eco-evolutionary framework for hosts and their microbiomes. *mSystems, 1*, e00028-00016.

Vazquez-Baeza, Y., Pirrung, M., Gonzalez, A., & Knight, R. (2013). EMPeror: a tool for visualizing high-throughput microbial community data. *Gigascience, 2*, 16.

Via, S., & Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution, 39*, 505-522.

Webster, N. S., & Reusch, T. B. H. (2017). Microbial contributions to the persistence of coral reefs. *The ISME Journal, 11*, 2167-2174.

West-Eberhard, M. (2003). *Developmental Plasticity and Evolution*. Oxford, UK: Oxford University Pres.

Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2014). PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics, 30*(5), 614-620.

Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Reviews, 32*(5), 723-735.

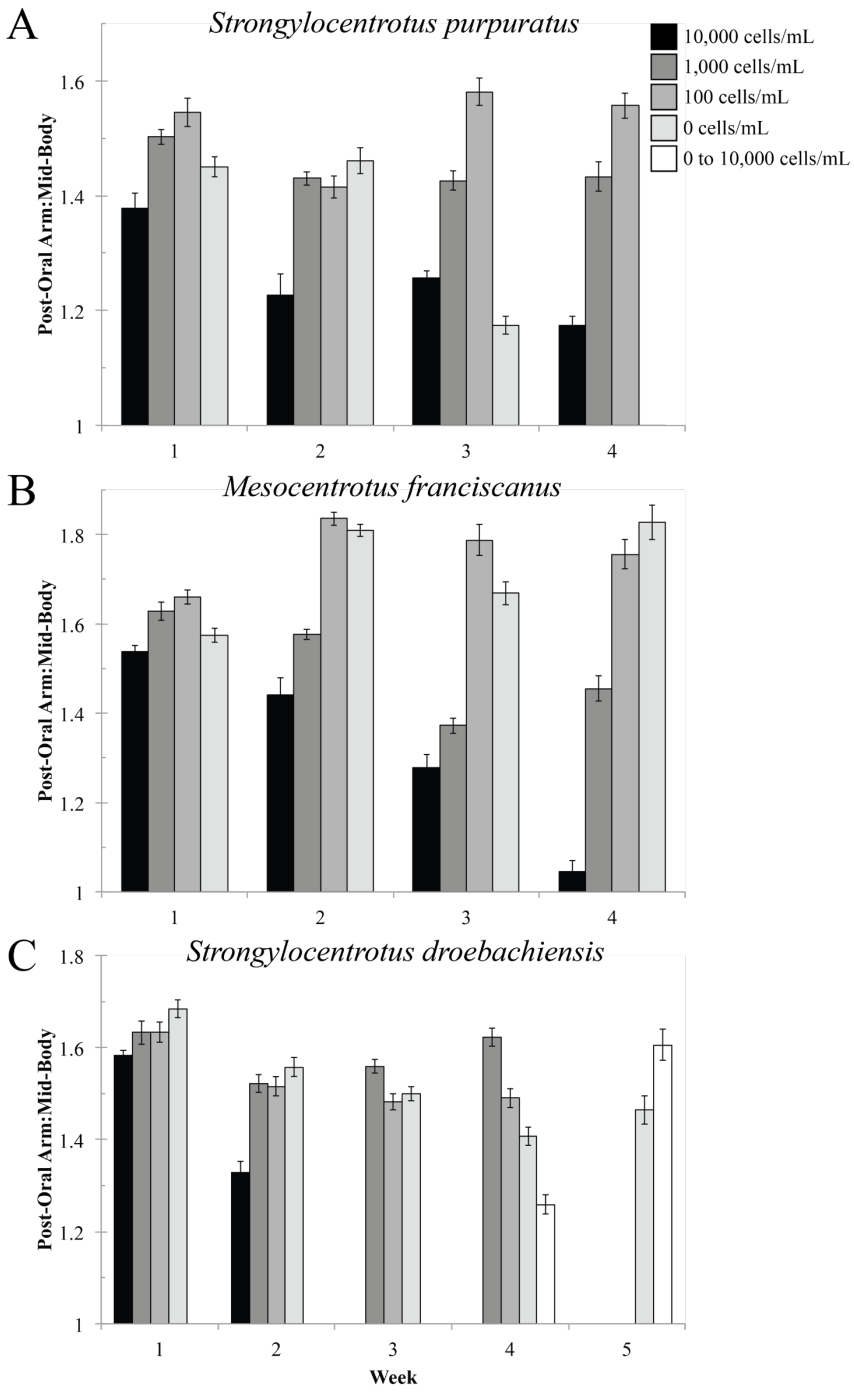



Figure 2.1. Three species of echinoid larvae alter phenotype to feeding environment. Ratio between the post-oral arm and mid body line (mean ± standard error; n=20; Supplementary Fig. 1D) for *Strongylocentrotus purpuratus* (A), *Mesocentrotus franciscanus* (B), and *S. droebachiensis* (C) larvae having been fed either 10,000 (black), 1,000 (dark grey), 100 (grey), and 0 cells•mL$^{-1}$ (light grey). For *S. droebachiensis*, larval phenotype was also manipulated (white) by being fed 0 cells•mL$^{-1}$ for three weeks then transferred to 10,000 cells•mL$^{-1}$ for three weeks (*i.e.*, until metamorphosis).

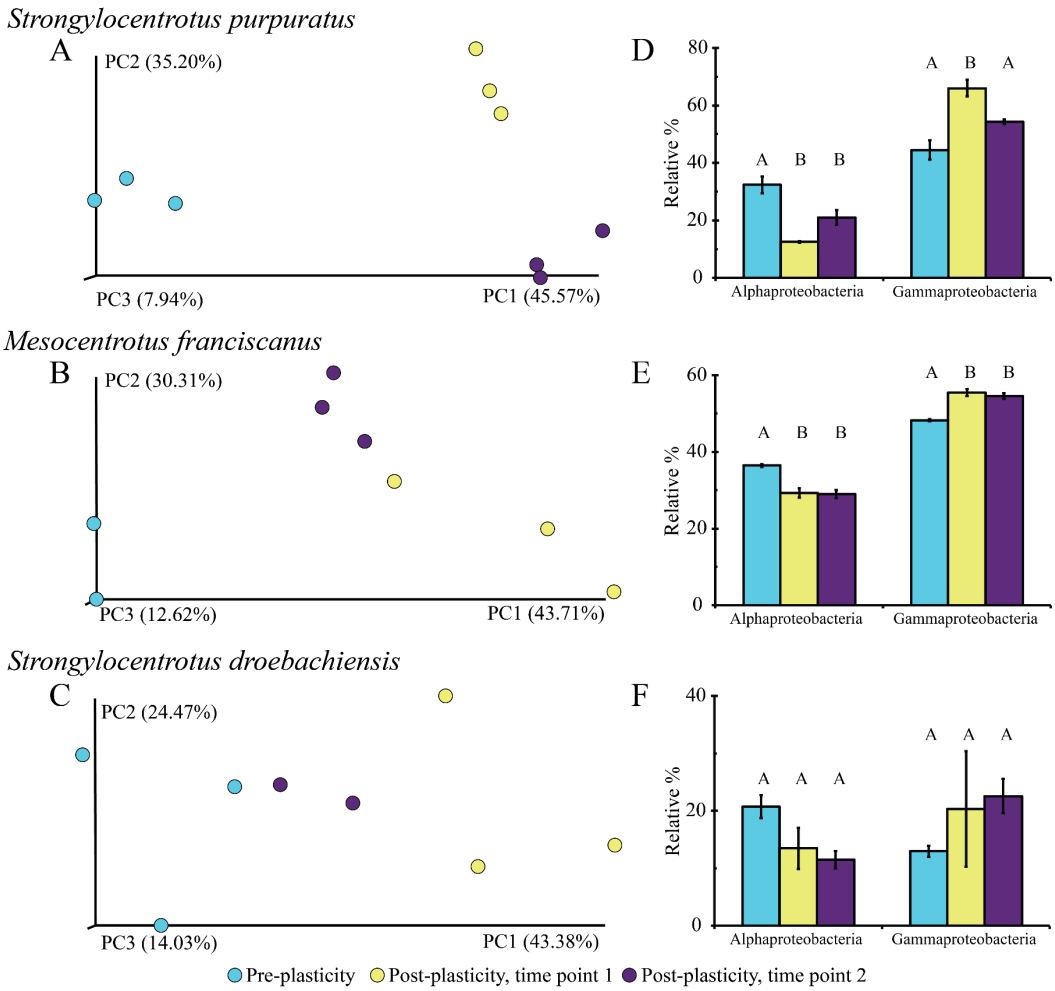



Figure 2.2. Similarity of the associated microbial community along a phenotypic continuum for three species of echinoid larvae. Community similarity of the associated microbiota for *Strongylocentrotus purpuratus* (A), *Mesocentrotus franciscanus* (B), and *S. droebachiensis* (C) prior to (blue) and post (purple and yellow) expression of phenotypic plasticity. Along this continuum, larvae differentially associate with α- and γ-proteobacteria pre- and post-expression of phenotypic plasticity, respectively (D, *S. purpuratus*; E, *M. franciscanus*; F, *S. droebachiensis*).

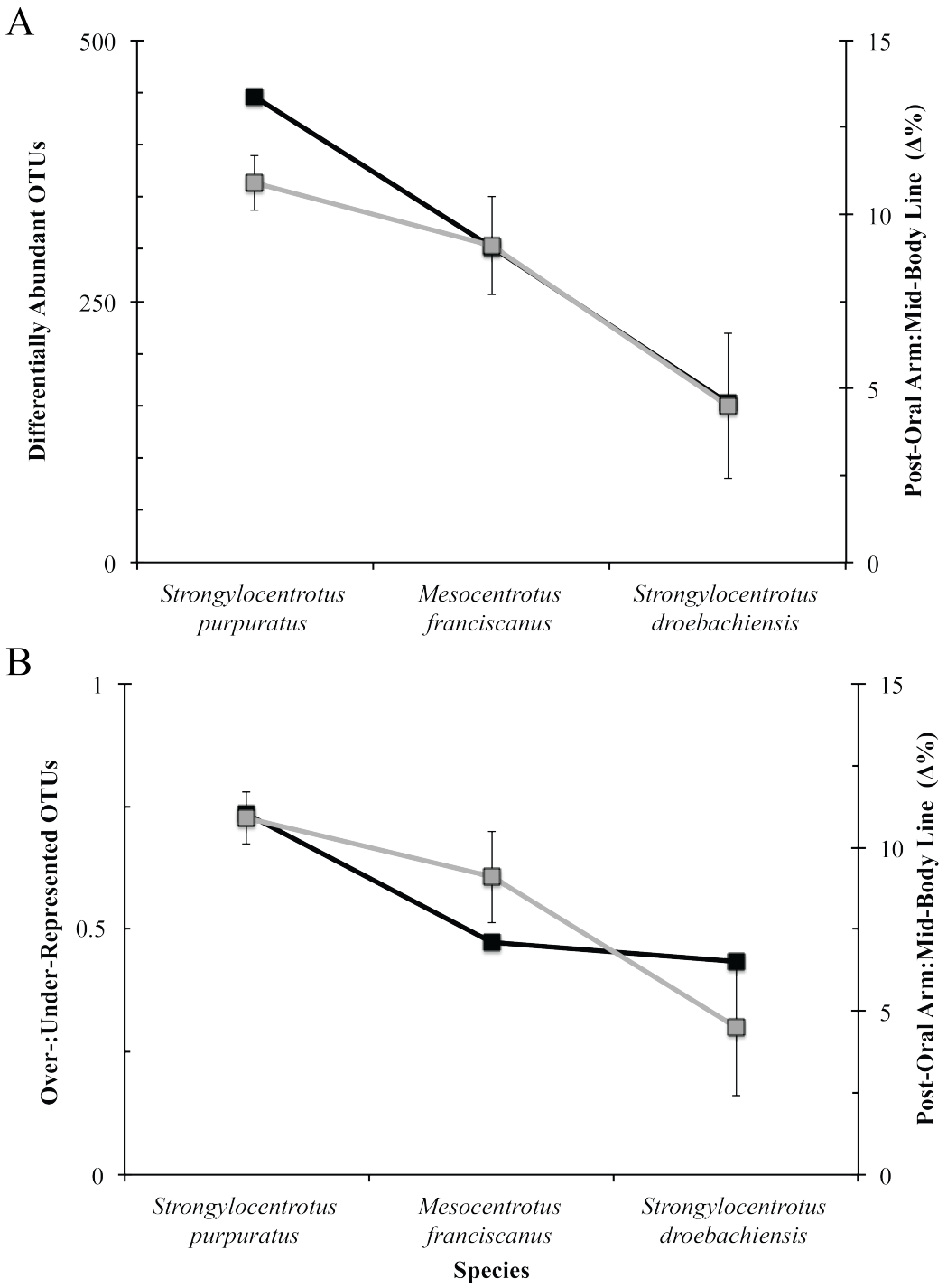



Figure 2.3. Differential abundance of OTUs along a morphological continuum for three species of echinoid larvae. Total (A) and ratio of (B) over- and under-represented OTUs associated with *Strongylocentrotus purpuratus*, *Mesocentrotus franciscanus*, and *S. droebachiensis* larvae following the expression of phenotypic plasticity (black) and in relation to the change in larval morphology (gray). Species on the x-axis are organized from least to most maternal investment, a direct correlate of the expression of phenotypic plasticity.

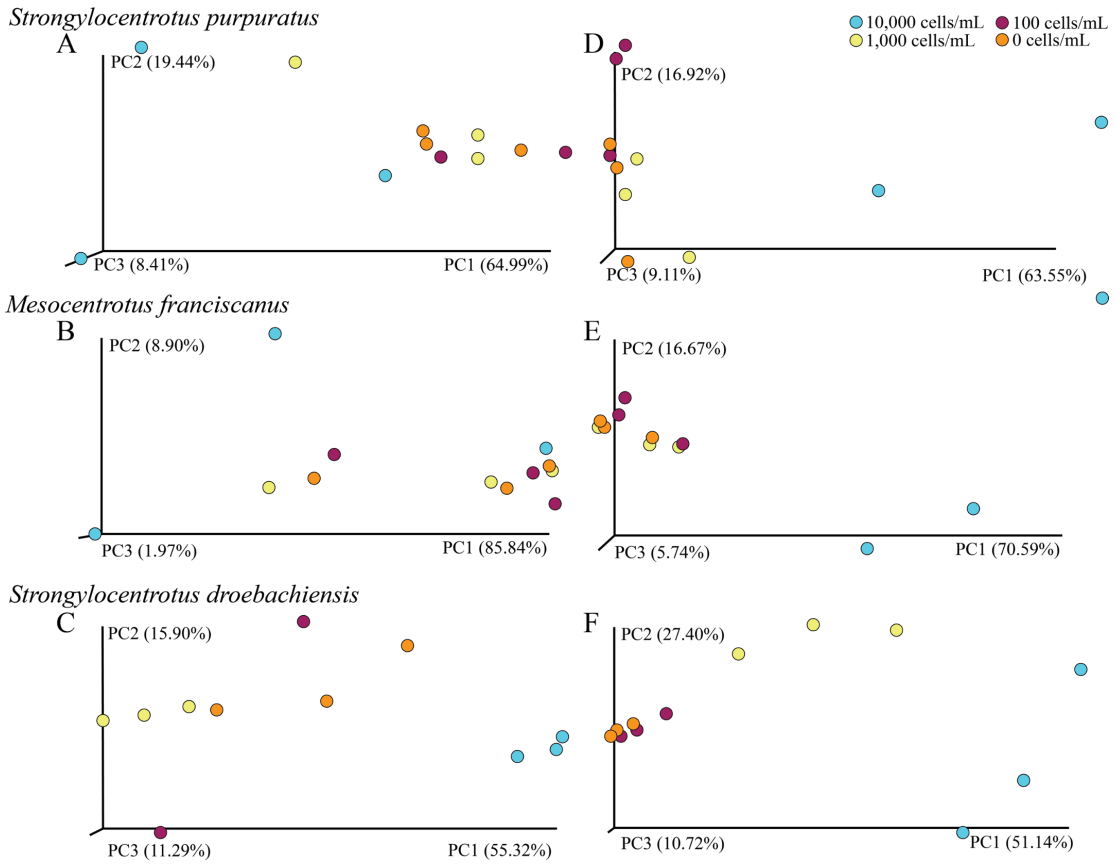



Figure 2.4. Induction of differential associations in the microbial community based on feeding environment for three species of echinoid larvae. Community similarity of the associated microbiota for *Strongylocentrotus purpuratus* (A, D), *Mesocentrotus franciscanus* (B, E), and *S. droebachiensis* (C, F) prior to (A-C) and post (D-F) expression of phenotypic plasticity, with larvae having been fed either 10,000 (blue), 1,000 (yellow), 100 (maroon), and 0 cells•mL$^{-1}$ (orange).

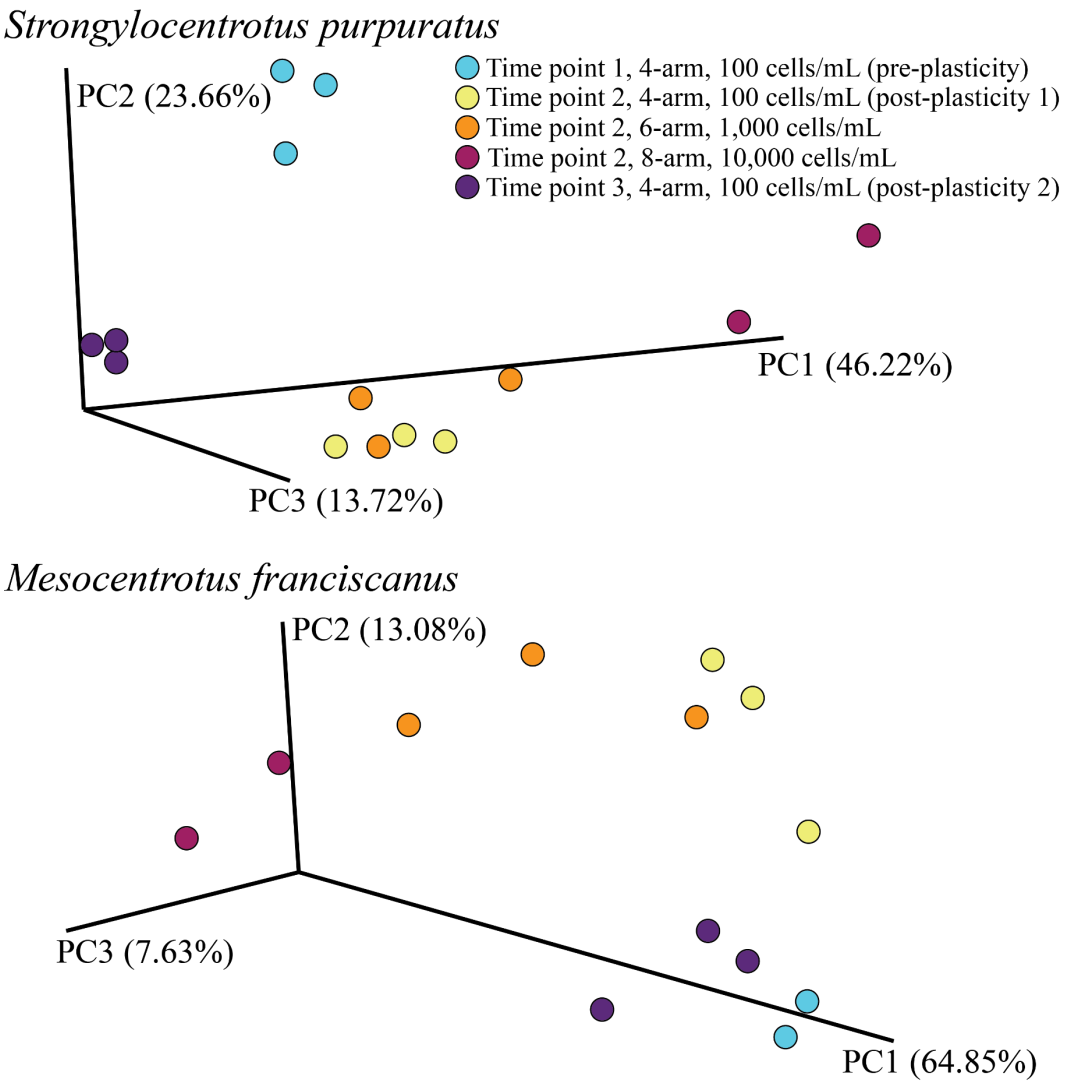



Figure 2.5. Decoupling phenotype-specific microbial communities from diet, development, and time for two species of echinoid larvae. Community similarity of the associated microbiota for *Strongylocentrotus purpuratus* (A) and *Mesocentrotus franciscanus* (B) larvae at the 8- (maroon), 6- (orange), and 4-arm (yellow) stage having been fed 10,000, 1,000, and 100 cells•mL$^{-1}$, respectively, in comparison with larvae pre (blue) and post-expression (purple and yellow) of phenotypic plasticity.

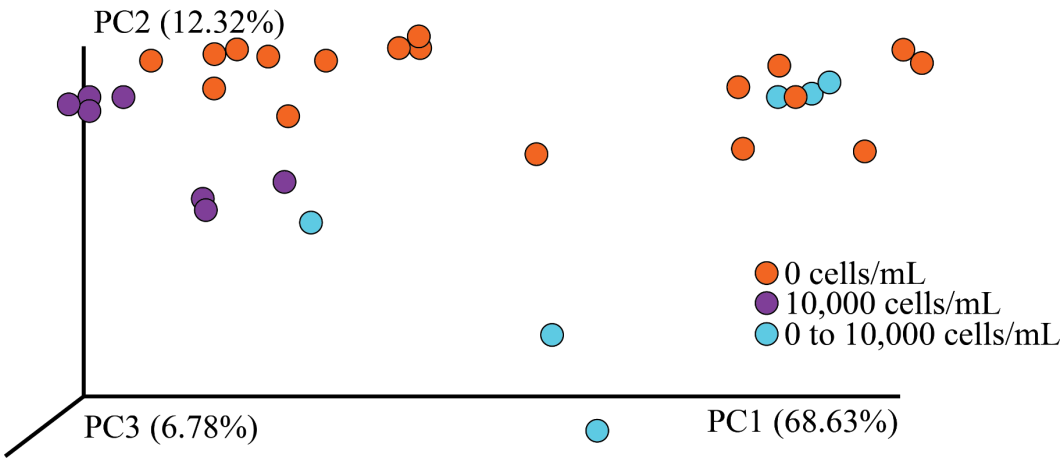
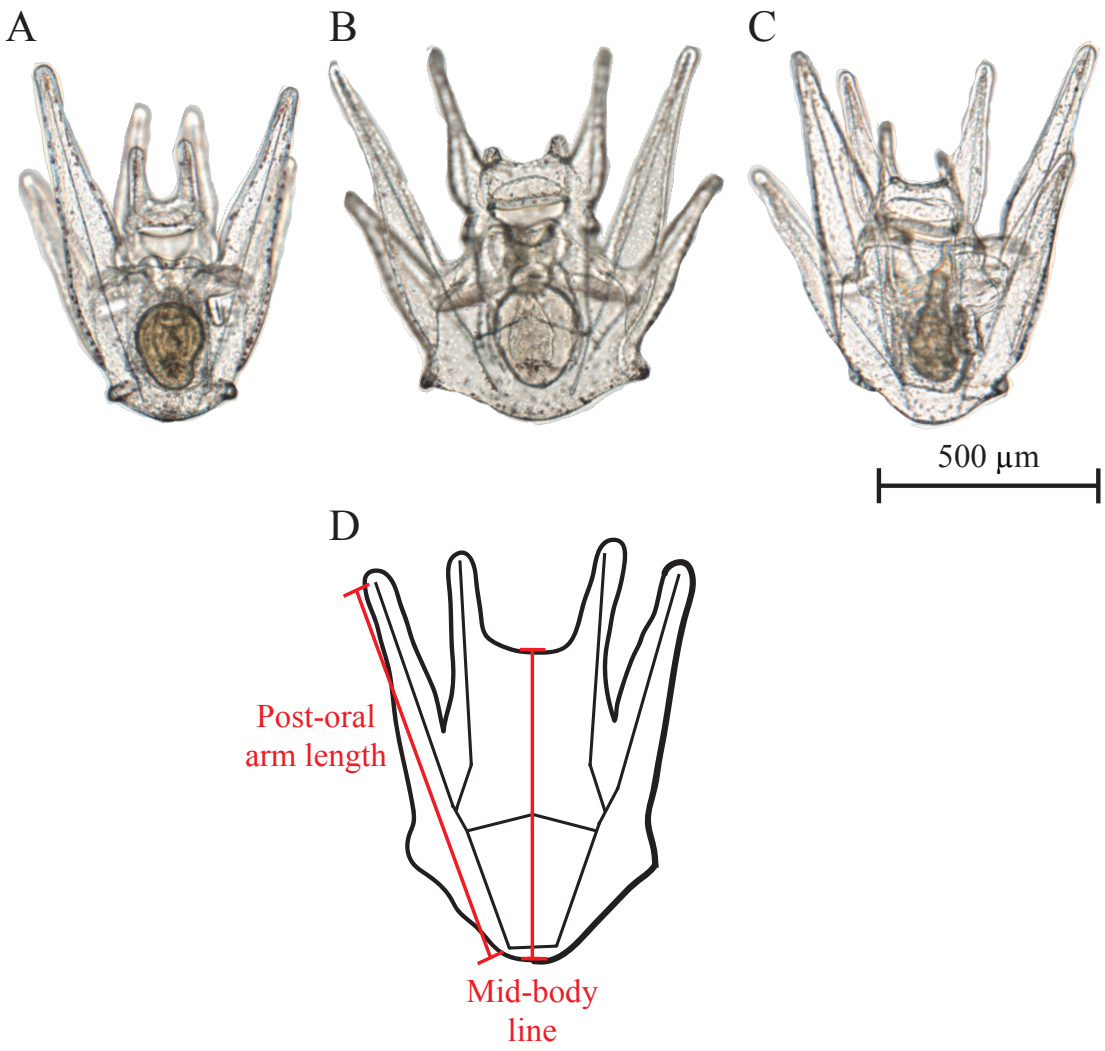
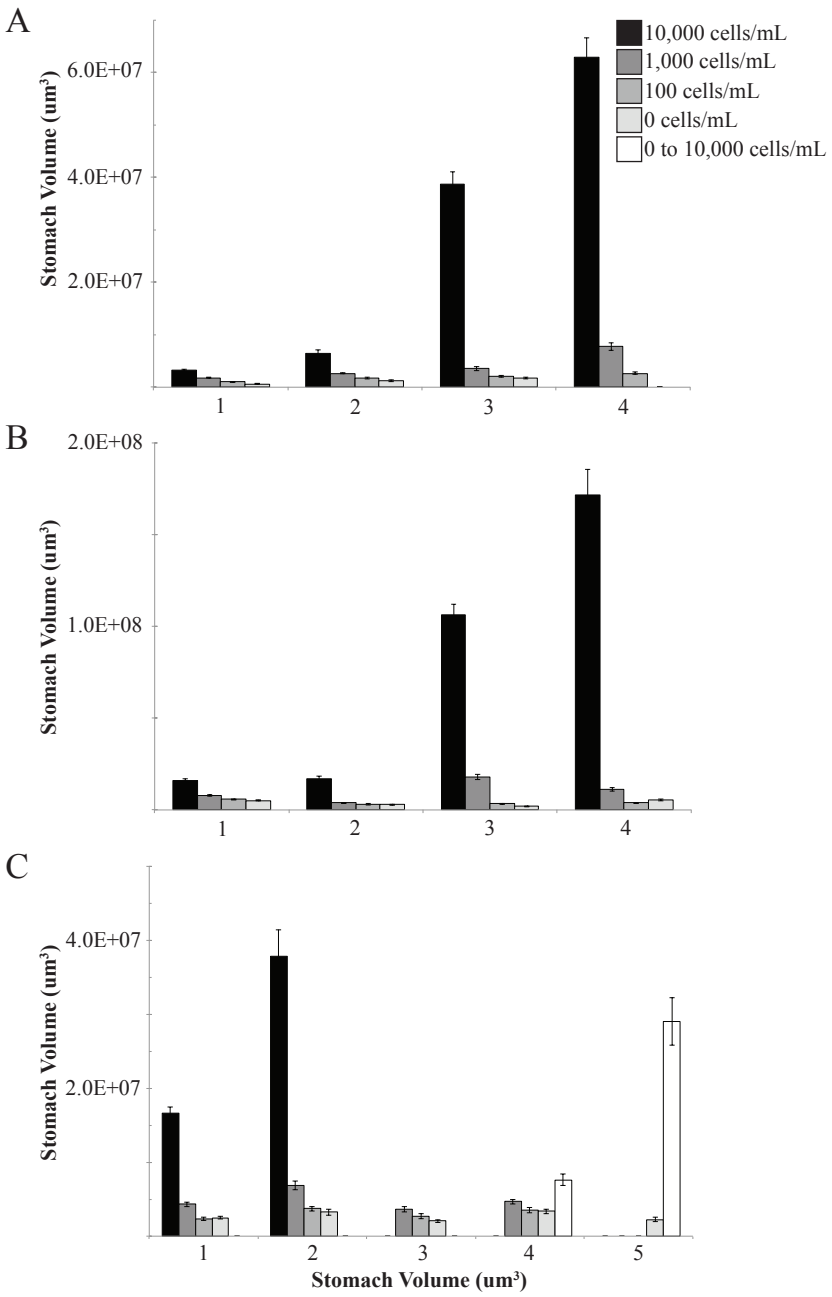


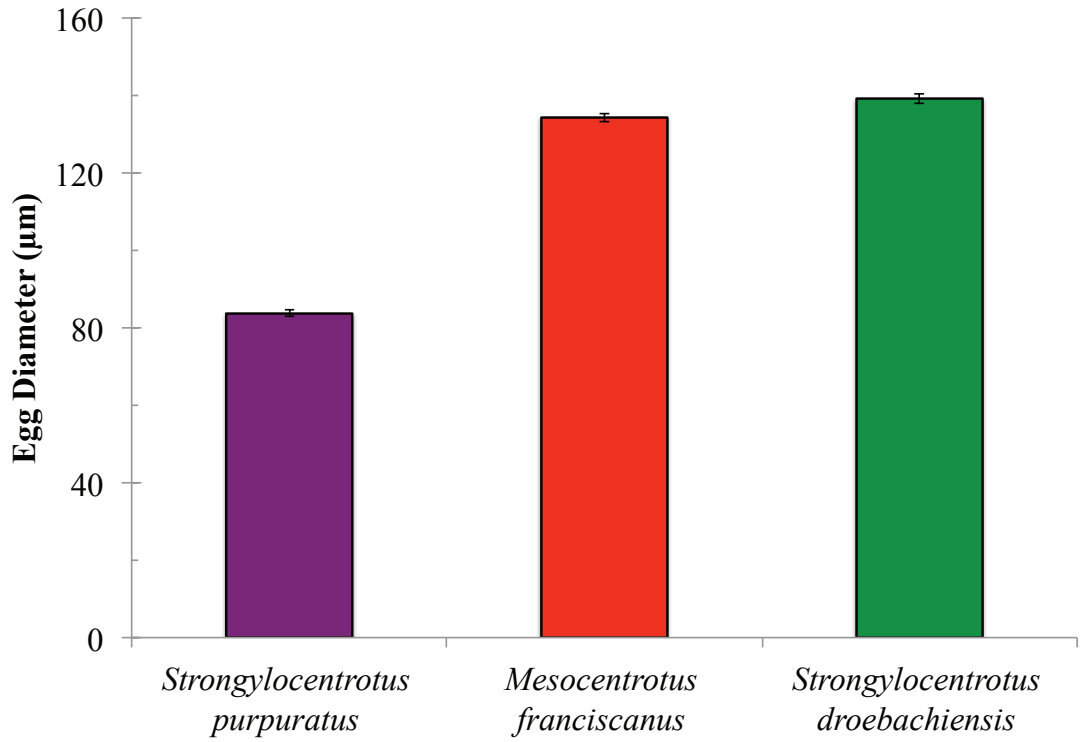

Figure 2.6. Bidirectional plasticity in the associated microbial communities for *Strongylocentrotus droebachiensis* larvae. Community similarity of the associated microbiota for *S. droebachiensis* larvae fed 10,000 cells•mL$^{-1}$ (purple) until metamorphosis and 0 cells•mL$^{-1}$ (orange) for three weeks, versus larvae fed 0 cells•mL$^{-1}$ for three weeks then switched to 10,000 cells•mL$^{-1}$ until metamorphosis (blue).



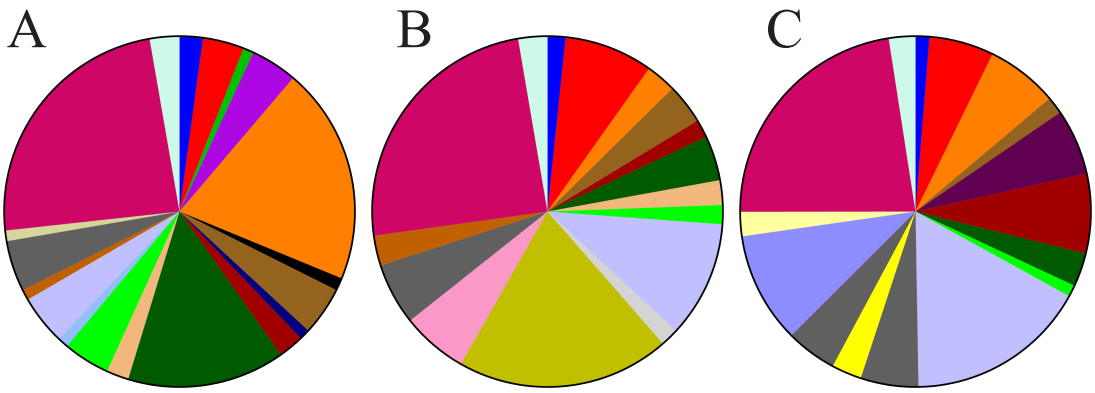

Supplemental Fig. 2.1. Photograph of each species of echinoid larvae and representative cartoon. Microphotograph of 8-arm *Strongylocentrotus purpuratus* (A), 6-arm *Mesocentrotus franciscanus* (B), and 8-arm *S. droebachiensis* (C) larva, and (D) cartoon of 4-arm echinoid larvae with pre-drawn line for post-oral arm and mid-body line measurements.




Supplemental Fig. 2.2. Three species of echinoid larvae alter stomach volume based on feeding environment. Post-oral arm to mid body line ratio (± standard error; n=20) for *Strongylocentrotus purpuratus* (A), *Mesocentrotus franciscanus* (B), and *S. droebachiensis* (C) larvae having been fed either 10,000 (black), 1,000 (dark grey), 100 (grey), and 0 cells•mL$^{-1}$ (light grey). For *S. droebachiensis*, larval stomach volume was also manipulated (white) by being fed 0 cells•mL$^{-1}$ for three weeks then transferred to 10,000 cells•mL$^{-1}$ for three weeks (*i.e.*, until metamorphosis). Data here correspond with Fig. 1.

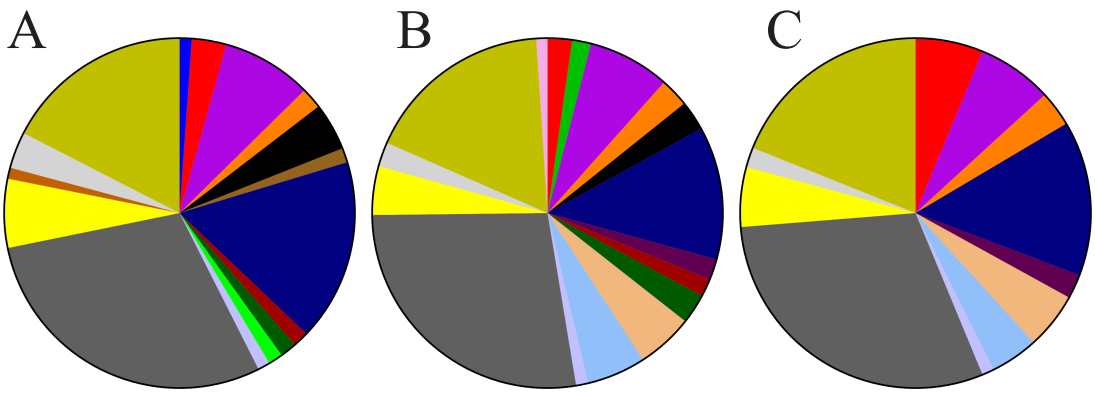



Supplemental Fig. 2.3. Egg diameter for three species of echinoid larvae. Mean egg size (± standard error; n=20) of unfertilized eggs for *Strongylocentrotus purpuratus*, *Mesocentrotus franciscanus*, and *S. droebachiensis*.



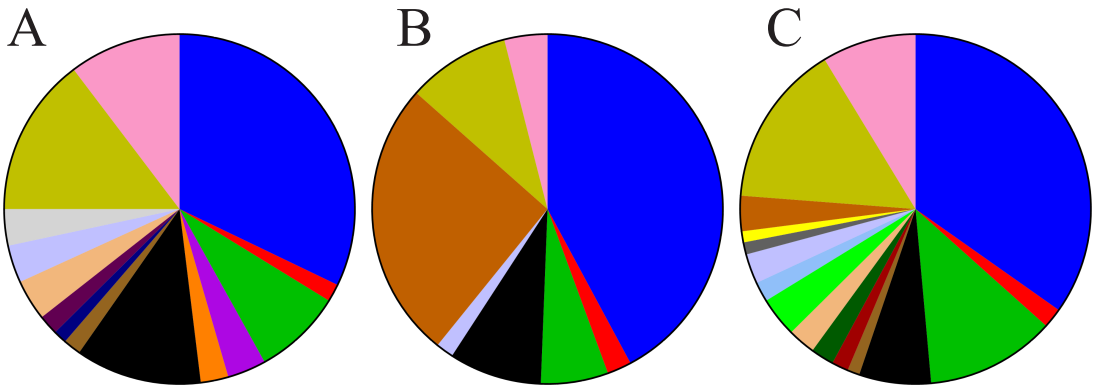

| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Bacteroidetes | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | N/A |
| Cyanobacteria | Chloroplast | Stramenopiles | N/A | N/A |
| Planctomycetes | Phycisphaerae | Phycisphaerales | N/A | N/A |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | N/A |
| | | Rhizobiales | Bradyrhizobiaceae | N/A |
| | | Rhodobacterales | Hyphomonadaceae | N/A |
| | | | Rhodobacteraceae | N/A |
| | | Sphingomonadales | N/A | N/A |
| | | | Sphingomonadaceae | *Sphingomonas* |
| | Betaproteobacteria | Methylophilales | Methylophilaceae | *Methylotenera* |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | N/A |
| | | | | *Alteromonas* |
| | | | | *ZD0117* |
| | | | | Other |
| | | | Colwelliaceae | N/A |
| | | | | *Colwellia* |
| | | | J115 | N/A |
| | | | | Other |
| | | Oceanospirillales | Oceanospirillaceae | *Oleibacter* |
| | | | | *Oleispira* |
| | | | | Other |
| | | Pseudomonadales | Moraxellaceae | *Perlucidibaca* |
| | | | Pseudomonadaceae | *Pseudomonas* |
| | | Xanthomonadales | Xanthomonadaceae | *Stenotrophomonas* |
| | | Other | Other | Other |
| Verrucomicrobia | Verrucomicrobiae | Verrucomicrobiales | Verrucomicrobiaceae | *Persicirhabdus* |
| Other | Other | Other | Other | Other |
| Unassigned | Unassigned | Unassigned | Unassigned | Unassigned |

Supplemental Fig. 2.4. Distribution of bacterial taxa associated with *Strongylocentrotus purpuratus* larvae along the phenotypic continuum. Genera associated with *S. purpuratus* larvae pre- (A) and post-expression (B, C) of phenotypic plasticity representing at least 1% of the community, with taxa less than 1% group as 'other.' Data correspond with Fig. 2A.



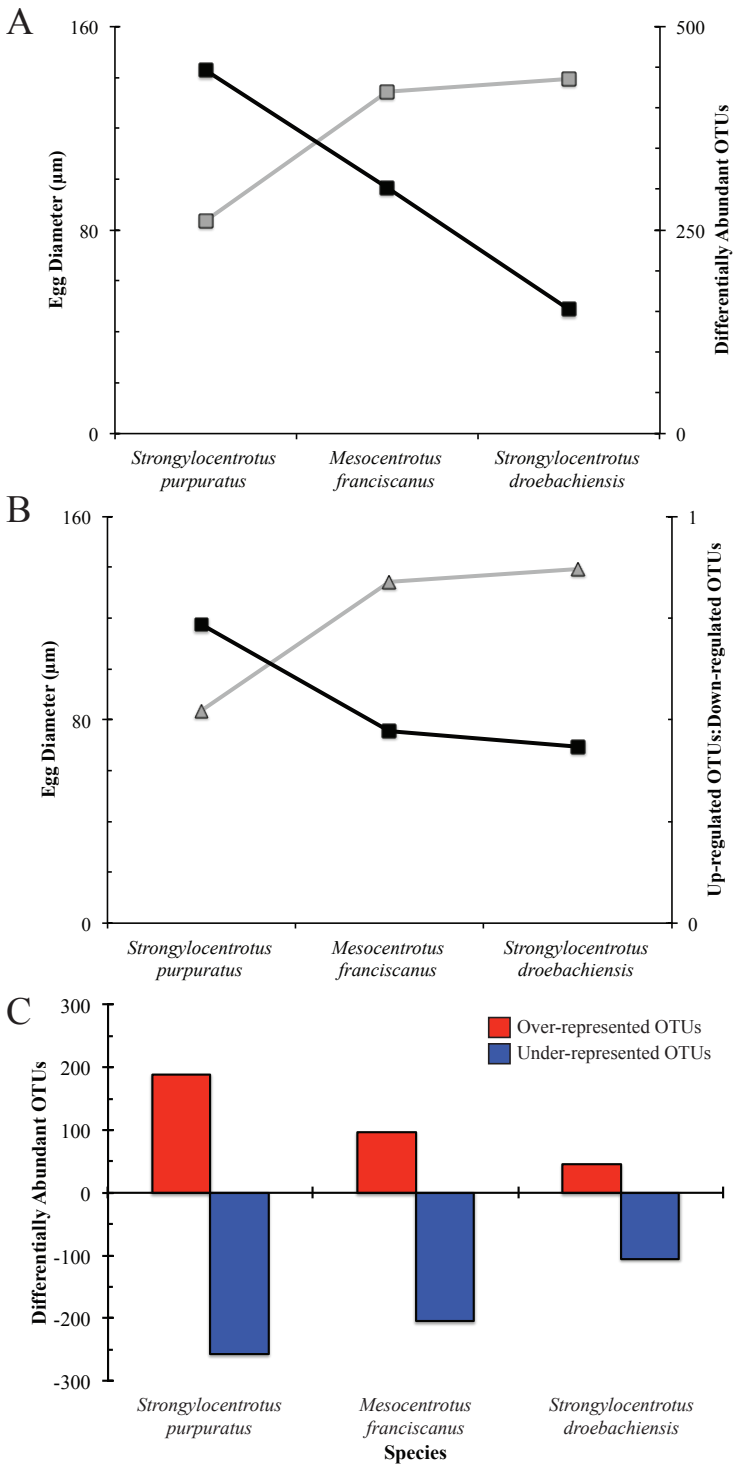

| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Bacteroidetes | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | N/A |
| Cyanobacteria | Chloroplast | Stramenopiles | N/A | N/A |
| Planctomycetes | Phycisphaerae | Phycisphaerales | | N/A |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | N/A |
| | | Rhizobiales | Bradyrhizobiaceae | N/A |
| | | Rhodobacterales | Rhodobacteraceae | N/A |
| | | | | *Octadecabacter* |
| | | Sphingomonadales | Sphingomonadaceae | *Sphingomonas* |
| | Betaproteobacteria | Methylophilales | Methylophilaceae | *Methylotenera* |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | N/A |
| | | | | *ZD0117* |
| | | | Colwelliaceae | N/A |
| | | Oceanospirillales | Halomonadaceae | *Candidatus Portiera* |
| | | Pseudomonadales | Moraxellaceae | *Perlucidibaca* |
| | | | Pseudomonadaceae | N/A |
| | | | | *Pseudomonas* |
| | | Xanthomonadales | Xanthomonadaceae | *Stenotrophomonas* |
| | | | | Other |
| Verrucomicrobia | Verrucomicrobiae | Verrucomicrobiales | Verrucomicrobiaceae | *Rubritalea* |
| Other | Other | Other | Other | Other |
| Unassigned | Unassigned | Unassigned | Unassigned | Unassigned |

Supplemental Fig. 2.5. Distribution of bacterial taxa associated with *Mesocentrotus franciscanus* larvae along the phenotypic continuum. Genera associated with *M. franciscanus* larvae pre- (A) and post-expression (B, C) of phenotypic plasticity representing at least 1% of the community, with taxa less than 1% group as 'other.' Data correspond with Fig. 2B.

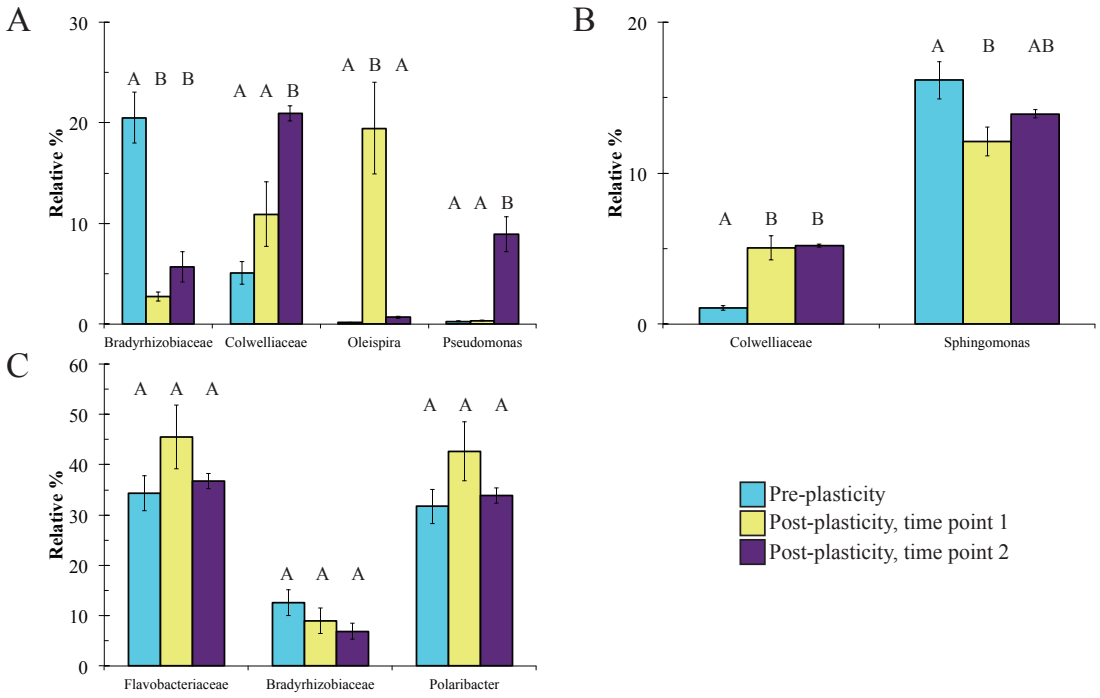



| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Bacteroidetes | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | *Polaribacter* |
| | | | | Other |
| Cyanobacteria | Chloroplast | Stramenopiles | N/A | N/A |
| Planctomycetes | Phycisphaerae | Phycisphaerales | | N/A |
| | vadinHA49 | | | N/A |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Bradyrhizobiaceae | N/A |
| | | Rhodobacterales | Rhodobacteraceae | N/A |
| | | | | *Marivita* |
| | | Sphingomonadales | Sphingomonadaceae | *Sphingomonas* |
| | Betaproteobacteria | Methylophilales | Methylophilaceae | *Methylotenera* |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | N/A |
| | | | | *ZD0117* |
| | | | Colwelliaceae | N/A |
| | | Enterobacteriales | Enterobacteriaceae | N/A |
| | | Oceanospirillales | Endozoicimonaceae | N/A |
| | | | Oceanospirillaceae | N/A |
| | | Pseudomonadales | Moraxellaceae | *Perlucidibaca* |
| | | Vibrionales | Vibrionaceae | *Vibrio* |
| Verrucomicrobia | Verrucomicrobiae | Verrucomicrobiales | Verrucomicrobiaceae | N/A |
| Other | Other | Other | Other | Other |
| Unassigned | Unassigned | Unassigned | Unassigned | Unassigned |

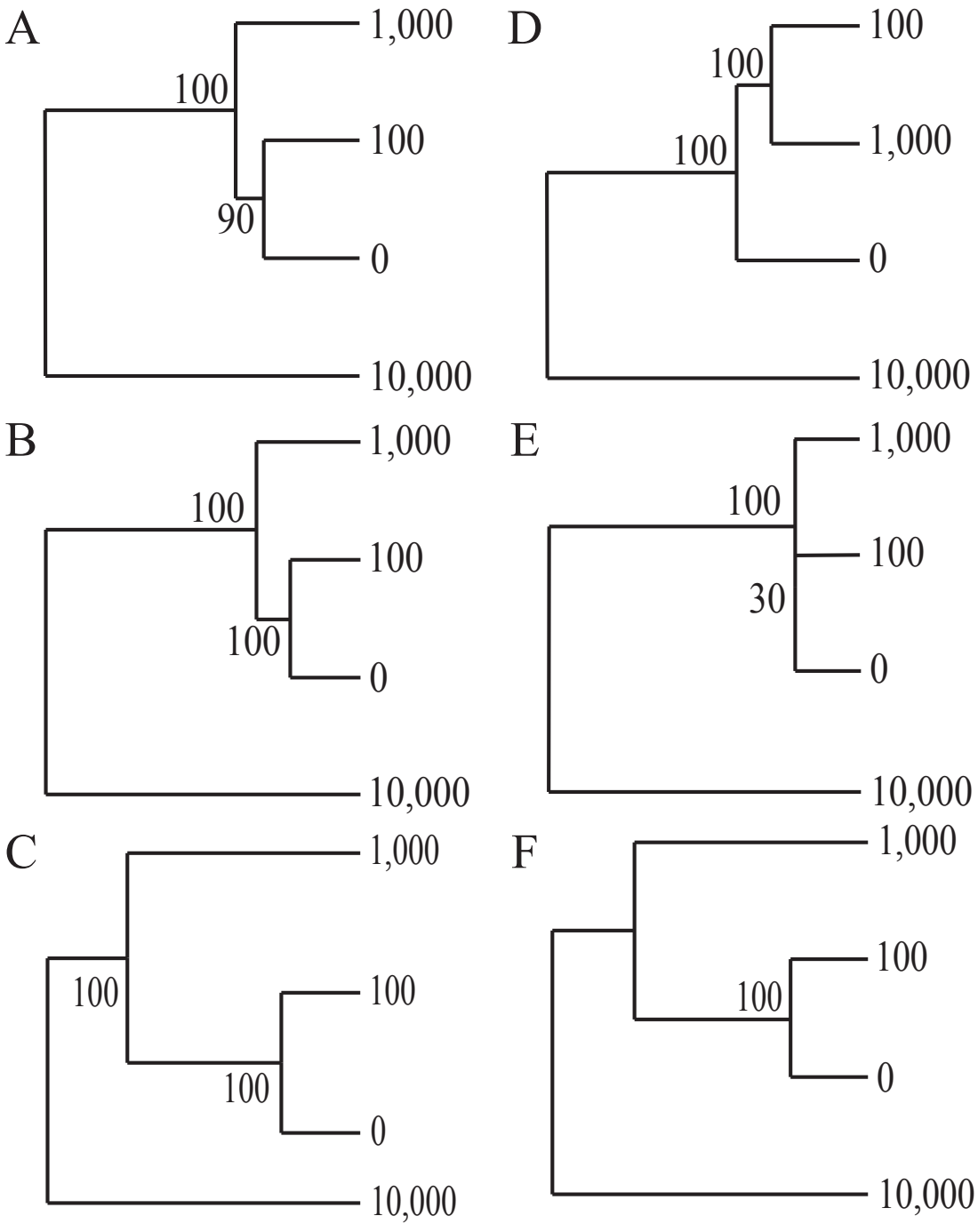
Supplemental Fig. 2.6. Distribution of bacterial taxa associated with *Strongylocentrotus droebachiensis* larvae along the phenotypic continuum. Genera associated with *S. droebachiensis* larvae pre- (A) and post-expression (B, C) of phenotypic plasticity representing at least 1% of the community, with taxa less than 1% group as 'other.' Data correspond with Fig. 2C.



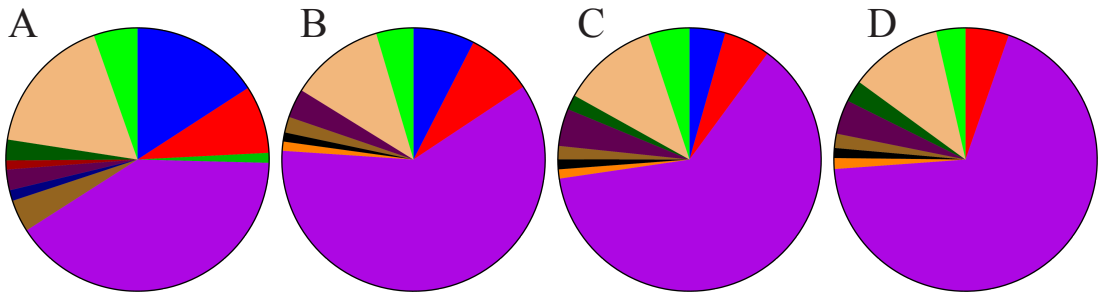

Supplemental Fig. 2.7. Differential abundance of OTUs in relation to egg size for three species of echinoid larvae. Total (A, B) and ration of (C) over- and under-represented OTUs associated with *Strongylocentrotus purpuratus*, *Mesocentrotus franciscanus*, and *S. droebachiensis* larvae following the expression of phenotypic plasticity (black) and in relation to egg size (gray).




Supplemental Fig. 2.8. Specific bacterial taxa within the proteobacteria differentially associated with larvae from three species of echinoid along the phenotypic continuum. Relative presence of specific bacterial taxa associated with *Strongylocentrotus purpuratus* (A), *Mesocentrotus franciscanus* (B), and *S. droebachiensis* (C) pre- (blue) and post-expression (yellow and purple) of phenotypic plasticity. Data correspond with Fig. 2D-F.

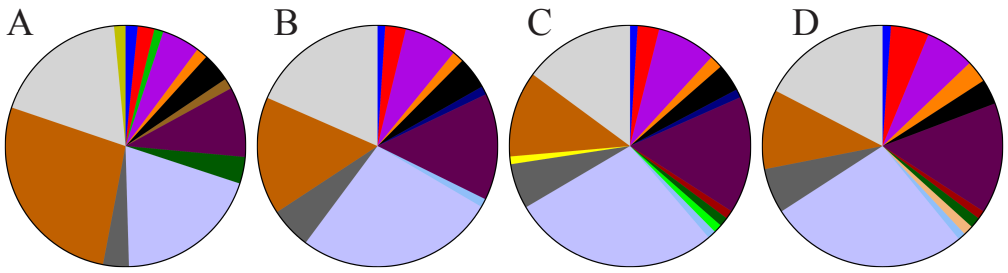



Supplemental Fig. 2.9. Similarity between associated microbial community for three species of differentially fed echinoid larvae. Microbial dendrograms of *Strongylocentrotus purpuratus* (A, D), *Mesocentrotus franciscanus* (B, E), and *S. droebachiensis* (C, F) differentially fed at time points pre- (A-C) and post-expression (D-F) of phenotypic plasticity. Data correspond with Fig. 4.



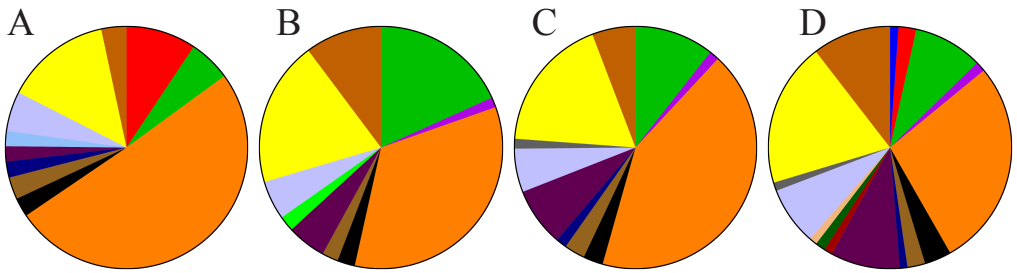

| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Bacteroidetes | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | N/A |
| Cyanobacteria | Chloroplast | Stramenopiles | N/A | N/A |
| Planctomycetes | Phycisphaerae | Phycisphaerales | | N/A |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Bradyrhizobiaceae | N/A |
| | | | | Other |
| | | | Other | Other |
| | | Rhodobacterales | Rhodobacteraceae | N/A |
| | | | | *Marivita* |
| | | Sphingomonadales | Sphingomonadaceae | *Sphingomonas* |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | N/A |
| | | | | *ZD0117* |
| Other | Other | Other | Other | Other |
| Unassigned | Unassigned | Unassigned | Unassigned | Unassigned |

Supplemental Fig. 2.10. Distribution of bacterial taxa associated with differentially fed *Strongylocentrotus purpuratus* larvae pre-expression of phenotypic plasticity. Genera associated with *S. purpuratus* larvae fed either 10,000 (A), 1,000 (B), 100 (C), and 0 cells•mL$^{-1}$ (D) following one week of differential feeding representing at least 1% of the community, with taxa less than 1% group as 'other.' Data correspond with Fig. 4A.



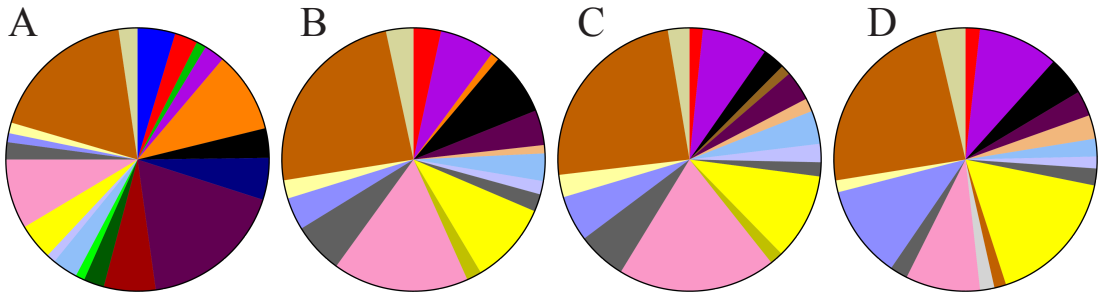

| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Bacteroidetes | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | N/A |
| Cyanobacteria | Chloroplast | Stramenopiles | N/A | N/A |
| Planctomycetes | Phycisphaerae | Phycisphaerales | | N/A |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | N/A |
| | | Rhizobiales | Bradyrhizobiaceae | N/A |
| | | Rhodobacterales | Rhodobacteraceae | N/A |
| | | | | *Marivita* |
| | | | | *Octadecabacter* |
| | | Sphingomonadales | Sphingomonadaceae | *Sphingomonas* |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | N/A |
| | | | | *ZD0117* |
| | | | Colwelliaceae | N/A |
| | | Oceanospirillales | Halomonadaceae | *Candidatus Portiera* |
| | | Pseudomonadales | Pseudomonadaceae | N/A |
| | | | | *Pseudomonas* |
| | | Xanthomonadales | Xanthomonadaceae | *Stenotrophomonas* |
| | | | | Other |
| Verrucomicrobia | Verrucomicrobiae | Verrucomicrobiales | Verrucomicrobiaceae | *Rubritalea* |
| Other | Other | Other | Other | Other |
| Unassigned | Unassigned | Unassigned | Unassigned | Unassigned |

Supplemental Fig. 2.11. Distribution of bacterial taxa associated with differentially fed *Mesocentrotus franciscanus* larvae pre-expression of phenotypic plasticity. Genera associated with *M. franciscanus* larvae fed either 10,000 (A), 1,000 (B), 100 (C), and 0 cells•mL$^{-1}$ (D) following one week of differential feeding representing at least 1% of the community, with taxa less than 1% group as 'other.' Data correspond with Fig. 4B.



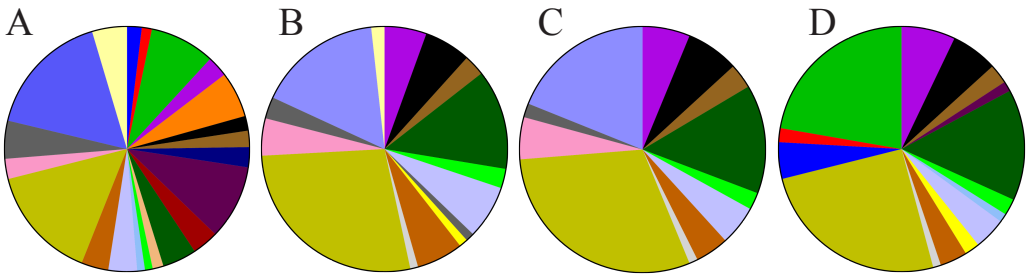

| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Bacteroidetes | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | N/A |
| | | | | *Polaribacter* |
| Cyanobacteria | Chloroplast | Stramenopiles | N/A | N/A |
| Planctomycetes | Planctomycetia | Pirellulales | Pirellulaceae | N/A |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Bradyrhizobiaceae | N/A |
| | | Rhodobacterales | Rhodobacteraceae | N/A |
| | | Sphingomonadales | Sphingomonadaceae | *Sphingomonas* |
| | Betaproteobacteria | Burkholderiales | Oxalobacteraceae | *Ralstonia* |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | ZD0117 |
| | | Enterobacteriales | Enterobacteriaceae | N/A |
| | | Oceanospirillales | Halomonadaceae | *Candidatus Portiera* |
| | | | Oceanospirillaceae | N/A |
| | | Pseudomonadales | Pseudomonadaceae | N/A |
| | | Vibrionales | Vibrionaceae | *Vibrio* |
| Verrucomicrobia | Verrucomicrobiae | Verrucomicrobiales | Verrucomicrobiaceae | N/A |
| Verrucomicrobia | | | | *Persicirhabdus* |
| Other | Other | Other | Other | Other |
| Unassigned | Unassigned | Unassigned | Unassigned | Unassigned |

Supplemental Fig. 2.12. Distribution of bacterial taxa associated with differentially fed *Strongylocentrotus droebachiensis* larvae pre-expression of phenotypic plasticity. Genera associated with *S. droebachiensis* larvae fed either 10,000 (A), 1,000 (B), 100 (C), and 0 cells•mL$^{-1}$ (D) following one week of differential feeding representing at least 1% of the community, with taxa less than 1% group as 'other.' Data correspond with Fig. 4C.



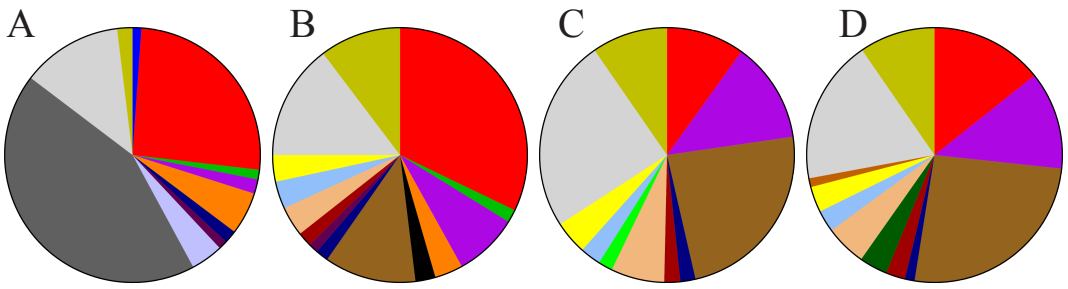

| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Bacteroidetes | [Rhodothermi] | [Rhodothermales] | [Balneolaceae] | *Balneola* |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | N/A |
| | | | | *Polaribacter* |
| Cyanobacteria | Chloroplast | Stramenopiles | N/A | N/A |
| Planctomycetes | Phycisphaerae | Phycisphaerales | | N/A |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Bradyrhizobiaceae | N/A |
| | | Rhodobacterales | Hyphomonadaceae | N/A |
| | | | | *Oceanicaulis* |
| | | | Rhodobacteraceae | N/A |
| | | | | *Marivita* |
| | | Other | Other | Other |
| | Betaproteobacteria | Methylophilales | Methylophilaceae | *Methylotenera* |
| | Gammaproteobacteria | N/A | N/A | N/A |
| | | Alteromonadales | Alteromonadaceae | N/A |
| | | | | *Alteromonas* |
| | | | | *ZD0117* |
| | | | Colwelliaceae | N/A |
| | | | J115 | N/A |
| | | | | Other |
| | | Oceanospirillales | Oceanospirillaceae | *Oleibacter* |
| | | | | *Oleispira* |
| | | | | Other |
| | | Pseudomonadales | Moraxellaceae | *Perlucidibaca* |
| | | Other | Other | Other |
| Other | Other | | | Other |
| Unassigned | Unassigned | Unassigned | Unassigned | Unassigned |

Supplemental Fig. 2.13. Distribution of bacterial taxa associated with differentially fed *Strongylocentrotus purpuratus* larvae post-expression of phenotypic plasticity. Genera associated with *S. purpuratus* larvae fed either 10,000 (A), 1,000 (B), 100 (C), and 0 cells•mL$^{-1}$ (D) following one week of differential feeding representing at least 1% of the community, with taxa less than 1% group as 'other.' Data correspond with Fig. 4D.



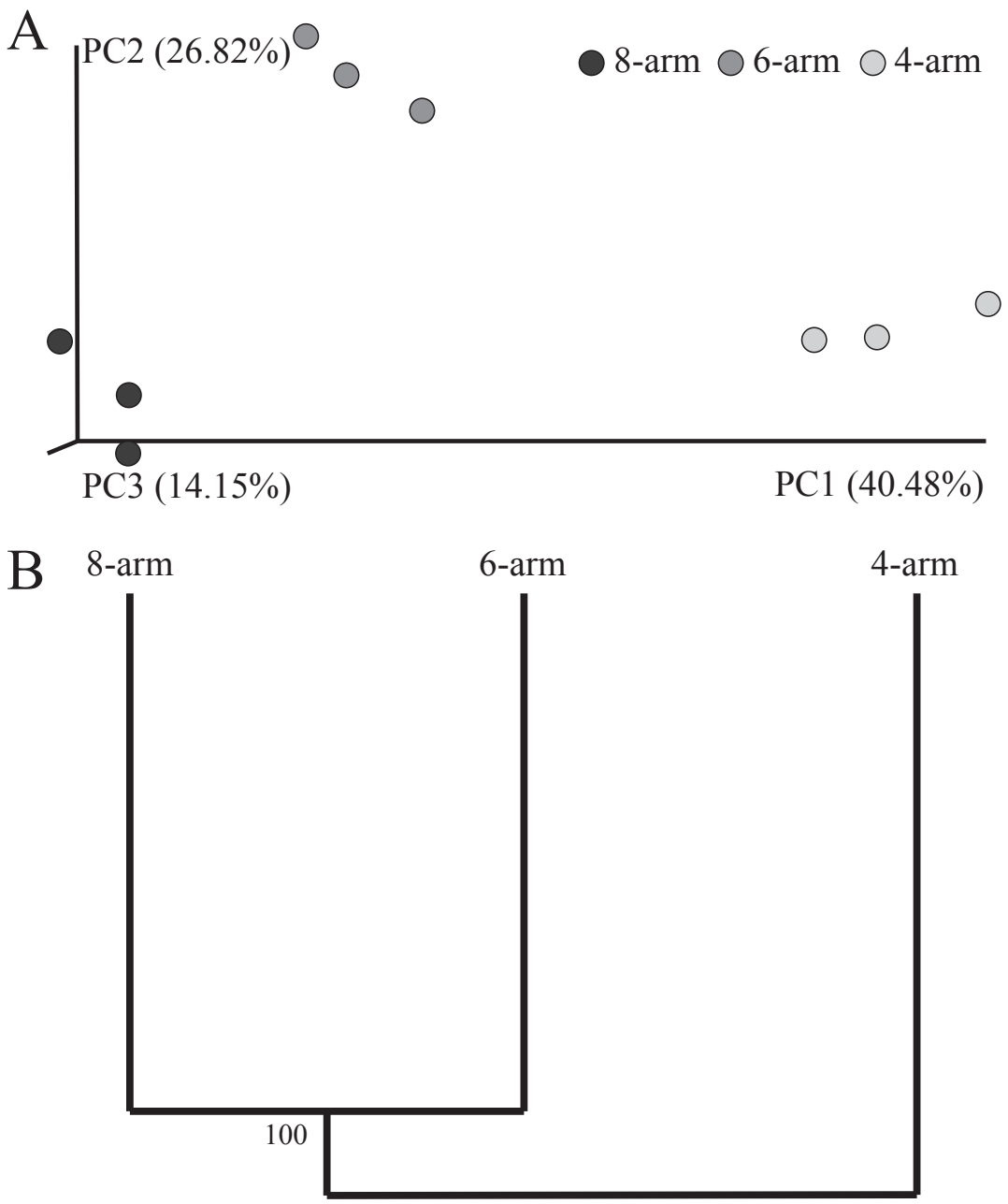

| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Bacteroidetes | [Rhodothermi] | [Rhodothermales] | [Balneolaceae] | *Balneola* |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | N/A |
| | | | | *Polaribacter* |
| Cyanobacteria | Chloroplast | Stramenopiles | N/A | N/A |
| Planctomycetes | Phycisphaerae | Phycisphaerales | | N/A |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | N/A |
| | | Rhizobiales | Bradyrhizobiaceae | N/A |
| | | Rhodobacterales | Hyphomonadaceae | *Oceanicaulis* |
| | | | Rhodobacteraceae | N/A |
| | | | | *Marivita* |
| | | Sphingomonadales | Sphingomonadaceae | *Sphingomonas* |
| | | Other | Other | Other |
| | Betaproteobacteria | Methylophilales | Methylophilaceae | *Methylotenera* |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | N/A |
| | | | Colwelliaceae | N/A |
| | | | J115 | N/A |
| | | Oceanospirillales | Oceanospirillaceae | *Oleispira* |
| | | Pseudomonadales | Moraxellaceae | *Perlucidibaca* |
| | | | Pseudomonadaceae | N/A |
| | | | | *Pseudomonas* |
| | | Xanthomonadales | Xanthomonadaceae | *Stenotrophomonas* |
| Verrucomicrobia | Verrucomicrobiae | Verrucomicrobiales | Verrucomicrobiaceae | *Rubritalea* |
| Other | Other | Other | Other | Other |
| Unassigned | Unassigned | Unassigned | Unassigned | Unassigned |

Supplemental Fig. 2.14. Distribution of bacterial taxa associated with differentially fed *Mesocentrotus franciscanus* larvae post-expression of phenotypic plasticity. Genera associated with *M. franciscanus* larvae fed either 10,000 (A), 1,000 (B), 100 (C), and 0 cells•mL$^{-1}$ (D) following one week of differential feeding representing at least 1% of the community, with taxa less than 1% group as 'other.' Data correspond with Fig. 4E.

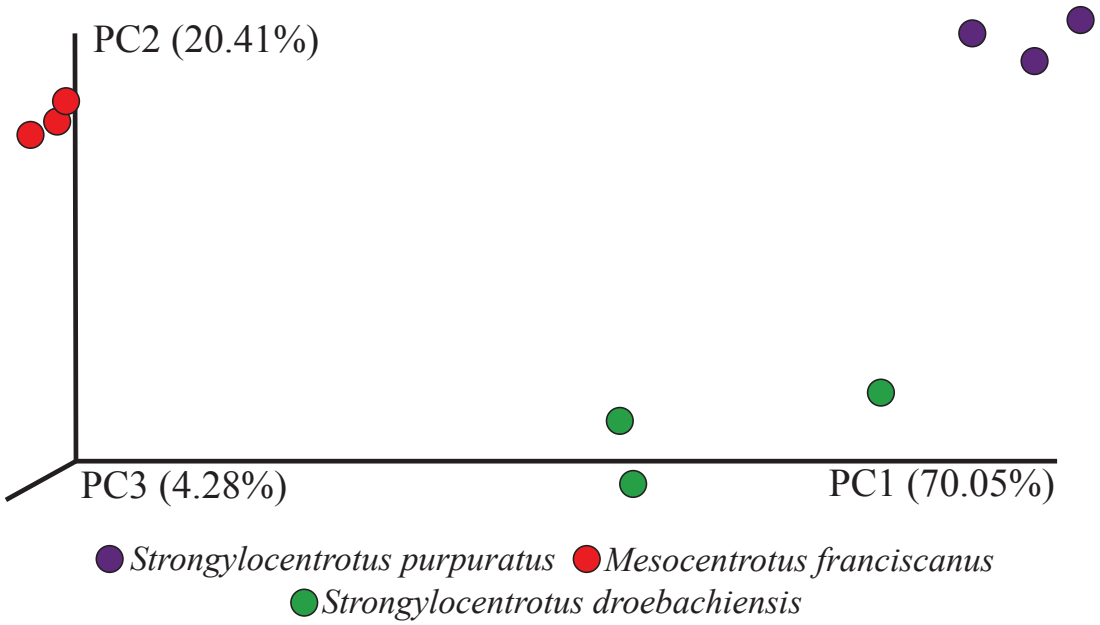



| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Bacteroidetes | [Saprospirae] | [Saprospirales] | Saprospiraceae | *Saprospira* |
| | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | *Polaribacter* |
| | | | | Other |
| Cyanobacteria | Chloroplast | Stramenopiles | N/A | N/A |
| Planctomycetes | Phycisphaerae | Phycisphaerales | | N/A |
| Planctomycetes | vadinHA49 | N/A | | N/A |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Bradyrhizobiaceae | N/A |
| | | Rhodobacterales | Rhodobacteraceae | N/A |
| | | | | *Marivita* |
| | | Sphingomonadales | Sphingomonadaceae | *Sphingomonas* |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | N/A |
| | | | | *ZD0117* |
| | | | Shewanellaceae | *Shewanella* |
| | | Oceanospirillales | Endozoicimonaceae | N/A |
| | | Vibrionales | Vibrionaceae | N/A |
| | | | | *Vibrio* |
| Verrucomicrobia | Verrucomicrobiae | Verrucomicrobiales | Verrucomicrobiaceae | N/A |
| | | | | *Persicirhabdus* |
| Other | Other | Other | Other | Other |
| Unassigned | Unassigned | Unassigned | Unassigned | Unassigned |

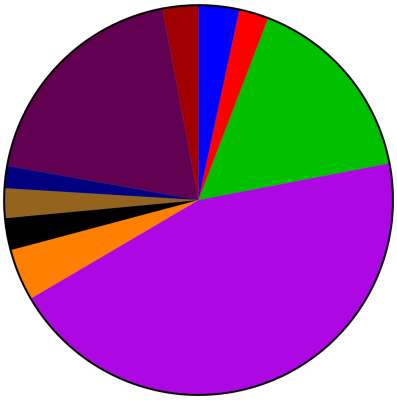
Supplemental Fig. 2.15. Distribution of bacterial taxa associated with differentially fed *Strongylocentrotus droebachiensis* larvae post-expression of phenotypic plasticity. Genera associated with *S. droebachiensis* larvae fed either 10,000 (A), 1,000 (B), 100 (C), and 0 cells•mL$^{-1}$ (D) following one week of differential feeding representing at least 1% of the community, with taxa less than 1% group as 'other.' Data correspond with Fig. 4F.




Supplemental Fig. 2.16. Developmental stage-specific microbial community for *Strongylocentrotus purpuratus* larvae. Community similarity of the associated microbiota for *Strongylocentrotus purpuratus* larvae at the 4- (light grey), 6- (grey), and 8-arm (black) stages (A), with respective microbial dendrogram.

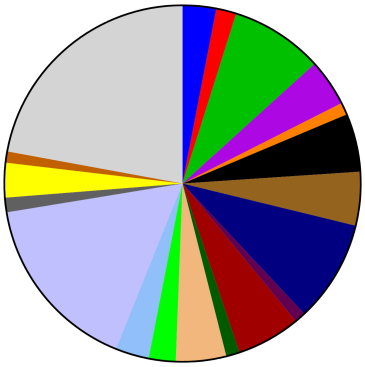



Supplemental Fig. 17. Species-specific associated microbial communities for three species of echinoid larvae. Community similarity of the associated microbiota for *Strongylocentrotus purpuratus* (purple), *Mesocentrotus franciscanus* (red), and *S. droebachiensis* (green) prior to feeding.



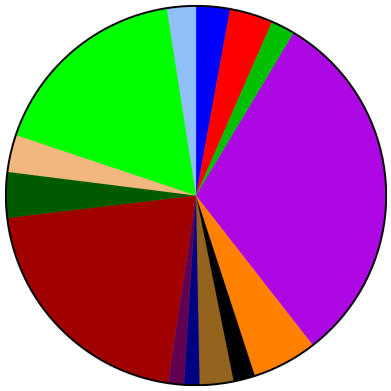
| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Bacteroidetes | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | N/A |
| | | | | *Polaribacter* |
| Cyanobacteria | Chloroplast | Stramenopiles | N/A | N/A |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Bradyrhizobiaceae | N/A |
| | | Rhodobacterales | Rhodobacteraceae | N/A |
| | | Sphingomonadales | Sphingomonadaceae | *Sphingomonas* |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | N/A |
| | | | Colwelliaceae | N/A |
| Other | Other | Other | Other | Other |
| Unassigned | Unassigned | Unassigned | Unassigned | Unassigned |

Supplemental Fig. 2.18. Bacterial taxa associated with prefeeding *Strongylocentrotus purpuratus* larvae. Genera associated with *S. purpuratus* larvae prior to feeding representing at least 1% of the community, with taxa less than 1% group as 'other.' Data correspond with Supplemental Fig. 18.



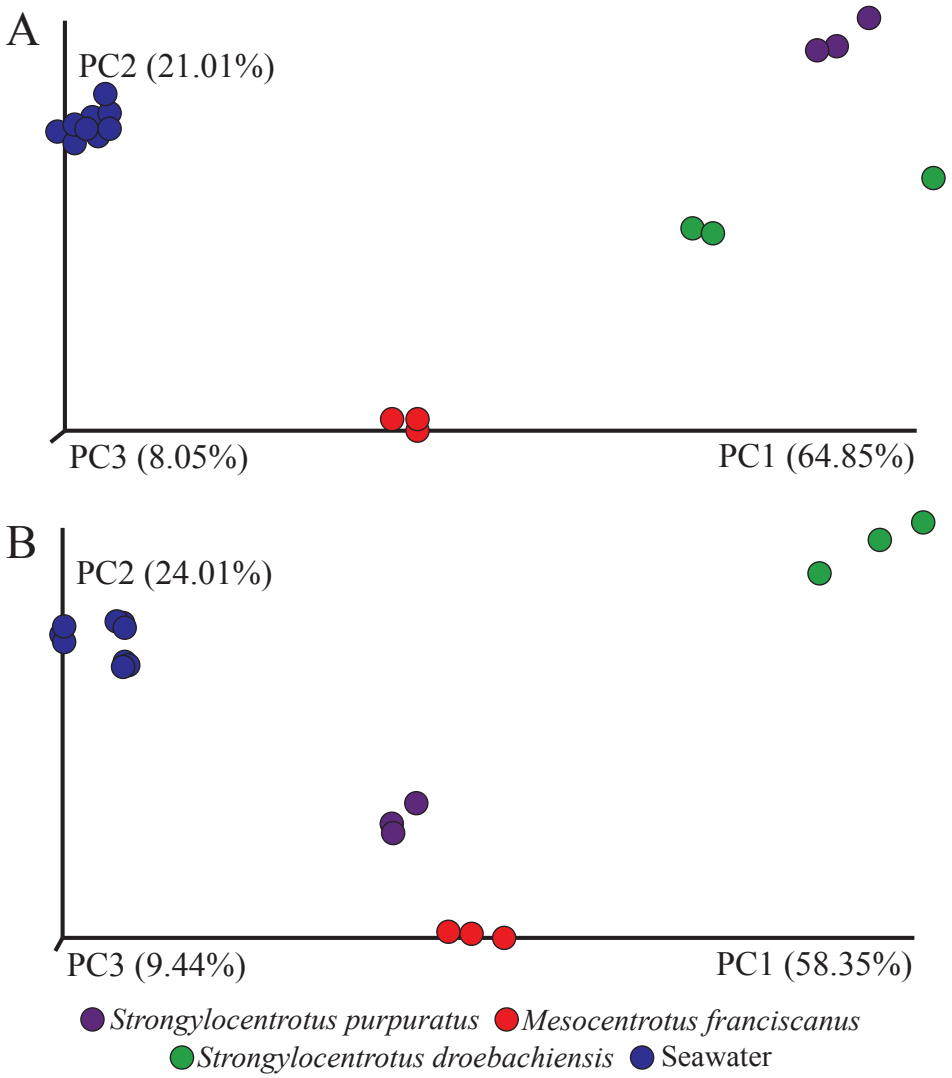
| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Bacteroidetes | [Saprospirae] | [Saprospirales] | Saprospiraceae | N/A |
| | Flavobacteriia | Flavobacteriales | Cryomorphaceae | *Crocinitomix* |
| | | | Flavobacteriaceae | N/A |
| | | | | *Polaribacter* |
| Cyanobacteria | Chloroplast | Stramenopiles | N/A | N/A |
| Proteobacteria | Alphaproteobacteria | Caulobacterales | Caulobacteraceae | N/A |
| | | Rhodobacterales | Rhodobacteraceae | N/A |
| | | Sphingomonadales | Sphingomonadaceae | *Sphingomonas* |
| | Gammaproteobacteria | Alteromonadales | N/A | N/A |
| | | | Alteromonadaceae | N/A |
| | | | | *Alteromonas* |
| | | | Colwelliaceae | N/A |
| | | Oceanospirillales | Oceanospirillaceae | *Marinomonas* |
| | | | | *Oleispira* |
| | | Pseudomonadales | Pseudomonadaceae | *Pseudomonas* |
| | | Vibrionales | Pseudoalteromonadaceae | *Pseudoalteromonas* |
| | | Xanthomonadales | Xanthomonadaceae | *Stenotrophomonas* |
| | | Other | Other | Other |
| Other | Other | | | Other |

Supplemental Fig. 2.19. Bacterial taxa associated with prefeeding *Mesocentrotus franciscanus* larvae. Genera associated with *M. franciscanus* larvae prior to feeding representing at least 1% of the community, with taxa less than 1% group as 'other.' Data correspond with Supplemental Fig. 18.

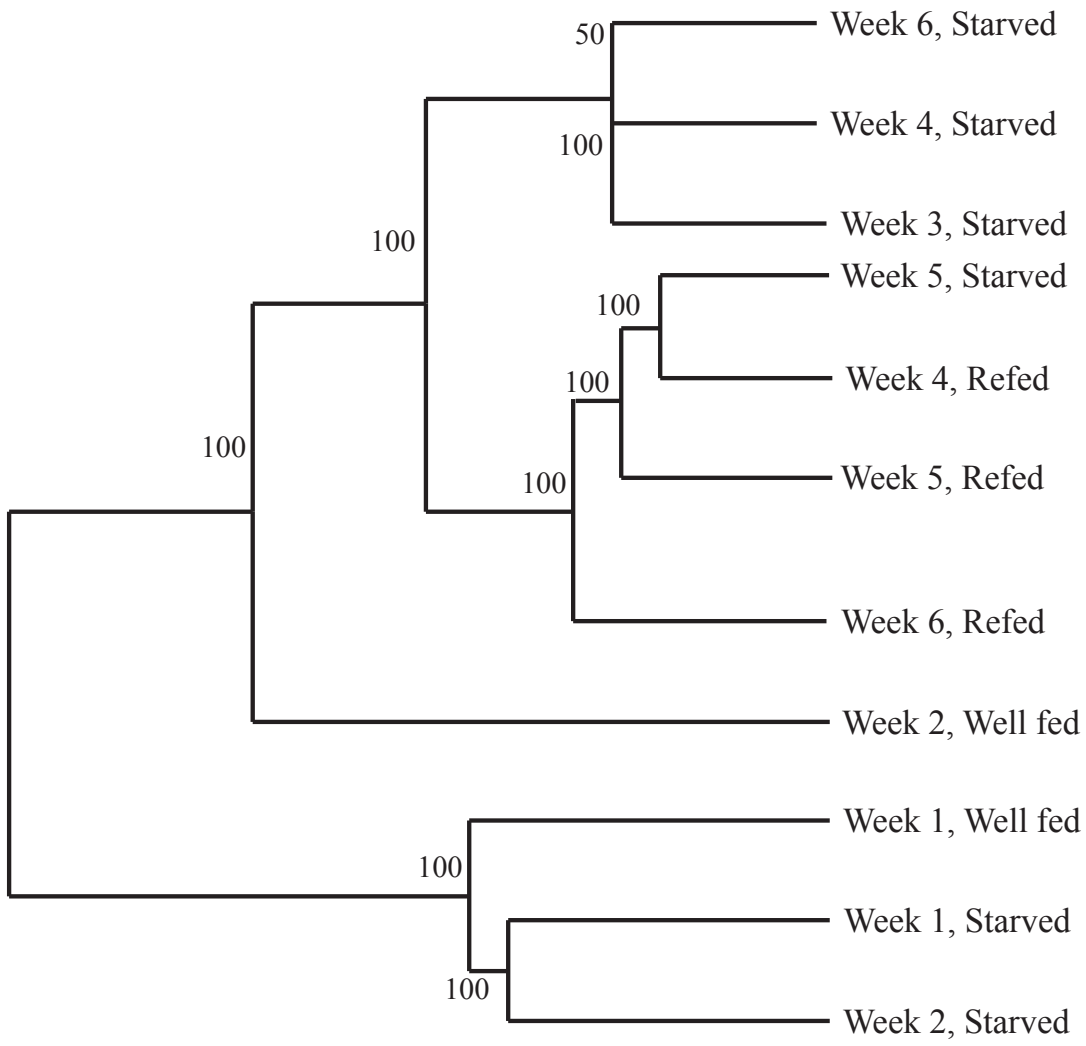


| Phylum | Class | Order | Family | Genus |
|---|---|---|---|---|
| Bacteroidetes | Flavobacteriia | Flavobacteriales | Flavobacteriaceae | N/A |
| | | | | *Polaribacter* |
| Cyanobacteria | Chloroplast | Stramenopiles | N/A | N/A |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Bradyrhizobiaceae | N/A |
| | | Rhodobacterales | Rhodobacteraceae | N/A |
| | | Sphingomonadales | Sphingomonadaceae | *Sphingomonas* |
| | Gammaproteobacteria | Alteromonadales | Alteromonadaceae | N/A |
| | | | Colwelliaceae | N/A |
| | | Oceanospirillales | Oceanospirillaceae | N/A |
| | | | | *Marinomonas* |
| | | Vibrionales | Vibrionaceae | N/A |
| | | | | Vibrio |
| Other | Other | Other | Other | Other |
| Unassigned | Unassigned | Unassigned | Unassigned | Unassigned |

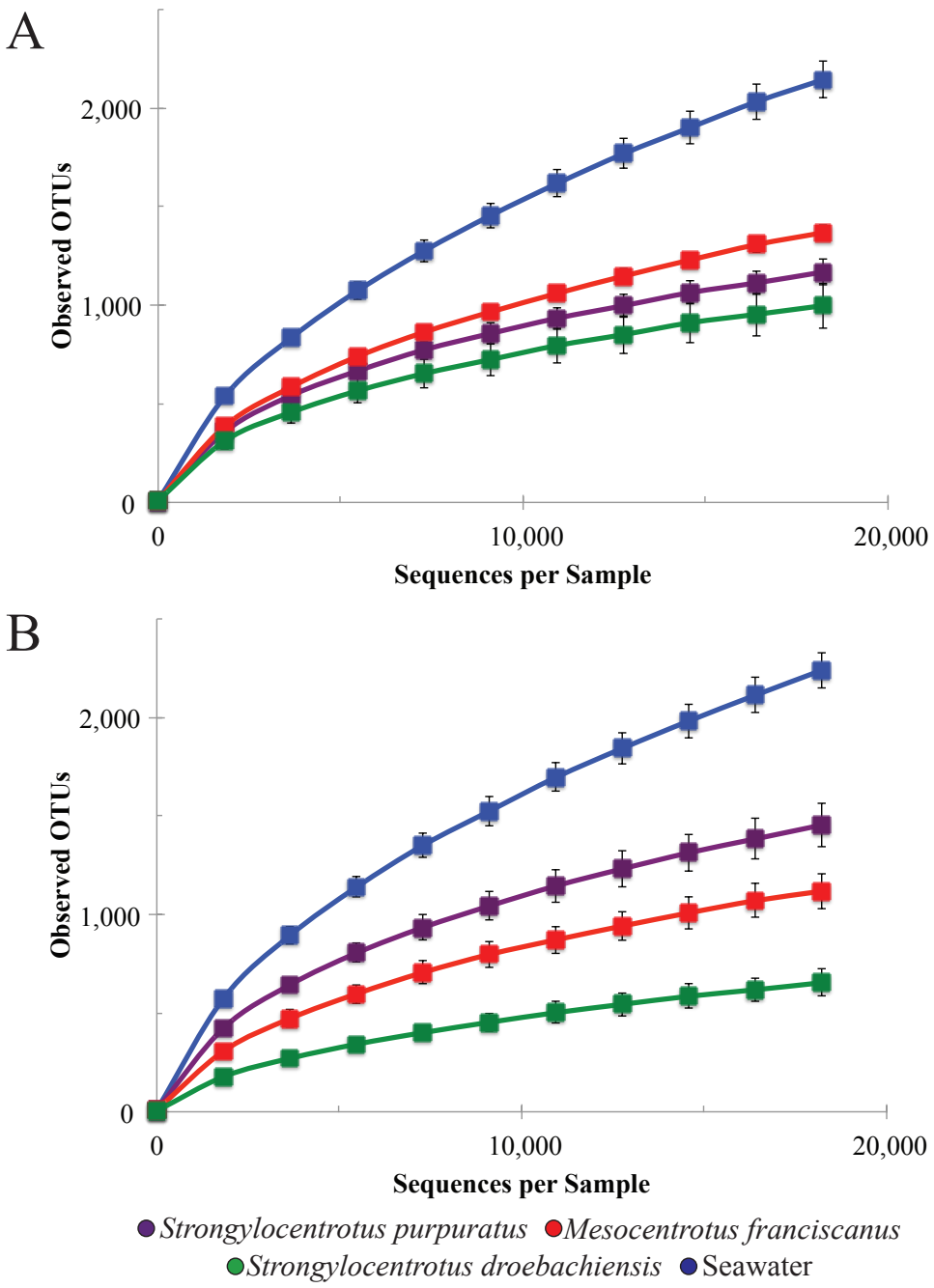
Supplemental Fig. 2.20. Bacterial taxa associated with prefeeding *Strongylocentrotus droebachiensis* larvae. Genera associated with *S. droebachiensis* larvae prior to feeding representing at least 1% of the community, with taxa less than 1% group as 'other.' Data correspond with Supplemental Fig. 18.




Supplemental Fig. 2.21. Similarity of the associated microbial community for three species of echinoids at two larval stages. Community similarity of the associated microbiota for *Strongylocentrotus purpuratus* (purple), *Mesocentrotus franciscanus* (red), and *S. droebachiensis* (green) and seawater (blue) pre- (A) and post-feeding (B).




Supplemental Fig. 2.22. Similarity between associated microbial communities for dietarily manipulated larvae. Microbial dendrograms of *Strongylocentrotus droebachiensis* larvae fed 10,000 cells•mL$^{-1}$ until metamorphosis and 0 cells•mL$^{-1}$ for three weeks, versus larvae fed 0 cells•mL$^{-1}$ for three weeks then switched to 10,000 cells•mL$^{-1}$ until metamorphosis. Data correspond with Fig. 6.




Supplemental Fig. 2.23. Alpha rarefaction curves for three species of echinoids at two larval stages and of the seawater. Alpha rarefaction curves for *Strongylocentrotus purpuratus* (purple), *Mesocentrotus franciscanus* (red), and *S. droebachiensis* (green) and seawater (blue) pre- (A) and post-feeding (B) based on rarefaction depth (*i.e.*, 18,225) used for all PCoA plots.


Supplemental Fig. 2.24. *in situ* measurements of sea surface temperature at Friday Harbor, WA. Sea surface temperature at Friday Harbor, WA (NOAA station, FRDW1; 48°32'43" N 123°0'44" W) for the entirety of larval experimentation (15 May 2016 – 15 July 2016).

# CHAPTER 3

# SHIFT IN BACTERIAL TAXA PRECEDES MORPHOLOGICAL PLASTICITY IN A LARVAL ECHINOID

Tyler J. Carrier and Adam M. Reitzel

Citation

**Carrier TJ, Reitzel, AM.** 2019. Shift in bacterial taxa precedes morphological plasticity in a larval echinoid. Marine Biology 166:164.

Abstract

Morphological plasticity is an adaptive response to heterogenous environments when a fitness advantage is conferred. Larval sea urchins, for example, are hypothesized to increase individual fitness in dilute feeding environments by elongating their feeding structure relative to their body size. Morphological plasticity for larval sea urchins is also coupled with significant shifts in the associated bacterial community, but whether this change to the associated microbiota occurs before, during, or following the expression of morphological plasticity is unclear. Using the sea urchin *Lytechinus variegatus*, we compare the temporal pattern of the microbial community and how it relates to the timing of morphological plasticity for larvae cultured in different food concentrations. From prefeeding through the expression of morphological plasticity, we observed that the microbiota associated with *L. variegatus* larvae exhibit a four-stage successional pattern, where changes in this community shift prior to the expression of the environment-specific morphology. The high food treatment, which results in the short-arm phenotype, deviated from the microbial trajectory of larval siblings prior to measurable morphological

plasticity. These data suggest that a holobiont may exhibit shifts in the associated bacterial community corresponding with variation in the feeding environment that could occur in anticipation of or prior to morphological plasticity.

## 3.1 Introduction

As the environment ebbs and flows, animals from diverse phylogenetic lineages may modify phenotypic traits to better match environmental conditions (Miner, Sultan, Morgan, Padilla, & Relyea, 2005; Sterns, 1989; West-Eberhard, 2003). When phenotypic plasticity—the potential to produce a range of phenotypes from a single genotype—is induced and confers a fitness advantage, the response is considered adaptive and an evolvable trait influenced by natural selection (Miner et al., 2005; Sterns, 1989; West-Eberhard, 2003). For morphological characters to exhibit plasticity favored by selection, abiotic or biotic stimuli must be reliable and detected at an appropriate time scale to elicit a developmental change that results in a phenotype-environment match (Levins, 1968; Miner et al., 2005; Padilla & Adolph, 1996). Plasticity in morphology and other life history characters are, thus, viewed as a product of host genotype and the environment (Agrawal, 2001; DeWitt, Sih, & Wilson, 1998; Miner et al., 2005; Sterns, 1989; West-Eberhard, 2003). Recent evidence, however, suggests that phenotypic state in morphologically plastic species also correlates with the composition of the symbiotic bacterial community (Bittleston et al., 2018; Carrier & Reitzel, 2018).

Host-associated microbial communities are fundamental to the metabolism, immunity, and development of the host (Gilbert, Sapp, & Tauber, 2012; McFall-Ngai et al., 2013; Zilber-Rosenberg & Rosenberg, 2008). Symbiotic relationships between host

and microbiota have deep evolutionary origins, often exhibit co-divergence over evolutionary time, and can be important for coping with environmental stressors (Brooks, Kohl, Brucker, van Opstal, & Bordenstein, 2016; Carrier & Reitzel, 2017; Kohl & Carey, 2016; McFall-Ngai et al., 2013; Moeller et al., 2016). When faced with heterogeneous environments that are known to induce phenotypic plasticity, an animal may recruit, expel, and/or shuffle the membership and relative proportion of associated microbiota to assemble a community with specific functional properties (*e.g.*, genes and metabolic pathways) for the environmental conditions (Bordenstein & Theis, 2015; Burke, Steinberg, Rusch, Kjelleberg, & Thomas, 2011; Louca et al., 2016; Roth-Schulze et al., 2018; Zilber-Rosenberg & Rosenberg, 2008). When faced with prolonged diet restriction, for example, the composition and diversity of invertebrate- and vertebrate-associated bacterial communities shifts considerably to buffer against reduced exogenous nutrients (Carrier & Reitzel, 2018; Kohl, Amaya, Passement, Dearing, & McCue, 2014).

Planktotrophic (feeding) larvae of benthic marine invertebrates inhabit coastal seas, where the abundance and distribution of exogenous nutrients are spatially and temporally heterogeneous (Olson & Olson, 1989). Several groups of planktotrophic larvae, including sea urchins (phylum Echinodermata), respond to heterogeneous feeding environments by exhibiting morphological plasticity (McAlister & Miner, 2018). To increase individual fitness in the face of diet restriction, larval sea urchins suppress development of the larval body (*i.e.*, mid-body line) and absorb stomach tissues to allocate energetic resources towards the feeding structures (*i.e.*, post-oral arms) that, in turn, increases the capacity to collect particulates (Hart & Strathmann, 1994; Miner, 2004; Sewell, 2005; Sewell, Cameron, & McArdle, 2004; Soars, Prowse, & Byrne, 2009;

Strathmann, Fenaux, & Strathmann, 1992). Moreover, morphological plasticity by echinoid larvae is coupled with physiological plasticity (Adams, Sewell, Angerer, & Angerer, 2011; Carrier, King, & Coffman, 2015; Rendleman, Rodriguez, Ohanian, & Pace, 2018), whereby diet restriction correlates with reduced expression of genes associated with growth and metabolism and higher expression of genes involved with neurogenesis, environmental sensing, immunity, and longevity (Carrier et al., 2015).

Larval sea urchins associate with bacterial communities that are diverse and dynamic yet specific to host species (Carrier & Reitzel, 2018; Carrier & Reitzel, 2019), variable between populations (Carrier, Dupont, & Reitzel, 2019), and distinct from the environmental microbiota (Carrier et al., 2019; Carrier & Reitzel, 2018; Carrier & Reitzel, 2019). Larval-associated bacterial communities shift over the course of embryonic development (Carrier & Reitzel, 2019), with disease (Carrier, Macrander, & Reitzel, 2018) and in response to food availability (Carrier et al., 2019; Carrier & Reitzel, 2018; T.J. Carrier et al., 2018). Specifically, the bacterial community associated with echinoid larvae exhibits bi-directional shifts that correlate with phenotype (Carrier & Reitzel, 2018). This correlation between morphological plasticity and the composition of the associated bacterial community, thus far, is unique to echinoid larvae, but recent data suggests similar responses in taxa from disparate clades (Bittleston et al., 2018; Parker & Brisson, 2019). What remains unclear is whether associating with a morphology-specific bacterial community occurs before, during, or following the expression of morphological plasticity.

The expression of morphological plasticity by temperate Strongylocentrotids requires a multi-week stimulus (Carrier et al., 2019; Carrier & Reitzel, 2018; Hart &

Strathmann, 1994; Miner, 2004), which is restrictive for defining a precise temporal succession by the holobiont during morphological plasticity. Tropical and subtropical sea urchins, on the other hand, develop at a more rapid pace and may express plasticity following a few days of differential feeding (Boidron-Metairon, 1988; Byrne, Sewell, & Prowse, 2008; McEdward & Herrera, 1999). This shortened developmental window permits a narrow time frame for changes in morphology and associations with bacteria that can be studied at a finer grain than species with prolonged larval durations. Using *Lytechinus variegatus*, a subtropical/tropical sea urchin known to exhibit morphological plasticity following four days of differential feeding during embryonic development (Boidron-Metairon, 1988; McAlister, 2007; McEdward & Herrera, 1999), we define this temporal succession and test whether shifts in the bacterial taxa occurs before, during, or following a morphological response. To test this, we performed a differential feeding experiment on *L. variegatus* larvae and used amplicon sequencing to profile the larval-associated bacterial communities from prefeeding though the expression of morphological plasticity.

## 3.2 Materials and Methods

### 3.2.1 Specimen collection and larval rearing

Adult *L. variegatus* were collected from populations in Back Sound, North Carolina (NC), USA in July 2017, were transferred to the Duke University Marine Laboratory (Beaufort, NC) within one hour, and were maintained in flow-through aquaria.

Within two days of collection adult sea urchins were spawned by a one to two mL intracoelomic injection of 0.50 M potassium chloride. Gametes from five males and five

females were pooled separately and the fertilization of eggs (diameter: 97.9 µm ± 1.07 µm; Figure 1A) and larval rearing followed Strathmann (1987), with the exception that embryos and larvae were reared using 5.0 µm filtered seawater (FSW) to include the environmental microbiota. Briefly, eggs were fertilized (verified using stereomicroscope) using dilute sperm in 100 mL of FSW at ambient temperature and salinity (Figure S3.1). After fertilization, embryos were transferred to jars with 3 L of FSW (n=4; Table S1) and diluted to a density of eight individuals•$mL^{-1}$.

For larval feeding, monocultures of the cryptophyte *Rhodomonas lens* were grown in f/2 media at room temperature with a combination of ambient and artificial lighting for 24 hours per day (Guillard, 1975).

### 3.2.2 Experimental feeding and tissue collection

At 24 hours post-fertilization, each jar of prism stage larvae was sub-divided into four replicates (n=16), diluted to two individuals•$mL^{-1}$ in 3 L of FSW, and, at random, were provided growth medium-free *R. lens* at either 10,000, 1,000, 100, or 0 cells•$mL^{-1}$ (n=4 per diet; Table S1). For the rest of the experiment, larval cultures had daily water changes of 90-95% volume and *R. lens* was replenished to the experimental density.

Larvae were differentially fed for five days and samples (n=100 larvae) were taken daily for each replicate of the four treatments. Immediately after sampling, larval samples were concentrated into a pellet using a microcentrifuge and the FSW was removed with a sterile glass pipette. Pelleted larvae were then preserved in RNAlater (Thermo Scientific, Massachusetts, USA) and stored at -20°C until extraction of nucleic acids.

### 3.2.3 Larval morphology

Morphological plasticity for larval echinoids has been commonly measured as the relative length of post-oral arms relative to the length of the body (as measured by the 'mid-body line'; (Hart & Strathmann, 1994; Miner, 2004). The developmental timing for morphological plasticity of *L. variegatus* has been previously reported in multiple previous studies (see, Boidron-Metairon, 1988; McAlister, 2007; McEdward & Herrera, 1999), where plasticity in larval morphology is induced following four days of differential feeding. To verify that our culturing resulted in morphological plasticity at this same time point, we sampled twenty larvae (n=20) from a single replicate of each dietary treatment at each time point.

Larvae were imaged using a compound microscope (Leitz Labovert; camera: Olympus DP71) and morphometrics (length of larval body, MBL; post-oral arms, POA; and stomach area; Figure 3.1) were collected using ImageJ (v. 1.9.2; Schneider, Rasband, & Eliceiri, 2012). We then calculated the ratio of POA to MBL (*i.e.*, POA:MBL) as a proxy for the expression of morphological plasticity (see, Miner, 2004). To test whether larval morphology (MBL, POA, and stomach area) differed significantly across time and between diets, we used a two-way analysis of variance (ANOVA, cut off p=0.05, JMP Pro v. 13). When statistical differences were detected, we then performed a Tukey's post-hoc test for pairwise comparisons.

### 3.2.4 Assaying bacterial communities

Total DNA was extracted from larval samples using the GeneJet Genomic DNA Purification Kit (Thermo Scientific), quantified using the NanoDrop 2000 UV-Vis

Spectrophotometer (Thermo Scientific), and diluted to 5 ng•μL$^{-1}$ using RNase/DNase-free water.

Bacterial DNA was amplified using primers for the V3/V4 regions of the 16S rRNA gene (Table S2; Klindworth et al., 2013). Products were purified using the Axygen AxyPrep Mag PCR Clean-up Kit (Axygen Scientific), indexed via PCR using the Nextera XT Index Kit V2 (Illumina Inc.), and then purified again. At each of these three clean up states, fluorometric quantitation was performed using a Qubit (Life Technologies) and libraries were validated using a Bioanalyzer High Sensitivity DNA chip (Agilent Technologies). Illumina MiSeq sequencing (v3, 2x300 bp paired-end reads) was performed at the University of North Carolina at Charlotte. PCR recipe and thermal profiles are available in Table S2.

### 3.2.5 Computational analysis

Raw reads, along with quality information, were imported into QIIME 2 (v. 2019.1; Bolyen et al., 2019), where forward and reverse reads were paired using VSEARCH (Rognes, Flouri, Nichols, Quince, & Mahé, 2016), filtered by quality score, and denoised using Deblur (Amir et al., 2017). QIIME 2-generated 'features' were grouped into operational taxonomic units (OTUs) based on a minimum 99% similarity and were assigned taxonomy using SILVA (v. 132; Quast et al., 2013). Sequences matching to Archaea as well as samples with less than 1,000 reads were discarded and the filtered biom table was rarified to 1,287 sequences (*i.e.*, the read count for the sample with the least remaining reads; Figure S3.2).

To test whether community membership and composition shift over time, in response to food availability, and relative to morphology, we calculated unweighted and weighted UniFrac values (Lozupone & Knight, 2005) and compared them using principal coordinate analyses (PCoA). Results from these analyses were then recreated and stylized using QIIME 1 (v. 1.9.1; Caporaso et al., 2010) and Adobe Illustrator CC. To test for differences in membership and composition over the course of the differential feeding experiment, we used a two-way PERMANOVA and, subsequently, performed pairwise comparisons. To complement UniFrac values, we also calculated several measures of alpha diversity (*i.e.*, total OTUs, phylogenetic distance, McIntosh evenness, and McIntosh dominance) over time and across diets, and compared these values with a two-way analysis of variance (ANOVA) and, subsequently, performed by a Tukey's post-hoc test for pairwise comparisons between diets and times. Lastly, we summarized the bacterial groups associated with *L. variegatus* larvae and determine which differ with diet and time using a two-way ANOVA.

The QIIME2 pipeline used to convert raw reads to OTUs for visualization of this data is presented in detail in Note S1, and the raw sequence reads as part of this dataset are available on the Dryad Digital Repository.

## 3.3 Results

Prior to differential feeding, prism-stage *L. variegatus* larvae associated with a bacterial community that was significantly different in membership and composition from all later larval stages (Figure 3.2; Tables S3-4). As prefeeding larvae, the associated microbiota consisted of fewer OTUs (~135 ± 5) from a phylogenetically restricted

community that was less taxonomically dominant (*i.e.*, more even) than the bacterial community at each subsequent day (Figure 3.3A-B, Figure S3.3).

Following one day of development and differential feeding, *L. variegatus* larvae from each diet developed into two-arm larvae (Table S5). Between these feeding treatments, larvae fed 10,000 and 0 cells•mL$^{-1}$ had significantly lower POA:MBL than those fed 1,000 and 100 cells•mL$^{-1}$ (Figure 3.1B; Tables S6). The membership, composition, phylogenetic diversity, and community evenness of the larval-associated bacterial community was, however, not significantly different across feeding regimes (Figures 3.2-3.4, S3.3; Table S7) while total OTUs and community dominance varied slightly between dietary treatments (Figures 3.2-3.4, S3.3; Table S7).

A second day of differential feeding resulted in more rapid development for larvae in the highest food concentration: larvae fed 10,000 cells•mL$^{-1}$ were four-arm larvae while those fed 1,000, 100, and 0 cells•mL$^{-1}$ were still two-arm larvae (Tables S5). Despite this difference in developmental stage, larvae fed 10,000 and 1,000 cells•mL$^{-1}$ had a similar POA:MBL, which was significantly lower than those fed 100 and 0 cells•mL$^{-1}$ (Figures 3.1; Tables S6). Dietary treatments also resulted in differences in the bacterial communities, where algal concentrations correlated with diet-specific bacterial communities in both membership and composition (Figures 3.2, 3.4; Tables S3-4). These community-level differences resulted in larval-associated microbiota with more OTUs and a higher phylogenetic diversity but similar community evenness and dominance (Figures 3.3A-B, S3.3; Table S7). Of the dietary treatments, larvae fed 100 cells•mL$^{-1}$ were the most diverse while those fed 10,000 and 1,000 cells•mL$^{-1}$ were the least diverse (Figure 3.3A-B, S3.3; Table S7).

Independent of diet quantity, *L. variegatus* larvae from each algal concentration at day three were four-armed and had statistically similar POA:MBL (Figure 3.1; Table S5, S6). Despite being developmentally and morphologically similar, the membership and composition of the associated bacterial communities were significantly different across diets (Figures 3.2, 3.4; Tables S3-4). Specifically, larvae fed 1,000, 100, and 0 cells•mL$^{-1}$ were similar to each other but distinct from those fed 10,000 cells•mL$^{-1}$ (Figure 3.2, 3.4; Tables S3-4). Between days two and three, larvae fed 1,000, 100, and 0 cells•mL$^{-1}$ maintained a bacterial community with a similar number of total OTUs and phylogenetic diversity (Figures 3.3, S3.3; Tables S7-8). Between these same timepoints, larvae fed 10,000 cells•mL$^{-1}$ exhibited a reduction in total OTUs and phylogenetic diversity that resulted in a more even and less taxonomically dominant community (Figures 3.3, S3.3; Table S7).

As expected based on previous literature for *L. variegatus* (Boidron-Metairon, 1988; McAlister, 2007; McEdward & Herrera, 1999), morphological plasticity was observed on the fourth day of differential feeding (Figure 3.1; Tables S5, S6), where larvae fed 1,000, 100, and 0 cells•mL$^{-1}$ had a higher POA:MBL (*i.e.*, post-oral arms relative to the larval body) than those fed 10,000 cells•mL$^{-1}$. Like the day prior, larvae fed 1,000, 100, and 0 cells•mL$^{-1}$ associated with bacterial communities that were similar in membership and composition but statistically different from larvae fed 10,000 cells•mL$^{-1}$ (Figure 3.2, 3.4; Tables S3-4). On the day that morphological plasticity was expressed by the larval host, the number of OTUs and the phylogenetic diversity of that taxa were similar for larvae fed 1,000, 100, and 0 cells•mL$^{-1}$ (Figure 3.3; Table S7). Larvae fed

10,000 cells•mL$^{-1}$, however, continued to associate with a community that was less taxonomically and phylogenetically rich as well as more even and less dominant.

Consistent with the previous day (*i.e.*, day 4 of differential feeding), *L. variegatus* larvae maintained different phenotypes with respect to POA:MBL rations, where larvae fed 1,000, 100, and 0 cells•mL$^{-1}$ were larger than those fed 10,000 cells•mL$^{-1}$ (Figure 3.1; Table S5-6). On fifth day of differential feeding, patterns of community membership and composition (Figures 3.2, 3.4; Tables S3-4) as well as total OTUs, phylogenetic distance, evenness, and dominance all reflected the differences observed in larval morphology (Figure 3.3; Table S7). Specifically, larvae fed 1,000, 100, and 0 cells•mL$^{-1}$ associated with statistically similar numbers of total OTUs and phylogenetic diversity that were significantly more diverse than larvae fed 10,000 cells•mL$^{-1}$ (Figure 3.3; Table S9).

*L. variegatus* larvae associated with bacterial communities that differed significantly between each timepoint (Figure 3.2; Table S9). Over the course of this development period, *L. variegatus* larvae primarily associated with three Bacteroidetes (23.5%) families and eleven Proteobacteria (64.0%) families (Figure S3.4). When morphological plasticity was expressed and reflective patterns were observed in the bacterial communities (*i.e.*, days 3-5 of differential feeding), we observed that the abundance of all 14 bacterial families from Bacteroidetes and Proteobacteria differed significantly with time while 11 of these 14 bacterial families differed across diets (Table S10). Moreover, we observed that the abundance of α- and γ-proteobacteria were inversely proportional (Figure 3.3C, S3.4; Table S11), such that α-proteobacteria increased and γ-proteobacteria decreased following the expression of morphological plasticity.

Across the same three days, larvae fed 10,000 cells•mL$^{-1}$ stably associated with 114 OTUs (43.7%) while 16-40 OTUs (6.1-17.6%) were specific to a single day and 19-34 OTUs (7.3-13.0%) were shared between successive days (Figure 3.3). Larvae fed 1,000, 100, and 0 cells•mL$^{-1}$, on the other hand, stably associated with an average of 190 OTUs (56.6%) while 15-19 OTUs (4.5-5.7%) were specific to a single day and 36-39 OTUs (10.7-11.6%) were shared between successive days (Figure 3.3). Despite the shifts in community membership, the larval stages of *L. variegatus* maintained a 'core' of 31 OTUs from the Bacteroidetes (12.9%), Epsilonbacteraeota (3.2%), and Proteobacteria (83.9%).

## 3.4 Discussion

Morphological plasticity is an adaptive response to heterogenous environments when a fitness advantage results relative to an individual with no response (Miner et al., 2005; Sterns, 1989; West-Eberhard, 2003). Larval sea urchins, in particular, are hypothesized to increase individual fitness in dilute feeding environments by elongating their feeding structure that, in turn, allows for a greater capacity to filter particulates and reduce development time (Boidron-Metairon, 1988; Byrne et al., 2008; Hart & Strathmann, 1994; McAlister & Miner, 2018; Miner, 2004; Soars et al., 2009). Morphological plasticity for larval sea urchins is also coupled with significant shifts in the associated bacterial community (Carrier et al., 2019; Carrier & Reitzel, 2018). The timing of the expression of morphological plasticity and associating with a phenotype-specific bacterial community, however, had previously remained unclear.

Daily profiling of the bacterial communities associated with *L. variegatus* larvae over the course of early development and through the expression of morphological plasticity supports three primary findings. First, based on our sampling points, we observed that a four-stage pattern is followed as larvae transition from a shared to a phenotype-specific bacterial community (Figure 3.4). Second, the relatedness of the larval-associated bacterial community matches morphological plasticity and does so prior to the expression of the phenotype. Third, relatedness of the bacterial communities prior to and following the expression of morphological plasticity implies that the composition and structure of early-stage larvae is maintained by the long-arm larval holobionts while the short-arm morphology deviates from the developmental trajectory.

From the initiation of differential feeding through the expression of morphological plasticity and associating with phenotype-specific bacterial communities, we observed four specific 'Stages' for *L. variegatus* larvae (Figure 3.4). First, 'Stage 1' followed one-day of differential feeding where the bacterial communities across diets were similar in membership and composition. Second, 'Stage 2' followed two-days of differential feeding where the membership and composition of the larval-associated bacterial communities were diet-specific. Third, 'Stage 3' was observed after three-days of differential feeding, where the membership and composition of the larval-associated bacterial communities that were separated between high food availability and all other diet-restricted treatments, although the morphologies remained statistically indistinguishable. Lastly, 'Stage 4' was observed following four- and five-days of differential feeding and was where the relatedness of the bacterial communities correlated with morphological plasticity.

The expression of morphological plasticity by temperate Strongylocentrotids (Carrier & Reitzel, 2018) show similar 'Stages' to the patterns observed with subtropical/tropical *L. variegatus* larvae. Specifically, over four weeks of differential feeding for Strongylocentrotids, it was observed that these larvae exhibit 'Stages 1, 2, and 4' (Carrier & Reitzel, 2018). The temporal pattern of these 'Stages' was, however, unclear due to the long time periods between samples. Timing by *L. variegatus* larvae provides a fine grain temporal organization to compare with Strongylocentrotid larvae as well as implies another stage that was potentially missed. Similarly, differential feeding of a larval sea star (*Acanthaster* sp.) showed both 'Stages 1 and 2' (Carrier et al., 2018), which suggests that these 'Stages' may be broadly conserved in these two distinct classes (Echinoidea and Asteroidea). Consistent observation of these stages across species may imply that these four 'Stages' (Figure 4) are common for echinoderm larvae. Whether these 'Stages' are observed for other taxonomic Classes known to also exhibit morphological plasticity (*e.g.*, Ophiuroidea and Holothuroidea; Podolsky & McAlister, 2005; Sun & Li, 2013) is unknown but merits testing.

Time lags between experiencing and responding to an environmental stimulus are common, potentially advantageous, and hypothesized to be under strong selection for species expressing morphological plasticity (Levins, 1968; Padilla & Adolph, 1996). For plasticity to be advantageous, time lags must be short relative to the time scale of the environmental variant but long enough to minimize false-positive expression (Levins, 1968; Padilla & Adolph, 1996; Palumbi, 1984). When faced with fine grain variability in food abundance, *L. variegatus* larvae are unable to match phenotype with feeding environment, as the lag for morphological responses exceeds two days (Miner & Vonesh,

2004). Despite a multi-day time lag, *L. variegatus* larvae are capable of associating with bacterial communities reflective of morphological plasticity prior to measurable evidence of the morphological trait. This, in principle, implies that instances where a time lag exceeds the morphological response (*e.g.*, Miner & Vonesh, 2004), a holobiont may exhibit shifts in the associated bacterial community corresponding with the environmental variation in absence or preceding a morphological response. The expression of morphological plasticity also comes with the inherent energetic cost of producing and maintaining an alternate phenotype (DeWitt et al., 1998); thus, shifts in the bacterial community may be energetically favored over morphological plasticity in heterogenous environments.

Provided the substantial energetic requirement for planktotrophic echinoids to undergo larval development (Mileikovsky, 1971), laboratory culturing has traditionally simulated conditions equivalent to phytoplankton blooms. This has instilled the general idea that morphological plasticity requires food deprivation and plasticity is the elongation of the feeding apparatus (McAlister & Miner, 2018; McEdward & Herrera, 1999; Miner, 2004). Field culturing, however, suggests the opposite; echinoid larvae are naturally food-limited (Fenaux, Strathmann, & Strathmann, 1994; Olson & Olson, 1989; Pauley, Boring, & Strathmann, 1985) and 'plasticity' is shortening the larval arm and expanding stomach volume in response to uncommon food-rich environments (Miner, 2004). The molecular mechanisms underlying morphological plasticity have suggested that algal chemosensations inhibit growth of the larval arms (Adams et al., 2011) and, thus, the long-arm phenotype would likely be the default under natural conditions.

Following the expression of morphological plasticity, the membership, composition, and structure of the bacterial communities associated with long-arm *L. variegatus* larvae mirrored larval siblings prior to plasticity. Simultaneously, the membership and composition of the bacterial communities associated with short-arm *L. variegatus* larvae differed significantly from both pre-plasticity and long-arm siblings. This community shift followed a reduction in total and short-arm–specific OTUs and phylogenetic diversity of a more even and less dominant community. This implies that well-fed (or short-arm) larvae deviate from the microbial trajectory of *L. variegatus* larvae having yet to express plasticity and that the associated bacterial community may play a role in regulating the short-arm phenotype.

Taken together, the data presented here suggests: (*i*) that phenotype-specific bacterial communities for larval *L. variegatus* follow a four-stage progression (Figure 4); (*ii*) that shift in bacterial taxa precedes morphological plasticity and occur during the time lag; and (*iii*) that the bacterial communities associated with the long-arm phenotype is most similar to pre-plasticity larval siblings and the short-arm phenotype correlates with a restructuring of the bacterial community. Determining if and how this community contributes to larval fitness during before, during, and following the expression of morphological plasticity merits future investigation and would require multi-omic comparisons (Williams & Carrier, 2018) between axenic and germ-rich siblings (*e.g.*, Bates et al., 2006; Manahan, Davis, & Stephens, 1993; Smith, McCoy, & Macpherson, 2007) as well as add-back experiments of individual bacterial taxa (*e.g.*, Fraune et al., 2015; Murillo-Rincon et al., 2017; Wein et al., 2018).

Acknowledgements

We thank Daniel Rittschof (Duke Univ.) for welcoming this project into his laboratory; Beatriz Orihuela (Duke Univ.) for endless logistical assistance; Josh Osterberg (Duke Univ.) for collecting adult urchins; Karen Lopez (UNC Charlotte) for technical assistance with sequencing; Daniel Janies (UNC Charlotte) for sequencing resources; Justin McAlister (College of the Holy Cross) and Jason Hodin (Univ. Washington) for discussions on morphological plasticity in echinoid larvae; and the reviewers to providing critical feedback to an earlier version of this manuscript. This work was supported by an NSF Graduate Research Fellowship to TJC, a Human Frontier Science Program Award to AMR (RGY0079/2016), and a North Carolina Sea Grant award to AMR and TJC (2016-R/MG-1604).

References

Adams, D. K., Sewell, M. A., Angerer, R. C., & Angerer, L. M. (2011). Rapid adaptation to food availability by a dopamine-mediated morphogenetic response. *Nature Communications, 2*, 592.

Agrawal, A. A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science, 294*, 321-326.

Amir, A., McDonald, D., Navas-Molina, J. A., Kopylova, E., Morton, J. T., Xu, Z. Z., . . . Knight, R. (2017). Deblur rapidly resolves single-nucleotide community sequence patterns. *mSystems, 2*, e00191-00116.

Bates, J. M., Mittge, E., Kuhlman, J., Baden, K. N., Cheesman, S. E., & Guillemin, K. (2006). Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. *Developmental Biology, 297*, 374-386.

Bittleston, L. S., Wolock, C. J., Yahya, B. E., Chan, X. Y., Chan, K. G., Pierce, N. E., & Pringle, A. (2018). Convergence between the microcosms of Southeast Asian and North American pitcher plants. *eLife, 7*, e36741.

Boidron-Metairon, I. F. (1988). Morphological plasticity in laboratory-reared echinoplutei of *Dendraster excentricus* (Eschscholtz) and *Lytechinus variegatus* (Lamarck) in response to food conditions. *Journal of Experimental Marine Biology and Ecology, 119*, 31-41.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C., Al-Ghalith, G. A., . . . Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*.

Bordenstein, S. R., & Theis, K. R. (2015). Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biology, 13*, e1002226.

Brooks, A. W., Kohl, K. D., Brucker, R. M., van Opstal, E. J., & Bordenstein, S. R. (2016). Phylosymbiosis: relationships and functional effects of microbial communities across host evolutionary history. *Plos Biology, 14*, e2000225.

Burke, B., Steinberg, P., Rusch, D., Kjelleberg, S., & Thomas, T. (2011). Bacterial community assembly based on functional genes rather than species. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 14288-14293.

Byrne, M., Sewell, M. A., & Prowse, T. A. A. (2008). Nutritional ecology of sea urchin larvae: influence of endogenous and exogenous nutrition on echinopluteal growth and phenotypic plasticity in *Tripneustes gratilla*. *Functional Ecology, 22*, 643-648.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., . . . Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods, 7*(5), 335-336.

Carrier, T. J., Dupont, S., & Reitzel, A. M. (2019). Geographic location and food availability offer differing levels of influence on the bacterial communities associated with larval sea urchins. *Fems Microbiology Ecology, 95*, fiz103.

Carrier, T. J., King, B. L., & Coffman, J. A. (2015). Gene expression changes associated with the developmental plasticity of sea urchin larvae in response to food availability. *Biological Bulletin, 228*, 171-180.

Carrier, T. J., Macrander, J., & Reitzel, A. M. (2018). A microbial perspective on the life-history evolution of marine invertebrate larvae: if, where, and when to feed. *Marine Ecology, 39*, e12490.

Carrier, T. J., & Reitzel, A. M. (2017). The hologenome across environments and the implications of a host-associated microbial repertoire. *Frontiers in Microbiology, 8*, 802.

Carrier, T. J., & Reitzel, A. M. (2018). Convergent shifts in host-associated microbial communities across environmentally elicited phenotypes. *Nature Communications, 9*, 952.

Carrier, T. J., & Reitzel, A. M. (2019). Bacterial community dynamics during embryonic and larval development of three confamilial echinoids. *Marine Ecology Progress Series, 611*, 179-188.

Carrier, T. J., Wolfe, K., Lopez, K., Gall, M., Janies, D. A., Byrne, M., & A.M., R. (2018). Diet-induced shifts in the crown-of-thorns (*Acanthaster* sp.) larval microbiome. *Marine Biology, 165*, 157.

DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution, 13*(2), 77-81.

Fenaux, L., Strathmann, M. F., & Strathmann, R. R. (1994). Five tests of food-limited growth of larvae in coastal waters by comparison of rates of development and form of echinoplutei. *Limnology and Oceanography, 39*, 84-98.

Fraune, S., Anton-Erxleben, F., Augustin, R., Franzenburg, S., Knop, M., Schröder, K., . . . Bosch, T. C. G. (2015). Bacteria-bacteria interactions within the microbiota of

the ancestral metazoan Hydra contribute to fungal resistance. *ISME Journal, 9*, 1543-1556.

Gilbert, S. F., Sapp, J., & Tauber, A. I. (2012). A symbiotic view of life: We have never been individuals. *Quarterly Review of Biology, 87*(4), 325-341.

Guillard, R. R. L. (Ed.) (1975). *Culture of phytoplankton for feeding marine invertebrates*. New York, USA: Plenum Press.

Hart, M. W., & Strathmann, R. R. (1994). Functional consequences of phenotypic plasticity in echinoid larvae. *Biological Bulletin, 186*(3), 291-299.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glockner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research, 41*(1), e1.

Kohl, K. D., Amaya, J., Passement, C. A., Dearing, M. D., & McCue, M. D. (2014). Unique and shared responses of the gut microbiota to prolonged fasting: a comparative study across five classes of vertebrate hosts. *Fems Microbiology Ecology, 90*(3), 883-894.

Kohl, K. D., & Carey, H. V. (2016). A place for host-microbe symbiosis in the comparative physiologist's toolbox. *Journal of Experimental Biology, 219*, 3496-3504.

Levins, R. (1968). *Evolution in Changing Environments*. Princeton, NJ: Princeton University Press.

Louca, S., Jacques, S. M. S., Pires, A. P. F., Leal, J. S., Srivastava, D. S., Parfrey, L. W., . . . Doebeli, M. (2016). High taxonomic variability despite stable functional structure across microbial communities. *Nature Ecology & Evolution, 1*, 15.

Lozupone, C., & Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology, 71*, 8228-8235.

Manahan, D. T., Davis, J. P., & Stephens, G. C. (1993). Bacteria-free sea urchin larvae: selective uptake of neutral amino acids from seawater. *Science, 220*, 204-206.

McAlister, J. S. (2007). *The long arm of the larva: evolutionary responses to resource availability*: University of North Carolina at Chapel Hill.

McAlister, J. S., & Miner, B. G. (2018). Phenotypic plasticity of feeding structures in marine invertebrate larvae. In T. J. Carrier, A. M. Reitzel, & A. Heyland (Eds.), *Evolutionary Ecology of Marine Invertebrate Larvae*. Oxford, UK: Oxford University Press.

McEdward, L. R., & Herrera, J. C. (1999). Body form and skeletal skeletal morphometrics during larval development of the sea urchin Lytechinus variegatus. *Journal of Experimental Marine Biology and Ecology, 232*, 151-176.

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C. G., Carey, H. V., Domazet-Loso, T., Douglas, A. E., . . . Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences of the United States of America, 110*(9), 3229-3236.

Mileikovsky, S. A. (1971). Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evalution. *Marine Biology, 10*, 193-213.

Miner, B. G. (2004). Evolution of feeding structure plasticity in marine invertebrate larvae: a possible trade-off between arm length and stomach size. *Journal of Experimental Marine Biology and Ecology, 315*, 117-125.

Miner, B. G., Sultan, S. E., Morgan, S. G., Padilla, D. K., & Relyea, R. A. (2005). Ecological consequences of phenotypic plasticity. *Trends in Ecology & Evolution, 20*, 685-692.

Miner, B. G., & Vonesh, J. R. (2004). Effects of fine grain environmental variability on morphological plasticity. *Ecology Letters, 7*, 794-801.

Moeller, A. H., Caro-Quintero, A., Mjungu, D., Georgiev, A. V., Lonsdorf, E. V., Muller, M. N., . . . Ochman, H. (2016). Cospeciation of gut microbiota with hominids. *Science, 353*, 380-382.

Murillo-Rincon, A. P., Klimovich, A., Pemöller, E., Taubenheim, J., Mortzfeld, B., Augustin, R., & Bosch, T. C. G. (2017). Spontaneous body contractions are modulated by the microbiome of *Hydra*. *Scientific Reports, 7*, 15937.

Olson, R. R., & Olson, M. H. (1989). Food limitation of planktotrophic marine invertebrate larvae: does it control recruitment success? *Annual Review of Ecology and Systematics, 20*, 225-247.

Padilla, D. K., & Adolph, S. C. (1996). Plastic inducible morphologies are not always adaptive: the importance of time delays in a stocastic environment. *Evolutionary Ecology, 10*, 105-117.

Palumbi, S. R. (1984). Tactics of acclimation: morphological changes of sponges in an unpredictable envrionment. *Science, 225*, 1478-1480.

Parker, B. J., & Brisson, J. A. (2019). A laterally transferred viral gene modifies aphid wing plasticity. *Current Biology, 29*, 2098-2103.

Pauley, G., Boring, L., & Strathmann, R. R. (1985). Food limited growth and development of larvae: experiments with natural sea water. *Journal of Experimental Marine Biology and Ecology, 93*, 1-10.

Podolsky, R. D., & McAlister, J. S. (2005). Developmental plasticity in *Macrophiothrix* brittlestars: are morphologically convergent larvae also convergently plastic? *Biological Bulletin, 209*, 127-138.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., . . . Glockner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research, 41*, 590-596.

Rendleman, A., Rodriguez, J., Ohanian, A., & Pace, D. (2018). More than morphology: differences in food ration drive physiological plasticity in echinoid larvae. *Journal of Experimental Marine Biology and Ecology, 501*, 1-15.

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ, 4*, e2584.

Roth-Schulze, A. J., Pintado, J., Zozaya-Valdés, E., Cremades, J., Ruiz, P., Kjelleberg, S., & Thomas, T. (2018). Functional biogeography and host specificity of bacterial communities associated with the marine green alga *Ulva* spp. *Molecular Ecology, 27*, 1952-1965.

Schneider, C., Rasband, W., & Eliceiri, K. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, 9*, 671-675.

Sewell, M. A. (2005). Utilization of lipids during early development of the sea urchin *Evechinus chloroticus*. *Marine Ecology Progress Series, 304*, 133-142.

Sewell, M. A., Cameron, M. J., & McArdle, B. H. (2004). Developmental plasticity in larval development in the echinometrid sea urchin *Evechinus chloroticus* with varying food rations. *Journal of Experimental Marine Biology and Ecology, 309*, 219-237.

Smith, K., McCoy, K. D., & Macpherson, A. J. (2007). Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Seminars in Immunology, 19*, 59-69.

Soars, N. A., Prowse, T. A. A., & Byrne, M. (2009). Overview of phenotypic plasticity in echinoid larvae, '*Echinopluteus transversus*' type vs. typical echinoplutei. *Marine Ecology Progress Series, 383*, 113-125.

Sterns, S. (1989). The evolutionary significance of phenotypic plasticity. *BioScience, 39*, 436-445.

Strathmann, M. F. (1987). *Reproduction and development of marine invertebrates of the northern Pacific coast: data and methods for the study of eggs, embryos, and larvae*: University of Washington Press.

Strathmann, R. R., Fenaux, L., & Strathmann, M. F. (1992). Heterochronic developmental plasticity in larval sea urchins and its implications for evolution of nonfeeding larvae. *Evolution, 46*, 972-986.

Sun, X.-J., & Li, Q. (2013). The effect of food availability on development and phenotypic plasticity in larvae of the sea cucumber (*Apostichopus japonicus*). *Invertebrate Reproduction and Development, 57*, 255-263.

Wein, T., Dagan, T., Fraune, S., Bosch, T. C. G., Reusch, T. B. H., & Hülter, N. F. (2018). Carrying capacity and colonization dynamics of *Curvibacter* in the *Hydra* host habitat. *Frontiers in Microbiology, 9*, 443.

West-Eberhard, M. (2003). *Developmental Plasticity and Evolution*. Oxford, UK: Oxford University Pres.

Williams, E. A., & Carrier, T. J. (2018). An -omics perspective on marine invertebrate larvae. In T. J. Carrier, A. M. Reitzel, & A. Heyland (Eds.), *Evolutionary Ecology of Marine Invertebrate Larvae* (pp. 288-304). Oxford, UK: Oxford University Press.

Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Reviews, 32*, 723-735.

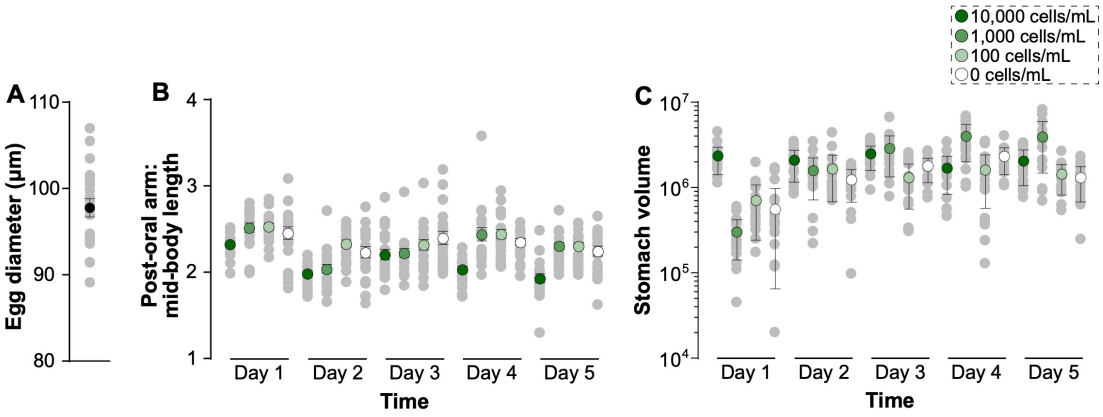



Figure 3.1. Egg size and morphometrics of *Lytechinus variegatus* larvae. (A) Mean egg diameter (± standard error) of unfertilized eggs. (B) Post-oral arm to mid body line ratio and (C) stomach volume (± standard error) of larvae having been fed either 10,000 (dark green), 1,000 (medium green), 100 (light green), and 0 cells•$mL^{-1}$ (white) of the cryptophyte *Rhodomonas lens* over the course of five days.

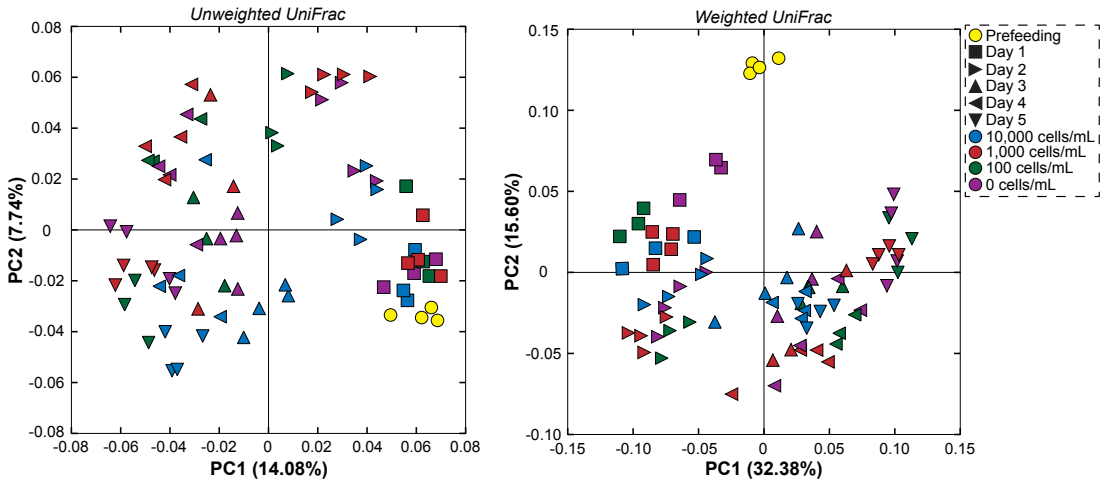



Figure 3.2. Dietary and temporal shifts in the bacterial community associated with *Lytechinus variegatus* larvae. Community similarity of *L. variegatus* larval-associated bacterial communities based on food availability (10,000, 1,000, 100, and 0 cells•mL$^{-1}$ of a phytoplankton represented by blue, red, green and purple, respectively) over a multi-day exposure (prefeeding were yellow-circles while day 1, 2, 3, 4, and 5 were represented by a square, rightward triangle, upward triangle, leftward triangle, and downward triangle, respectively). Comparisons between food availability and over time are based on unweighted (left) and weighted (right) UniFrac values.

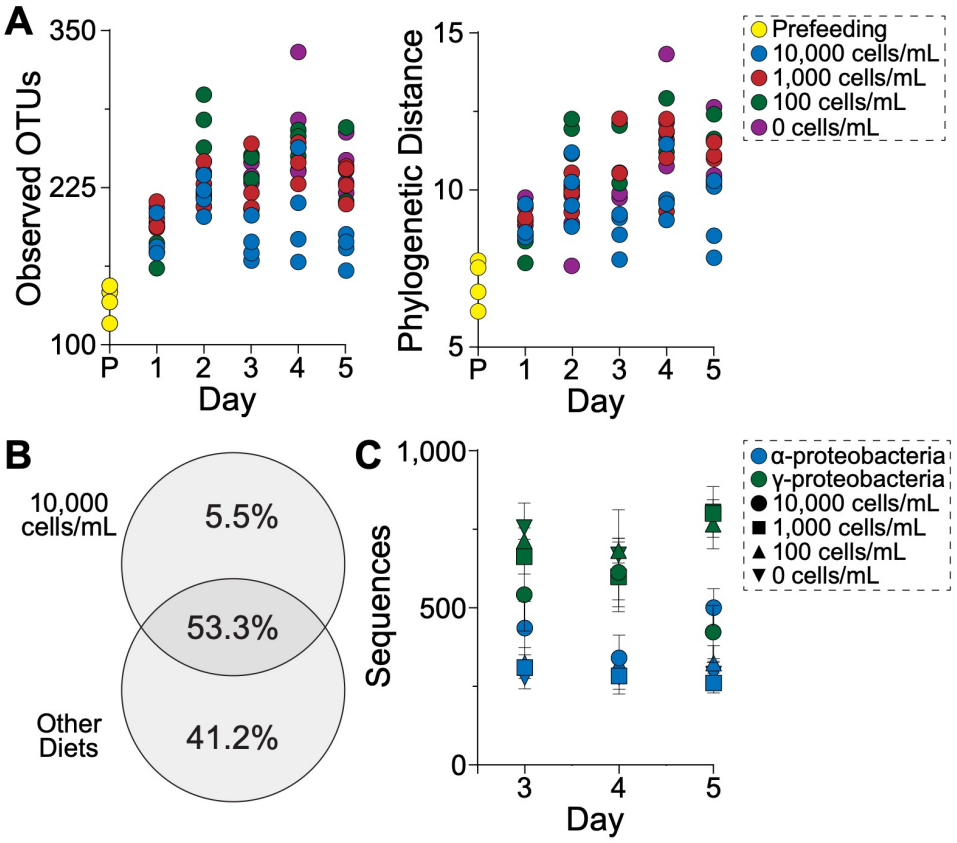



Figure 3.3. Changes to the bacterial community associated with *Lytechinus variegatus* larvae. (A) Enumeration of operational taxonomic units (OTUs) and phylogenetic distance of those OTUs for *L. variegatus* larvae from prefeeding through five days of feeding on either (10,000, 1,000, 100, and 0 cells•mL$^{-1}$ of a phytoplankton represented by blue, red, green and purple, respectively). (B) Percent of total bacterial OTUs for *L. variegatus* larvae from either of the larval phenotypes, and the (C) total sequences (from the rarefied table) of α- and γ-proteobacteria for these time points and diets.

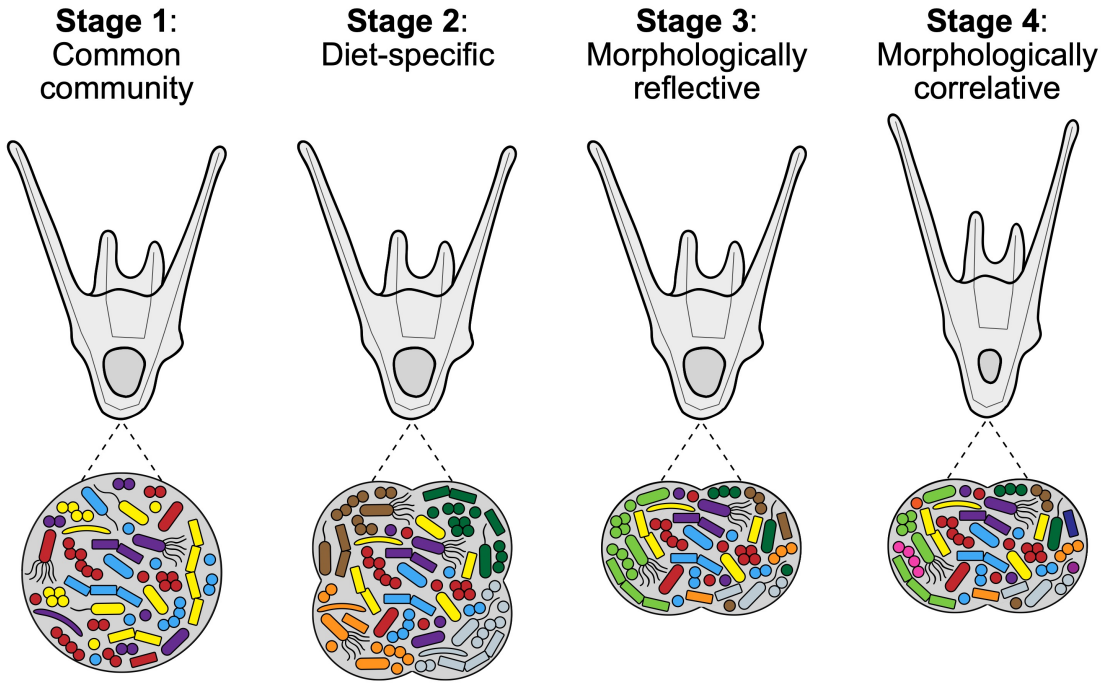



Figure 3.4. Visual model of the larval phenotypes and bacterial community during morphological plasticity. Visual model representation of morphological plasticity for echinoid larvae and the four successive stages towards associations with a phenotype-specific bacterial community. From the initiation of differential feeding through the expression of morphological plasticity and associating with phenotype-specific bacterial communities, we observed four ‘Stages’ (or phenotype-microbial community pairs) for *Lytechinus variegatus*. First, 'Stage 1' (or 'common community') was where the bacterial communities across diets were similar in membership and composition. Second, ‘Stage 2’ (or 'diet-specific') was where the membership and composition of the larval-associated bacterial communities were specific to food availability. Third, ‘Stage 3’ (or 'morphologically reflective') was where the membership and composition of the larval-associated bacterial communities reflected the two larval phenotypes while the host had yet to express these phenotypes. Lastly, ‘Stage 4’ (or 'morphologically correlative') was where the relatedness of the bacterial communities correlated with the expression of morphological plasticity.

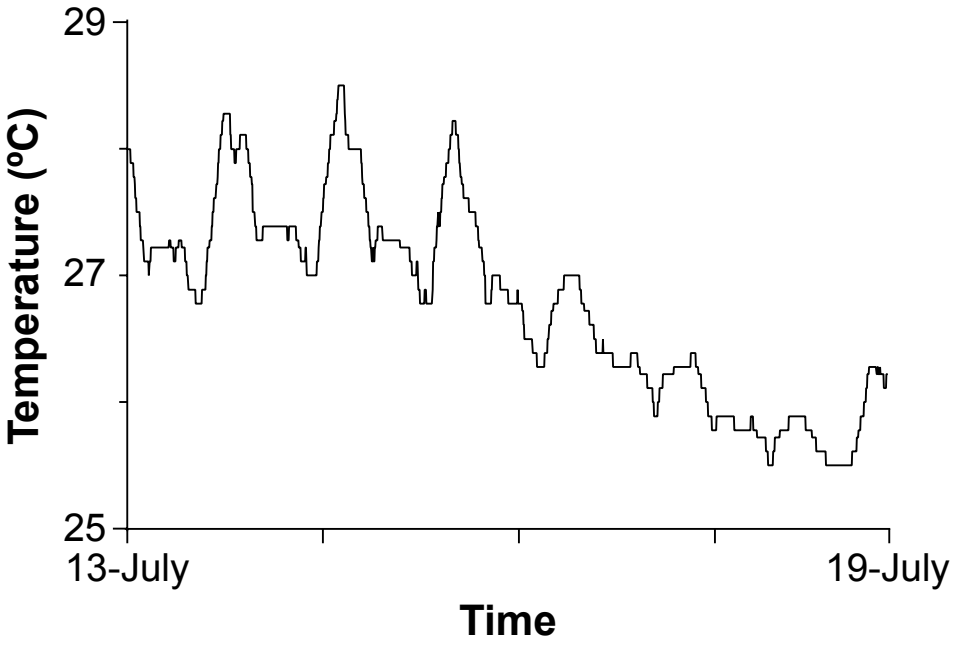



Figure S3.1. *in situ* measurements of sea surface temperature at the Duke University Marine Laboratory. Sea surface temperature at Beaufort, NC (NOAA station, BFTN7) for the entirety of larval experimentation (13-19 July 2017).

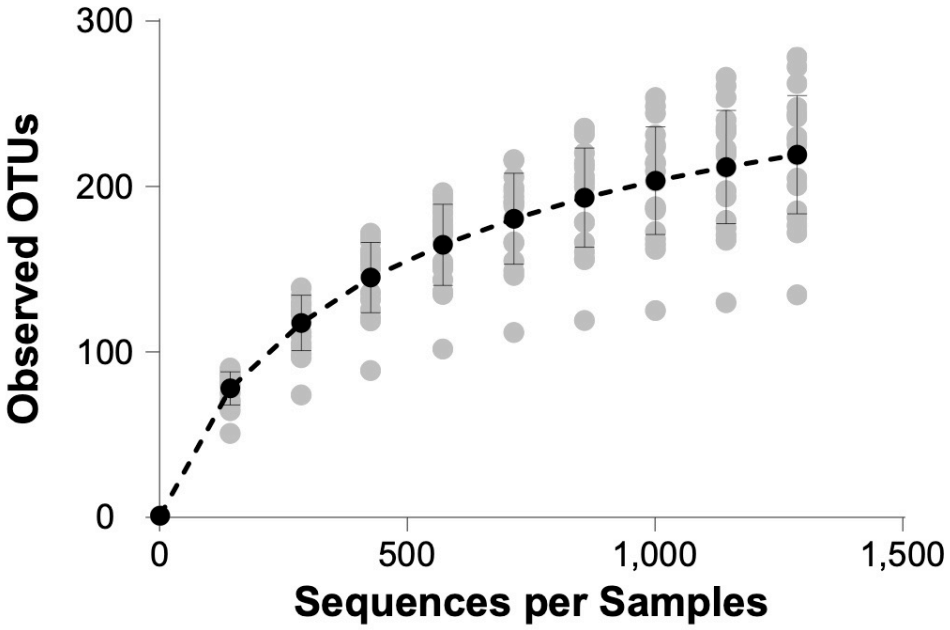



Figure S3.2. Alpha rarefaction curve for *Lytechinus variegatus* larvae. Alpha rarefaction curve for the associated bacteria with for *L. variegatus* from prefeeding through five days of feeding on either 10,000, 1,000, 100, and 0 cells•mL$^{-1}$ of a phytoplankton based on the rarefaction depth (1,287 sequences) used for all analyses.

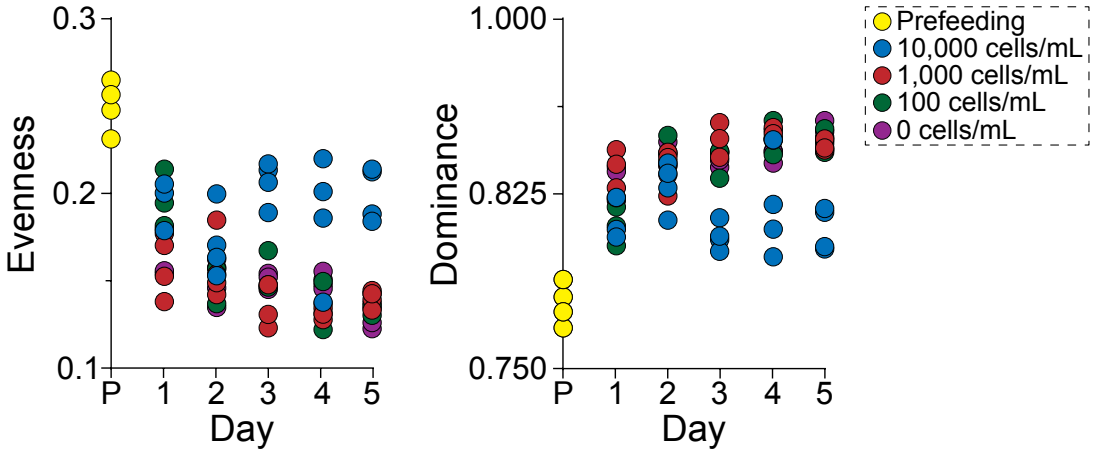



Figure S3.3. Changes in alpha diversity metrics to the bacterial community associated with *Lytechinus variegatus* larvae. Evenness and dominance of bacterial taxa for *L. variegatus* larvae from prefeeding through five days of feeding on either 10,000, 1,000, 100, and 0 cells•mL$^{-1}$ of a phytoplankton (represented by blue, red, green and purple, respectively).

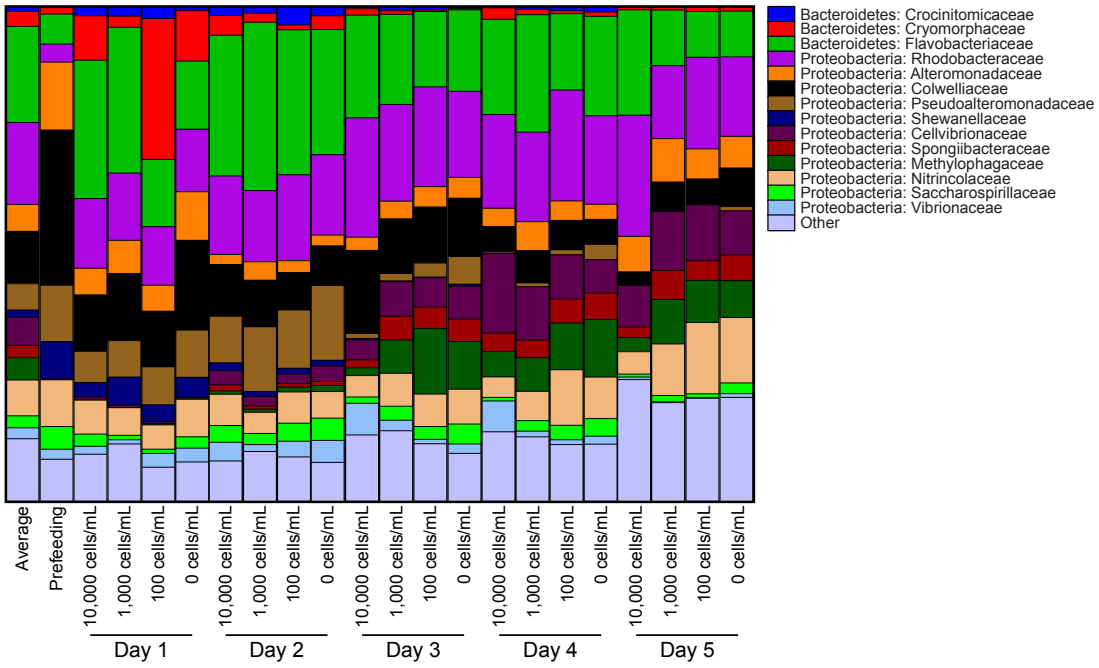



Figure S3.4. Bacterial taxa associated with differentially fed *Lytechinus variegatus* larvae. Bacterial family associated with *L. variegatus* larvae fed either fed either 10,000, 1,000, 100, or 0 cells $mL^{-1}$ over the course of five days of differential feeding.

# CHAPTER 4

# GEOGRAPHIC LOCATION AND FOOD AVAILABILITY OFFER DIFFERING LEVELS OF INFLUENCE ON THE BACTERIAL COMMUNITIES ASSOCIATED WITH LARVAL SEA URCHINS

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## Citation

**Carrier TJ, Dupont, S, Reitzel, AM.** 2019. Geographic location and food availability offer differing levels of influence on the bacterial communities associated with larval sea urchins. FEMS Microbiology Ecology **95:** fiz103.

## Abstract

Determining the factors underlying the assembly, structure, and diversity of symbiont communities remains a focal point of animal-microbiome research. Much of these efforts focus on taxonomic variation of microbiota within or between animal populations, but rarely test the proportional impacts of ecological components that may affect animal-associated microbiota. Using larvae from the sea urchin *Strongylocentrotus droebachiensis* from the Atlantic and Pacific Oceans, we test the hypothesis that, under natural conditions, inter-population differences in the composition of larval-associated bacterial communities are larger than intra-population variation due to a heterogeneous feeding environment. Despite significant differences in bacterial community structure within each *S. droebachiensis* larval population based on food availability, development, phenotype, and time, variation in OTU membership and community composition correlated more strongly with geographic location. Moreover, 20-30% of OTUs associated with larvae were specific to a single location while less than 10% were shared.

Taken together, these results suggest that inter-populational variation in symbiont communities may be more pronounced than intra-populational variation, and that this difference may suggest that broad scale ecological variables (*e.g.*, across ocean basins) may mask smaller scale ecological variables (*e.g.*, food availability).

## 4.1 Introduction

Acclimating to environmental variability through morphological, developmental, and/or physiological plasticity is a common trait of animals (Boidron-Metairon, 1988; Bradshaw, 1965; DeWitt, Sih, & Wilson, 1998; Miner, Sultan, Morgan, Padilla, & Relyea, 2005; Schlichting & Smith, 2002; Sterns, 1989; West-Eberhard, 2003). Over the past decade, the appreciation for the role that animal-associated microbiota play in responding to environmental stressors has grown profoundly (Apprill, 2017; Carrier & Reitzel, 2017, 2018; Kohl & Carey, 2016; Macke, Tasiemski, Massol, Callens, & Decaestecker, 2016; Shapira, 2016; Theis et al., 2016). When experiencing heterogeneous environments, an animal may recruit, expel, and/or shuffle the relative proportion of associated microbiota (Bordenstein & Theis, 2015; Zilber-Rosenberg & Rosenberg, 2008), resulting in a community with particular molecular pathways for the environmental conditions (Burke, Steinberg, Rusch, Kjelleberg, & Thomas, 2011; Louca et al., 2016; Roth-Schulze, Pintado, Zozaya-Valdés, et al., 2018).

Microbial communities associated with animals often vary in response to a multitude of abiotic and biotic factors, including temperature, salinity, diet quality and quantity, season, and habitat-type (see reviews by Carrier & Reitzel, 2017; Kohl & Carey, 2016). Of these, dietary responses are best studied and shown to have a significant impact

on the composition and function of this community (David et al., 2014; K. D. Kohl & Dearing, 2012; Rosenberg & Zilber-Rosenberg, 2016; Sonnenburg et al., 2016). When experiencing a prolonged restriction in food availability, the composition and diversity of microbiota associated with both invertebrate and vertebrate hosts shift considerably (Carrier & Reitzel, 2018; Kohl, Amaya, Passement, Dearing, & McCue, 2014), a response hypothesized to buffer against reduced exogenous nutrients.

Microbial communities associated with animals are also species-specific (*e.g.*, Carrier & Reitzel, 2018; Fraune & Bosch, 2007; Schmitt et al., 2012) and taxonomically variable across the geographical distribution of the host (Dishaw et al., 2014; Huang, Chang, Huang, Gao, & Liao, 2018; Llewellyn et al., 2016; Marino, Pawlik, López-Legentil, & Erwin, 2017; Marzinelli et al., 2015; Mortzfeld et al., 2015). Population-specific microbial communities are partially dependent on environmental conditions (Pantos, Bongaerts, Dennis, Tyson, & Hoegh-Guldberg, 2015) and dispersal limitations (Moeller et al., 2017). Despite this taxonomic variation, microbial communities can remain functionally similar due to shared genes across bacterial taxa (Louca et al., 2018; Roth-Schulze, Pintado, Zozaya-Valdés, et al., 2018). The microbial communities of the bromeliad *Aechmea nudicaulis*, for example, are taxonomically dynamic but, as a community, are functionally consistent between individuals (Louca et al., 2016). Similarly, the bacterial community associated with the green alga *Ulva* spp. are too spatially and temporally variable to define a ‘core’ community; however, nearly 70% of the microbial genes are conserved across the broad geographic distribution of *Ulva* spp. (Roth-Schulze, Pintado, Zozaya-Valdés, et al., 2018).

Efforts to classify the principles underlying the assembly of host-associated microbial communities have primarily been within (*e.g.*, Carrier & Reitzel, 2018; Carrier et al., 2018; David et al., 2014; Lokmer & Wegner, 2015; Webster, Botte, Soo, & Whalan, 2011; Webster, Soo, Cobb, & Negri, 2011) or between (*e.g.*, Dishaw et al., 2014; Huang et al., 2018; Llewellyn et al., 2016; Marino et al., 2017; Marzinelli et al., 2015; Mortzfeld et al., 2015; Yatsunenko et al., 2012) populations (but see Webster, Consuegra, Hitchings, & de Leaniz, 2018). Despite the known effects of host and environmental factors on symbiont community composition, the relevance of each factor on community assembly and structure is lesser known. The fields of sensory, behavioral, and circadian biology have shown that organismal responses to ecological variables are unequal and may be masked based on their spatiotemporal scale (Beauchard, Veríssimo, Queirós, & Herman, 2017; Hodin, Ferner, Heyland, & Gaylord, 2017; Kerr & Kemp, In Press; Li et al., 2018; Yang et al., 2018). Factors within and between populations in distinct geographic locations, therefore, may have differential impacts on the composition of host-associated microbiota.

Planktotrophic (feeding) larvae are one biological system to compare ecological components and how these factors influence the composition of animal-associated microbiota. Many planktotrophic larvae (*e.g.*, the pluteus of sea urchins) inhabit heterogeneous nutritional environments and are morphologically and physiologically plastic in response to food availability (Adams, Sewell, Angerer, & Angerer, 2011; Boidron-Metairon, 1988; Byrne, Sewell, & Prowse, 2008; Carrier, King, & Coffman, 2015; Hart & Strathmann, 1994; McAlister & Miner, 2018; B.G. Miner, 2004; B. G. Miner, 2011; Soars, Prowse, & Byrne, 2009). Larvae of the echinoid *Strongylocentrotus*

*droebachiensis*, for example, associate with phenotype-, diet-, and developmental stage-specific bacterial communities (Carrier & Reitzel, 2018; Carrier & Reitzel, 2019). *S. droebachiensis* has an Arctic-boreal distribution composed of multiple ocean basins with limited gene flow (Addison & Hart, 2004, 2005; Scheibling & Hatcher, 2013) and evidence for potential local adaptation (Manier & Palumbi, 2008; Marks, Biermann, Eanes, & Kryvi, 2008).

Using the circumpolar distribution of *S. droebachiensis* and the ability of their larvae to acclimate to heterogeneous feeding environments, we test the hypothesis that, under natural conditions, inter-population differences in the composition of animal-associated bacterial communities are more pronounced than intra-population variation. To test this, we performed differential feeding experiments on *S. droebachiensis* larvae from three locations—one in the Pacific and two in the Atlantic (Fig. 4.1)—and used amplicon sequencing to profile the larval-associated bacterial communities.

## 4.2 Materials and Methods

### 4.2.1 Adult urchin collection and larval rearing

Adult *S. droebachiensis* were collected from populations in the North Sea in March 2015, the Salish Sea in April 2016, and the Gulf of Maine in February 2017 (Fig. 4.1). Individuals from the North Sea were collected by divers in Drøbak, Norway (59°39’ N, 10°37’ E) and transported in cold, aerated seawater to the Sven Lovén Centre for Marine Infrastructure (Kristineberg, Sweden). Urchins were maintained in raw seawater and, to sustain a natural diet, were fed *ad libitum* on a live mix of *Ulva lactuca* and *Laminaria* spp. collected from the Kristineberg shoreline. Urchins from the Salish Sea

were hand-collected at low tide at Cattle Point, San Juan Island, USA (48°27' N, 122°57' W), transferred to the Friday Harbor Laboratories (FHL; Friday Harbor, WA, USA) within one hour, suspended in sub-tidal cages off the dock at FHL, and fed *Nereocystis* spp. *ad libitum*. Lastly, individuals from the Gulf of Maine were collected by divers in Frenchman Bay, Maine (44°25' N 68°12' W), shipped overnight to the Darling Marine Center (Walpole, ME, USA), and were maintained in flow-through aquaria and fed *Saccharina latissima ad libitum*.

Within two weeks of collections, adult urchins were spawned by a one to two mL intracoelomic injection of 0.50 M potassium chloride. For each population, gametes from three males and females were pooled separately. For experiments at each location, we used ambient, 5.0-µm filtered seawater (FSW) (see, Fig. S4.1) to avoid artificial stress on the host and microbiota that may occur if cultured under identical conditions (*e.g.*, temperature and salinity). Specifically, the ambient conditions during these larval culturing experiments differed between locations, with the North Sea, Gulf of Maine, and Salish Sea being the coolest (3.8 ± 0.9 °C), intermediate (7.9 ± 0.5 °C), and warmest (10.5 ± 0.2 °C), respectively, and, based on available buoy data, the Gulf of Maine and North Sea were less (29.7 ± 0.2 PSU) and more saline (32.5 ± 0.5 PSU), respectively (see, Fig. S4.1).

The fertilization of eggs and larval rearing followed Strathmann (1987), whereby eggs were fertilized (as verified using stereomicroscope) in 100 mL of FSW under ambient conditions. Seawater was decanted two hours post-fertilization to remove excess sperm, and embryos were then transferred to four 3 L jars and diluted to a density of six

individuals•mL$^{-1}$. Larval cultures were subsequently diluted to one larva per two mL at advanced developmental stages and were given 90-95% water changes every other day.

Beginning at the prism stage, larvae were provided monocultures of *Rhodomonas lens*, which was replenished during each water change and was set to the respective feeding concentrations. *R. lens* was cultured at room temperature in f/2 media with a combination of ambient and artificial lighting for 24 hours per day (Guillard, 1975). The f/2 growth media used for algal growth was removed prior to introduction to larval cultures by centrifugation of algae into pellets followed by resuspension in FSW.

### 4.2.2 Experimental feeding and larval morphometrics

At 48 hours post-fertilization, prism-stage larvae for each population were divided into twelve replicate jars (with a larval density of two individuals•mL$^{-1}$) that were sub-divided into four experimental feeding treatments: 10,000, 1,000, 100, or 0 cells•mL$^{-1}$ of *R. lens*. For each of the four-week experiments, larvae fed 10,000 cells•mL$^{-1}$ were reared through metamorphosis while diet-restricted (1,000 and 100 cells•mL$^{-1}$) and unfed larvae were cultured until developmental stasis (Table S1). Larvae (n=100) from each replicate for each treatment were sampled weekly. Immediately after sampling, larval samples were concentrated into a pellet using a microcentrifuge, the FSW was removed with a sterile glass pipette, and pelleted larvae were then preserved in RNAlater (Thermo Scientific, Massachusetts, USA) and stored at -20 °C before DNA extractions.

Complementary to sampling *S. droebachiensis* larvae, the environmental microbiota from the seawater was also sampled. When larval cultures were sampled, triplicate ~1-L of 5.0 μm FSW was filtered onto a 0.22-μm Millipore filter to retain the

environmental microbiota. Full filter disks were then preserved in RNAlater and stored at -20 °C before DNA extractions.

In addition to sampling larvae to assay the associated bacterial communities, twenty larvae (n=20) from a single replicate of each dietary treatment were sampled for morphometric analysis. Larvae were imaged using a compound microscope (Salish Sea: Nikon Eclipse E600; camera: QImaging MicroPublisher 5.0 RTV; Gulf of Maine: Zeiss Primo Star HD digital microscope; North Sea: Leica stereomicroscope) and morphometrics (length of larval body, post-oral arms, and stomach area; Figs. 4.2, S4.2-4.3) were performed using ImageJ (v. 1.9.2; Schneider, Rasband, & Eliceiri, 2012). Statistical differences (where $p<0.05$) in larval morphology and stomach volume were compared using two separate tests. First, a one-way analysis of variance (ANOVA; JMP Pro v. 13) was used to test whether larval morphology differed between populations, which was followed by a Tukey's post-hoc test for pairwise comparisons between populations. Second, a two-way ANOVA was used to test for differences over time and across feeding conditions, and a linear contrast analysis was used to compare variation for each diet and time point.

### 4.2.3 Assaying microbial communities

Total DNA was extracted from larval samples using the GeneJet Genomic DNA Purification Kit (Thermo Scientific, Massachusetts, USA). For FSW samples, eDNA was extracted using the FastDNA Spin Kit for Soil (MP Biomedical, Illkirch, France). DNA was then quantified using the NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, Massachusetts, USA) and diluted to 5 $ng \bullet \mu L^{-1}$ using RNase/DNase-free water.

Bacterial sequences were amplified using universal primers for the V3/V4 regions of the 16S rRNA gene (Forward: 5′ CCTACGGGNGGCWGCAG, Reverse: 5′ GACTACHVGGGTATCTAATCC; Klindworth et al., 2013). The ~450 bp product were purified using the Axygen AxyPrep Mag PCR Clean-up Kit (Axygen Scientific, New York, USA), indexed via PCR using the Nextera XT Index Kit V2 (Illumina, California, USA), and then purified again. At each of these three steps, fluorometric quantitation was performed using a Qubit (Life Technologies, California, USA) and libraries were validated using a Bioanalyzer High Sensitivity DNA chip (Agilent Technologies, California, USA). Illumina MiSeq sequencing (v3, 2x300 bp paired-end reads) was performed at the University of North Carolina at Charlotte. See, Table S2 for PCR recipe and thermal profiles.

Forward and reverse sequences (that are freely available on the Dryad Digital Repository) were paired and trimmed using PEAR (Zhang, Kobert, Flouri, & Stamatakis, 2014) and Trimmomatic (Bolger, Lohse, & Usadel, 2014), respectively, and converted from fastq to fasta (see, Note S1). Chimeric sequences were then detected using USEARCH (Edgar, Haas, Clemente, Quince, & Knight, 2011) and removed using filter_fasta.py prior to analysis of bacterial 16S rRNA sequences. Using QIIME 1.9.1 (Caporaso et al., 2010) and Greengenes (v. 13.5), bacterial 16S rRNA sequences were analyzed and grouped into operational taxonomic units (OTUs) based on a minimum 97% similarity. The biom table generated by pick_open_reference_otus.py was filtered of OTUs with ten or less sequences as well as sequences matching the cryptophytes (*i.e.*, *R. lens*; as per Carrier & Reitzel, 2018).

Using the filtered biom table and "biom summarize-table" to count total sequences per sample, the rarefaction depth of 3,193 reads was determined and applied to all subsequent analyses (Fig. S4.4). Alpha diversity (*i.e.*, Fisher's alpha, Shannon equitability, Faith's phylogenetic distance, and observed OTUs) was calculated using alpha_diversity.py and compared statistically using one-way ANOVAs in JMP. Beta diversity was calculated using the unweighted and weighted UniFrac (Lozupone & Knight, 2005) as part of jackknifed_beta_diversity.py. These values were compared using principal coordinate analyses (PCoA), recreated using make_2d_plots.py, and stylized for presentation in Adobe Illustrator CS6. Community similarity across phenotypes, dietary states, developmental stages, and biogeography were compared statistically using an analysis of similarity (ANOSIM), permutational multivariate analysis of variance using distance matrices (ADONIS), and permutational multivariate analysis of variance (PERMANOVA) using compare_categories.py. Shared OTUs between urchin populations were determined using compute_core_microbiome.py and shared_phylotypes.py. Average bacterial community for each urchin population as well as across populations and the shared community was generated using summarize_taxa_through_plots.py, visualized with Prism 7 (GraphPad Software), and stylized for presentation in Adobe Illustrator CS6.

A step-by-step listing of QIIME scripts used to convert raw reads to OTUs for visualization of these data is located in Note S1.

## 4.3 Results

### 4.3.1 Morphological plasticity of larval sea urchin

Diet-induced morphological plasticity was observed for *S. droebachiensis* larvae from each location (Figs. S4.2-4.3; Tables S3-4), where the extent and direction of expression was location-specific (ANOVA, $p<0.0001$; Fig. 4.2). Diet-restricted larvae from the Salish Sea and Gulf of Maine, in general, exhibited a higher post-oral arm to mid-body line ratio than *ad libitum* counterparts (ANOVA, $p<0.0001$; Figs. S4.2-4.3; Tables S3-4), even though analyses of Gulf of Maine larvae were partially confounded by developmental stage (Table S1). Larvae from the North Sea, however, exhibited the opposite response: *ad libitum* feeding induced a higher post-oral arm to mid-body line ratio than diet-restricted individuals (ANOVA, $p<0.0001$; Fig. S4.2-4.3; Tables S3-4).

### 4.3.2 Comparisons of bacterial communities within populations

*S. droebachiensis* larvae cultured at each location associated with a diet-specific bacterial community (Figs. 4.3A-C, S4.5-4.8; Table S6). Larvae from the Salish Sea and Gulf of Maine exhibited similar diet-specific community-level patterns in both alpha- and beta-diversity (Figs. 4.3A-B, S4.5-4.8; Table S6), where the bacterial community associated with food restricted larvae were more similar to each other than to well-fed counterparts. Larvae from the North Sea, on the other hand, exhibited the opposite response (Figs. 4.3C, S4.5, S4.8; Table S6), where diet-specific bacterial communities were observed (Table S6) except that all food concentrations were more similar to each other than to starved larvae (Fig. S4.8; Table S6).

The bacterial communities associated with larvae from each sea urchin population also varied temporally in both alpha- and beta-diversity (Figs. 4.3D-F, S4.5, S4.9-4.11; Table S6). Temporal changes in community diversity (*i.e.*, alpha-diversity) were reflective

of culturing environment: bacteria associated with larvae from the Atlantic Ocean increased in weeks one through three and declined in week four while those from the Pacific Ocean decreased in the first three weeks and increased in week four (Table S5). Compositional changes (*i.e.*, beta-diversity) in the associated bacterial communities for each larval population, on the other hand, experienced similar time-dependent shifts from week one though four (Figs. 4.3D-F, S4.5).

Complementary to dietary and temporal shifts in community structure and composition, larval-associated bacterial communities for *S. droebachiensis* varied significantly with larval morphology (Fig. S4.12; Table S7) and developmental stage (Figs. S4.9-4.11; Table S7). Variation in the larval-associated bacterial communities across food availability, time, morphology, and development, however, appeared to not co-depend on the bacterial taxa observed in the seawater, as the composition of larval-associated and environmental microbiota were significantly different (Fig. S4.13; Table S7).

### 4.3.3 Population-specific bacterial communities

Despite differences in bacterial community structure for *S. droebachiensis* larvae from each population, variation in OTU membership and composition correlated best with location (ANOSIM, unweighted UniFrac, $p<0.001$; ANOSIM, weighted UniFrac, $p<0.001$; Figs. 4.4A-B, S4.14-4.16; Table S8). Community relatedness of larval-associated microbiota in the Western and Eastern Atlantic Ocean was more similar to each other than to the Pacific Ocean population (Fig. 4.4 A-B). Moreover, bacterial community diversity and structure were significantly different between these three populations

(Fisher's alpha: F-ratio = 142.2, $p<0.001$; Shannon equitability: F-ratio = 110.1, $p<0.001$; Faith's phylogenetic distance: F-ratio = 159.2, $p<0.001$; observed OTUs: F-ratio = 124.2, $p<0.001$; Fig. 4.4C; Table S8). For each of the tested indices, bacterial communities of Gulf of Maine larvae were taxonomically richest and most diverse while Salish Sea larvae were the least rich and diverse, leaving North Sea larvae as intermediate (Tukey's test, $p<0.05$ for each; Fig. 4.4C; Table S8).

*S. droebachiensis* larvae from these locations primarily associated with seven bacterial classes from three phyla: Saprospirae and Flavobacteriia (Bacteroidetes), Alpha-, Beta-, Delta-, and Gammaproteobacteria (Proteobacteria), and Verrucomicrobiae (Verrucomicrobia) (Fig. 4.5A). Of these classes, Flavobacteriia, Alpha- and Gammaproteobacteria represented at least 10% of the relative community for each population, where Gammaproteobacteria was the most abundant clade at 20-50% (Fig. 4.5A). Of the hundreds of OTUs associated with *S. droebachiensis* larvae (Fig. S4.4), less than 10% were shared across populations, with 20-30% being specific to a single population and 3-11% being shared between two populations (Fig. 4.5B). This shared community was similar to the full communities for each population, in that it was composed primarily by Flavobacteriia (24.7%), Alpha- (28.1%) and Gammaproteobacteria (34.9%) (Fig. 4.5A).

## 4.4 Discussion

Principles underlying the assembly and diversity of host-associated microbial communities remains a focal point of host-microbiome research (*e.g.*, Bordenstein & Theis, 2015; McFall-Ngai et al., 2013; Zilber-Rosenberg & Rosenberg, 2008). These

previous studies show that evolutionary history (Brooks, Kohl, Brucker, van Opstal, & Bordenstein, 2016), physiology (*e.g.,* Buckley & Rast, 2017; Kohl et al., 2016; Kohl & Carey, 2016), and environmental conditions (*e.g.*, Carrier & Reitzel, 2017) are primary drivers for compositional differences. Much of this work, however, focuses either within or between host populations, with few studies comparing the intra- and inter-population processes underlying microbiome assembly.

Comparing the bacterial communities associated with *S. droebachiensis* larvae across feeding environments and geographical locations supports two primary findings. First, larvae from each of these three populations associate with bacterial communities that correlate with food availability, time, development, and phenotype. Second, despite variation in the microbiota within each population of urchin larvae, the composition of *S. droebachiensis* larval-associated bacterial communities best correlated with the location of the host population when cultured under natural conditions.

Larval marine invertebrates associate with microbial communities that are diverse and dynamic yet specific to host species and distinct from the environmental microbiota (Carrier, Macrander, & Reitzel, 2018; Carrier & Reitzel, 2018; Carrier & Reitzel, 2019; Carrier et al., 2018; Webster, Soo, et al., 2011). The bacterial community associated with larval echinoderms, in particular, shifts with food availability (Carrier & Reitzel, 2018; Carrier et al., 2018), embryonic and larval development (Carrier & Reitzel, 2018; Carrier & Reitzel, 2019), phenotype (Carrier & Reitzel, 2018), disease (Carrier et al., 2018), and physiology (Buckley & Rast, 2017). Common approaches for investigating larval-microbe interactions involve studies at single locations for species with broad geographic distributions (see Carrier & Reitzel, 2018; Carrier & Reitzel, 2019; Carrier et al., 2018;

Webster, Soo, et al., 2011). The data presented here suggest that community-level shifts in response to biotic (development and phenotype) and abiotic (food availability and time) factors occur at multiple locations across a population with limited gene flow. Three widely-distributed sea urchin species (including *S. droebachiensis*) have previously been shown to associate with food-elicited phenotype-specific bacterial communities when cultured under identical conditions (Carrier & Reitzel, 2018). It may, therefore, be hypothesized that phenotype-specific microbiota for these and, potentially, other species of larval echinoderms (*e.g.*, *Acanthaster* sp. in the Pacific Ocean, including on the Great Barrier Reef; Carrier et al., 2018; K Wolfe, Graba-Landry, Dworjanyn, & Byrne, 2015; Wolfe, Graba-Landry, Dworjanyn, & Byrne, 2015; Wolfe, Graba-Landry, Dworjanyn, & Byrne, 2017) occur throughout the geographical distribution of the species.

Marine invertebrate larvae are not unique in their ability to respond to environmental variability by exhibiting shifts in the associated bacterial community (Carrier & Reitzel, 2017; Kohl & Carey, 2016). Several groups of marine and terrestrial vertebrates (*e.g.*, mammals, reptiles, and fish) respond to diet variability and abiotic stress by restructuring their gut microbiota that, in turn, are likely to differ in community function (*e.g.*, Kohl et al., 2016; Kohl et al., 2018; Muegge et al., 2011). Similar to marine invertebrate larvae, marine and terrestrial vertebrates can be widely-distributed and, thus, genetically distinct populations may exhibit similar community-levels shifts in microbiota at multiple locations across these populations. To our knowledge, however, parallel responses between populations for vertebrates and other taxa have not yet been documented.

Inter-location variation in the composition of *S. droebachiensis*-associated bacterial communities was more pronounced than intra-location differences. While the mechanisms driving this result are unknown, genetic and ecological differences between *S. droebachiensis* populations are likely contributing factors (Moeller et al., 2017; Pantos et al., 2015). *S. droebachiensis* from the Salish Sea, Gulf of Maine, and North Sea have some degree to genetic isolation (Addison & Hart, 2004, 2005; Norderhaug et al., 2016). Each urchin population also inhabits environments that differ in physical (Otto et al., 1990; Pettigrew et al., 2005; Sutherland & MacCready, 2011) and biological (McQuatters-Gollop et al., 2007; Thomas, Townsend, & Weatherbee, 2003; Winter, Banse, & Anderson, 1975) factors.

The ambient conditions during these larval culturing experiments differed between locations, with the North Sea, Gulf of Maine, and Salish Sea being the coolest (3.8 ± 0.9 °C), intermediate (7.9 ± 0.5 °C), and warmest (10.5 ± 0.2 °C), respectively, and, based on available buoy data, the Gulf of Maine and North Sea were less (29.7 ± 0.2 PSU) and more saline (32.5 ± 0.5 PSU), respectively. Moreover, during the pelagic period, echinoid larvae from the Gulf of Maine experience a pronounced seasonal phytoplankton bloom (Starr, Himmelman, & Therriault, 1990), those from the Salish Sea encounter seasonal upwelling events (Sutherland & MacCready, 2011), and North Sea counterparts may deal with seasonal oligotrophication (Otto et al., 1990). Inter-location variation in the bacterial communities associated with *S. droebachiensis* larvae may, therefore, have been due to differences in the oceanographic environment.

Population-specific bacterial communities for *S. droebachiensis* larvae are consistent with the hypothesis that host-associated microbial communities are

taxonomically variable across the geographic distribution of the host (Dishaw et al., 2014; Huang et al., 2018; Marino et al., 2017; Marzinelli et al., 2015; Mortzfeld et al., 2015). Studies using diverse animal taxa (*e.g.*, sponges, corals, fish, and humans) have shown that the environment, not genotype, principally drives community differences (Burgsdorf et al., 2014; Pantos, Bongaerts, Dennis, Tyson, & Hoegh-Guldberg, 2015; Rothschild et al., 2018; Sullam et al., 2012). Genetic comparison of *S. droebachiensis* indicates that individuals from the Salish Sea and Gulf of Maine sub-populations are more related to each other than to the North Sea sub-population (Addison & Hart, 2004, 2005; Scheibling & Hatcher, 2013). Microbial communities from these populations, however, suggest the contrary: larvae from the Gulf of Maine and North Sea are more similar to each other than to Salish Sea larvae. How much of this difference is explained by the abiotic differences (*i.e.*, temperature), the time of year of the experiments, the local microbiota, or host genetics remains unclear. This result reinforces the hypothesis that environment may be a larger influence on community assembly than genetic similarity.

By comparing host-associated bacterial communities across feeding environments and geographic locations in larval sea urchins, we provide initial evidence that inter-location variation in symbiont communities can be more pronounced than intra-location variation. This difference may suggest that larger scale ecological variables that correspond to different geographic locations may mask more local scale ecological variables (*e.g.*, feeding, time, development, and phenotype). Explicit testing of this is merited and would benefit from common culturing of geographically distant population with restricted gene flow and functional characterization from animal taxa with diverse evolutionary histories.

Acknowledgements

We thank the staffs of the Friday Harbor Laboratories, Darling Marine Center, and Sven Lovén Centre for Marine Infrastructure for facility access and logistical assistance; Jason Hodin and Billie Swalla for providing laboratory space; Richard Strathmann for collection assistance; Daniel Janies for sequencing resources; Karen Lopez for technical assistance with sequencing; and Jason Macrander for bioinformatics assistance.

T.J.C. was supported by an NSF Graduate Research Fellowship, Charles Lambert Memorial Endowment fellowship from the Friday Harbor Laboratories, and a Sigma Xi Grants-in-Aid of Research grant; S.D. was funded by the Centre for Marine Evolutionary Biology and supported by a Linnaeus grant from the Swedish Research Councils VR and Formas; and A.M.R. was supported by Human Frontier Science Program Award RGY0079/2016.

References

Adams, D. K., Sewell, M. A., Angerer, R. C., & Angerer, L. M. (2011). Rapid adaptation to food availability by a dopamine-mediated morphogenetic response. *Nature Communications, 2*, 592.

Addison, J. A., & Hart, M. W. (2004). Analysis of population genetic structure of the green sea urchin (*Strongylocentrotus droebachiensis*) using microsatellites. *Marine Biology, 144*, 243-251.

Addison, J. A., & Hart, M. W. (2005). Colonization, dispersal, and hybridization influence phylogeography of North Atlantic sea urchins (*Strongylocentrotus droebachiensis*). *Evolution, 59*, 532-543.

Apprill, A. (2017). Marine animal microbiomes: toward understanding host-microbiome interactions in a changing ocean. *Frontiers in Marine Science, 4*, 222.

Beauchard, O., Veríssimo, H., Queirós, A. M., & Herman, P. M. J. (2017). The use of multiple biological traits in marine community ecology and its potential in ecological indicator development. *Ecological Indicators, 76*, 81-96.

Boidron-Metairon, I. F. (1988). Morphological plasticity in laboratory-reared echinoplutei of *Dendraster excentricus* (Eschscholtz) and *Lytechinus variegatus* (Lamarck) in response to food conditions. *Journal of Experimental Marine Biology and Ecology, 119*, 31-41.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics, 30*(15), 2114-2120.

Bordenstein, S. R., & Theis, K. R. (2015). Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biology, 13*, e1002226.

Bradshaw, A. (1965). Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics, 13*, 115-155.

Brooks, A. W., Kohl, K. D., Brucker, R. M., van Opstal, E. J., & Bordenstein, S. R. (2016). Phylosymbiosis: relationships and functional effects of microbial communities across host evolutionary history. *Plos Biology, 14*, e2000225.

Buckley, K. M., & Rast, J. P. (2017). An organismal model for gene expression networks in the gut-associated immune response. *Frontiers in Immunology, 8*, 1297.

Burgsdorf, I., Erwin, P. M., López-Legentil, S., Cerrano, C., Haber, M., Frenk, S., & Steindler, L. (2014). Biogeography rather than association with cyanobacteria structures symbiotic microbial communities in the marine sponge *Petrosia ficiformis*. *Frontiers in Microbiology, 5*, 529.

Burke, B., Steinberg, P., Rusch, D., Kjelleberg, S., & Thomas, T. (2011). Bacterial community assembly based on functional genes rather than species. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 14288-14293.

Byrne, M., Sewell, M. A., & Prowse, T. A. A. (2008). Nutritional ecology of sea urchin larvae: influence of endogenous and exogenous nutrition on echinopluteal growth and phenotypic plasticity in *Tripneustes gratilla*. *Functional Ecology, 22*, 643-648.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., . . . Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods, 7*(5), 335-336.

Carrier, T. J., King, B. L., & Coffman, J. A. (2015). Gene expression changes associated with the developmental plasticity of sea urchin larvae in response to food availability. *Biological Bulletin, 228*, 171-180.

Carrier, T. J., Macrander, J., & Reitzel, A. M. (2018). A microbial perspective on the life-history evolution of marine invertebrate larvae: if, where, and when to feed. *Marine Ecology, 39*, e12490.

Carrier, T. J., & Reitzel, A. M. (2017). The hologenome across environments and the implications of a host-associated microbial repertoire. *Frontiers in Microbiology, 8*, 802.

Carrier, T. J., & Reitzel, A. M. (2018). Convergent shifts in host-associated microbial communities across environmentally elicited phenotypes. *Nature Communications, 9*, 952.

Carrier, T. J., & Reitzel, A. M. (2019). Bacterial community dynamics during embryonic and larval development of three confamilial echinoids. *Marine Ecology Progress Series, 611*, 179-188.

Carrier, T. J., Wolfe, K., Lopez, K., Gall, M., Janies, D. A., Byrne, M., & A.M., R. (2018). Diet-induced shifts in the crown-of-thorns (*Acanthaster* sp.) larval microbiome. *Marine Biology, 165*, 157.

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., . . . Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature, 505*(7484), 559-564.

DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution, 13*(2), 77-81.

Dishaw, L. J., Flores-Torres, J., Lax, S., Gemayel, K., Leigh, B., Melillo, D., . . . Gilbert, J. A. (2014). The gut of geographically disparate *Ciona intestinalis* harbors a core microbiota. *PLoS ONE, 9*, e93386.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics, 27*(16), 2194-2200.

Fraune, S., & Bosch, T. C. G. (2007). Long-term maintenance of species-specific bacterial microbiota in the basal metazoan *Hydra*. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 13146-13151.

Guillard, R. R. L. (Ed.) (1975). *Culture of phytoplankton for feeding marine invertebrates*. New York, USA: Plenum Press.

Hart, M. W., & Strathmann, R. R. (1994). Functional consequences of phenotypic plasticity in echinoid larvae. *Biological Bulletin, 186*(3), 291-299.

Hodin, J., Ferner, M. C., Heyland, A., & Gaylord, B. (2017). I feel that! Fluid dynamics and sensory aspects of larval settlement across scales. In T. J. Carrier, A. M. Reitzel, & A. Heyland (Eds.), *Evolutionary Ecology of Marine Invertebrate Larvae*. Oxford, UK: Oxford University Press.

Huang, B.-H., Chang, C.-W., Huang, C.-W., Gao, J., & Liao, P.-C. (2018). Composition and functional specialists of the gut microbiota of frogs reflect habitat differences and agricultural activity. *Frontiers in Microbiology, 8*, 2670.

Kerr, J. R., & Kemp, P. S. (In Press). Masking a fish's detection of environmental stimuli: application to improving downstream migration at river infrastructure. *Journal of Fish Biology*, 1-10.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glockner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research, 41*(1), e1.

Kohl, K. D., Amaya, J., Passement, C. A., Dearing, M. D., & McCue, M. D. (2014). Unique and shared responses of the gut microbiota to prolonged fasting: a comparative study across five classes of vertebrate hosts. *FEMS Microbiology Ecology, 90*(3), 883-894.

Kohl, K. D., Brun, A., Magallanes, M., Brinkerhoff, J., Laspiur, A., Acosta, J. C., . . . Caviedes-Vidal, E. (2016). Physiological and microbial adjustments to diet quality permit facultative herbivory in an omnivorous lizard. *Journal of Experimental Biology, 219*, 1903-1912.

Kohl, K. D., & Carey, H. V. (2016). A place for host-microbe symbiosis in the comparative physiologist's toolbox. *Journal of Experimental Biology, 219*, 3496-3504.

Kohl, K. D., & Dearing, M. D. (2012). Experience matters: prior exposure to plant toxins enhances diversity of gut microbes in herbivores. *Ecology Letters, 15*(9), 1008-1015.

Kohl, K. D., Oakeson, K. F., Orr, T. J., Miller, A. W., Forbey, J. S., Phillips, C. D., . . . Dearing, M. D. (2018). Metagenomic sequencing provides insights into microbial detoxification in the guts of small mammalian herbivores (*Neotoma* spp.). *Fems Microbiology Ecology, 94*, fiy184.

Li, Y., He, N., Hou, J., Xu, L., Liu, C., Zhang, J., . . . Wu, X. B. (2018). Factors influencing leaf chlorophyll content in natural forests at the biome scale. *Frontiers in Ecology and the Environment, 6*, 64.

Llewellyn, M. S., McGinnity, P., Dionne, M., Letourneau, J., Thonier, F., Carvalho, G., . . . Derome, N. (2016). The biogeography of the atlantic salmon (*Salmo salar*) gut microbiome. *ISME Journal, 10*, 1280-1284.

Lokmer, A., & Wegner, K. M. (2015). Hemolymph microbiome of Pacific oysters in response to temperature, temperature stress and infection. *The ISME Journal, 9*, 670-682.

Louca, S., Jacques, S. M. S., Pires, A. P. F., Leal, J. S., Srivastava, D. S., Parfrey, L. W., . . . Doebeli, M. (2016). High taxonomic variability despite stable functional structure across microbial communities. *Nature Ecology & Evolution, 1*, 15.

Louca, S., Polz, M. F., Mazel, F., Albright, M. B. N., Huber, J. A., O'Connor, M. I., . . . Parfrey, L. W. (2018). Function and functional redundancy in microbial systems. *Nature Ecology & Evolution, 2*, 936-943.

Lozupone, C., & Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology, 71*(12), 8228-8235.

Macke, E., Tasiemski, A., Massol, F., Callens, M., & Decaestecker, E. (2016). Life history and eco-evolutionary dynamics in light of the gut microbiota. *Oikos*.

Manier, M. K., & Palumbi, S. R. (2008). Intraspecific divergence in sperm morphology of the green sea urchin, *Strongylocentrotus droebachiensis*: implications for selection in broadcast spawners. *BMC Evolutionary Biology, 8*, 283.

Marino, C. M., Pawlik, J. R., López-Legentil, S., & Erwin, P. M. (2017). Latitudinal variation in the microbiome of the sponge *Ircinia campana* correlates with host haplotype but not anti-predatory chemical defense. *Marine Ecology Progress Series, 565*, 53-66.

Marks, J. A., Biermann, C. H., Eanes, W. F., & Kryvi, H. (2008). Sperm polymorphism within the sea urchin *Strongylocentrotus droebachiensis*: divergence between Pacific and Atlantic Oceans. *Biological Bulletin, 215*, 115-125.

Marzinelli, E. M., Campbell, A. H., Valdes, E. Z., Vergés, A., S., N., Wernberg, T., . . . Steinberg, P. D. (2015). Continental-scale variation in seaweed host-associated bacterial communities is a function of host condition, not geography. *Environmental Microbiology, 17*, 4078-4088.

McAlister, J. S., & Miner, B. G. (2018). Phenotypic plasticity of feeding structures in marine invertebrate larvae. In T. J. Carrier, A. M. Reitzel, & A. Heyland (Eds.), *Evolutionary Ecology of Marine Invertebrate Larvae*. Oxford, UK: Oxford University Press.

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C. G., Carey, H. V., Domazet-Loso, T., Douglas, A. E., . . . Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences of the United States of America, 110*, 3229-3236.

McQuatters-Gollop, A., Raitsos, D. E., Edwards, M., Pradhan, Y., Mee, L. D., Lavender, S. J., & Attrill, M. J. (2007). A long-term chlorophyll dataset reveals regime shift in North Sea phytoplankton biomass unconnected to nutrient levels. *Limnology and Oceanograhpy, 52*, 635-648.

Miner, B. G. (2004). Evolution of feeding structure plasticity in marine invertebrate larvae: a possible trade-off between arm length and stomach size. *Journal of Experimental Marine Biology and Ecology, 315*, 117-125.

Miner, B. G. (2011). Mechanisms underlying feeding structure plasticity in echinoderm larvae. In T. Flatt & A. Heyland (Eds.), *Mechanisms of Life History Evolution* (pp. 221-229): Oxford University Press.

Miner, B. G., Sultan, S. E., Morgan, S. G., Padilla, D. K., & Relyea, R. A. (2005). Ecological consequences of phenotypic plasticity. *Trends in Ecology & Evolution, 20*, 685-692.

Moeller, A. H., Suzuki, T. A., Lin, D., Lacey, E. A., Wasser, S. K., & Nachman, M. W. (2017). Dispersal limitation promotes the diversification of the mammalian gut microbiota. *Proceedings of the National Academy of Sciences of the United States of America, 114*, 13768-13773.

Mortzfeld, B. M., Urbanski, S., Reitzel, A. M., Kunzel, S., Technau, U., & Fraune, S. (2015). Response of bacterial colonization in *Nematostella vectensis* to development, environment and biogeography. *Environmental Microbiology, 18*(6), 1764-1781.

Muegge, B. D., Kuczynski, J., Knights, D., Clemente, J. C., Gonzalez, A., Fontana, L., . . . Gordon, J. I. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science, 332*, 970-974.

Norderhaug, K. M., Anglès d'Auriac, M. B., FagerliH. Gundersen, C. W., Christie, H., Dahl, K., & Hobæk, A. (2016). Genetic diversity of the NE Atlantic sea urchin

*Strongylocentrotus droebachiensis* unveils chaotic genetic patchiness possibly linked to local selective pressure. *Marine Biology, 163*, 36.

Otto, L., Zimmerman, J. T. F., Furnes, G. K., Mork, M., Saetre, R., & Becker, G. (1990). Review of the physical oceanography of the North Sea. *Netherlands Journal of Sea Research, 26*, 161-238.

Pantos, O., Bongaerts, P., Dennis, P. G., Tyson, G. W., & Hoegh-Guldberg, O. (2015). Habitat-specific environmental conditions primarily control the microbiomes of the coral *Seriatopora hystrix*. *ISME Journal, 9*, 1916-1927.

Pettigrew, N. R., Churchill, J. H., Janzen, C. D., Mangum, L. J., Signell, R. P., Thomas, A. C., . . . Xue, H. (2005). The kinematic and hydrographic structure of the Gulf of Maine Coastal Current. *Deep Sea Research Part II: Topical Studies in Oceanography, 52*, 2369-2391.

Rosenberg, E., & Zilber-Rosenberg, I. (2016). Interaction between the microbiome and diet: the hologenome concept. *Journal of Nutrition & Food Sciences, 6*, 5.

Roth-Schulze, A. J., Pintado, J., Zozaya-Valdés, E., Cremades, J., Ruiz, P., Kjelleberg, S., & Thomas, T. (2018). Functional biogeography and host specificity of bacterial communities associated with the marine green alga *Ulva* spp. *Molecular Ecology, 27*, 1952-1965.

Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D., . . . Segal, E. (2018). Environment dominates over host genetics in shaping human gut microbiota *Nature, 555*, 210-215.

Scheibling, R. E., & Hatcher, B. G. (2013). *Strongylocentrotus droebachiensis*. In J. M. Lawrence (Ed.), *Sea Urchins: Biology and Ecology*. San Diego, CA: Acedemic Press.

Schlichting, C. D., & Smith, H. (2002). Phenotypic plasticity: linking molecular mechanisms with evolutionary outcomes. *Evolutionary Ecology, 16*(3), 189-211.

Schmitt, S., Tsai, P., Bell, J., Fromont, J., Ilan, M., Lindquist, N., . . . Taylor, M. W. (2012). Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges. *ISME Journal, 6*, 564-576.

Schneider, C., Rasband, W., & Eliceiri, K. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, 9*, 671-675.

Shapira, M. (2016). Gut microbiotas and host evolution: scaling up symbiosis. *Trends in Ecology & Evolution, 31*, 539-549.

Soars, N. A., Prowse, T. A. A., & Byrne, M. (2009). Overview of phenotypic plasticity in echinoid larvae, '*Echinopluteus transversus*' type vs. typical echinoplutei. *Marine Ecology Progress Series, 383*, 113-125.

Sonnenburg, E. D., Smits, S. A., Tikhonov, M., Higginbottom, S. K., Wingreen, N. S., & Sonnenburg, J. L. (2016). Diet-induced extinctions in the gut microbiota compound over generations. *Nature, 529*(7585), 208-212.

Starr, M., Himmelman, J., & Therriault, J. (1990). Direct coupling of marine invertebrate spawning with phytoplankton blooms. *Science, 247*, 1071-1074.

Sterns, S. (1989). The evolutionary significance of phenotypic plasticity. *BioScience, 39*(7), 436-445.

Strathmann, M. F. (1987). *Reproduction and development of marine invertebrates of the northern Pacific coast: data and methods for the study of eggs, embryos, and larvae*: University of Washington Press.

Sullam, K. E., Essinger, S. D., Lozupone, C. A., O'Connor, M. P., Rosen, G. L., Knight, R., . . . Russell, J. A. (2012). Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. *Molecular Ecology, 21*, 3363-3378.

Sutherland, D. A., & MacCready, P. (2011). A model study of the Salish Sea estuarine circulation. *Journal of Physical Oceanography, 41*, 1125-1143.

Theis, K. R., Dheilly, N. M., Klassen, J. L., Brucker, R. M., Baines, J. F., Bosch, T. C. G., . . . Bordenstein, S. R. (2016). Getting the hologenome concept right: an eco-evolutionary framework for hosts and their microbiomes. *mSystems, 1*(2), e00028-00016.

Thomas, A. C., Townsend, D. W., & Weatherbee, R. (2003). Satellite-measured phytoplankton variability in the Gulf of Maine. *Continental Shelf Research, 23*, 971-989.

Webster, N. S., Botte, E. S., Soo, R. M., & Whalan, S. (2011). The larval sponge holobiont exhibits high thermal tolerance. *Environmental Microbiology Reports, 3*(6), 756-762.

Webster, N. S., Soo, R., Cobb, R., & Negri, A. P. (2011). Elevated seawater temperature causes a microbial shift on crustose coralline algae with implications for the recruitment of coral larvae. *ISME Journal, 5*(4), 759-770.

Webster, T. M. U., Consuegra, S., Hitchings, M., & de Leaniz, C. G. (2018). Inter-population variation in the Atlantic salmon microbiome reflects environmental and genetic diversity. *Applied and Environmental Microbiology, 84*, e00691-00618.

West-Eberhard, M. (2003). *Developmental Plasticity and Evolution*. Oxford, UK: Oxford University Pres.

Winter, D. F., Banse, K., & Anderson, G. C. (1975). The dynamics of phytoplankton blooms in puget sound a fjord in the Northwestern United States. *Marine Biology, 29*, 139-176.

Wolfe, K., Graba-Landry, A., Dworjanyn, S., & Byrne, M. (2015). Larval phenotypic plasticity in the boom-and-bust crown-of-thorns seastar, *Acanthaster planci*. *Marine Ecology Progress Series, 539*, 179-189

Wolfe, K., Graba-Landry, A., Dworjanyn, S., & Byrne, M. (2015). Larval starvation to satiation: influence of nutrient regime on the success of *Acanthaster planci*. *PLoS ONE*, e0122010.

Wolfe, K., Graba-Landry, A., Dworjanyn, S., & Byrne, M. (2017). Superstars: Assessing nutrient thresholds for enhanced larval success of *Acanthaster planci*, a review of the evidence. *Marine Pollution Bulletin, 116*, 307-314.

Yang, Y., Niu, H., Xiao, L., Lin, Q., Han, B.-P., & Naselli-Flores, L. (2018). Spatial heterogeneity of spring phytoplankton in a large tropical reservoir: could mass effect homogenize the heterogeneity by species sorting? *Hydrobiologia, 819*, 109-122.

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., . . . Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature, 486*(7402), 222-227.

Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2014). PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics, 30*(5), 614-620.

Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Reviews, 32*(5), 723-735.

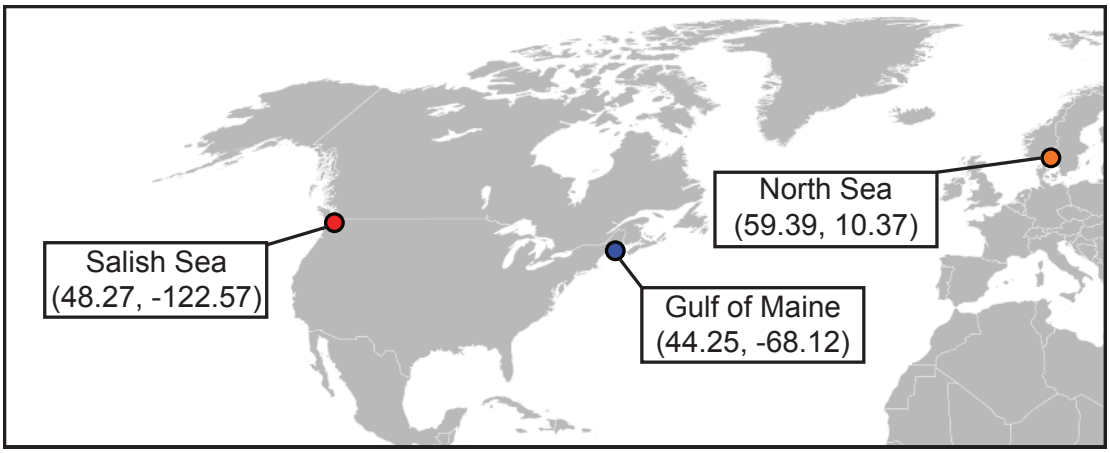


Figure 4.1. Location of *Strongylocentrotus droebachiensis* experiments. Cartoon representation for where the adult populations were sampled and where differential feeding experiments were conducted.

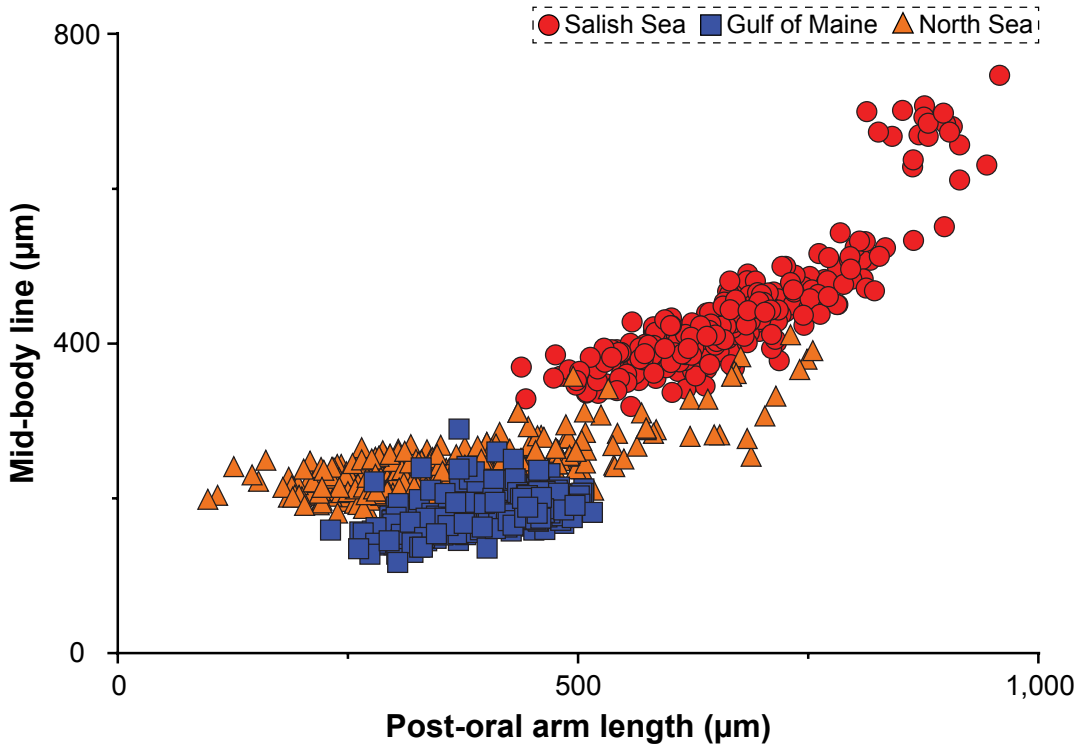


Figure 4.2. Morphology of *Strongylocentrotus droebachiensis* larvae from three populations. Post-oral arm and mid-body line lengths for *S. droebachiensis* larvae from the Salish Sea, Gulf of Maine, and North Sea, with feeding quantity, developmental stage, and time collapsed (see, Figs. S1-2 for the sub-division of larval features.)

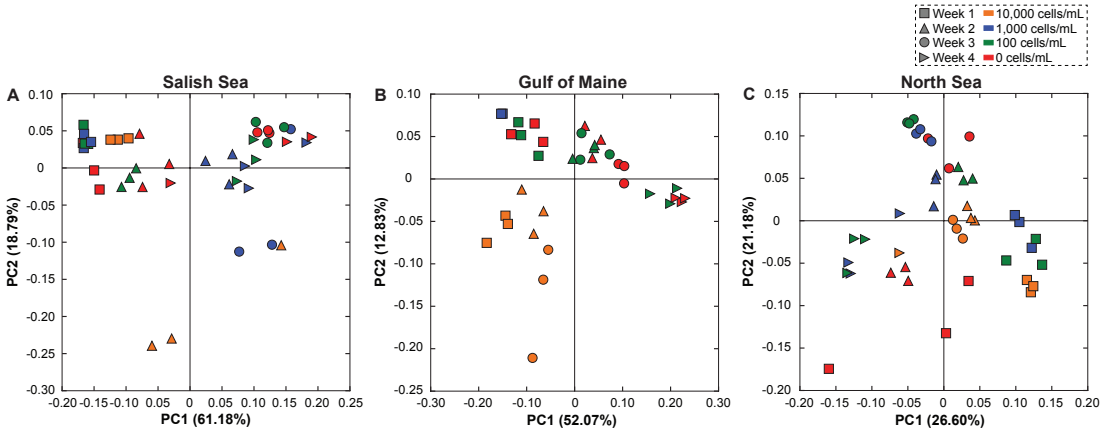


Figure 4.3. Dietary and temporal shifts in the bacterial communities associated with *Strongylocentrotus droebachiensis* larvae. Community similarity of weighted UniFrac based on *S. droebachiensis* larval-associated bacterial communities based on food availability (10,000, 1,000, 100, and 0 cells•mL$^{-1}$ represented by orange, blue, green, and red, respectively) over a multi-week exposure (Week 1, 2, 3, and 4 represented by a square, triangle, circle, and sideways-triangle, respectively) for populations from the Salish Sea (A), Gulf of Maine (B), and North Sea (C).

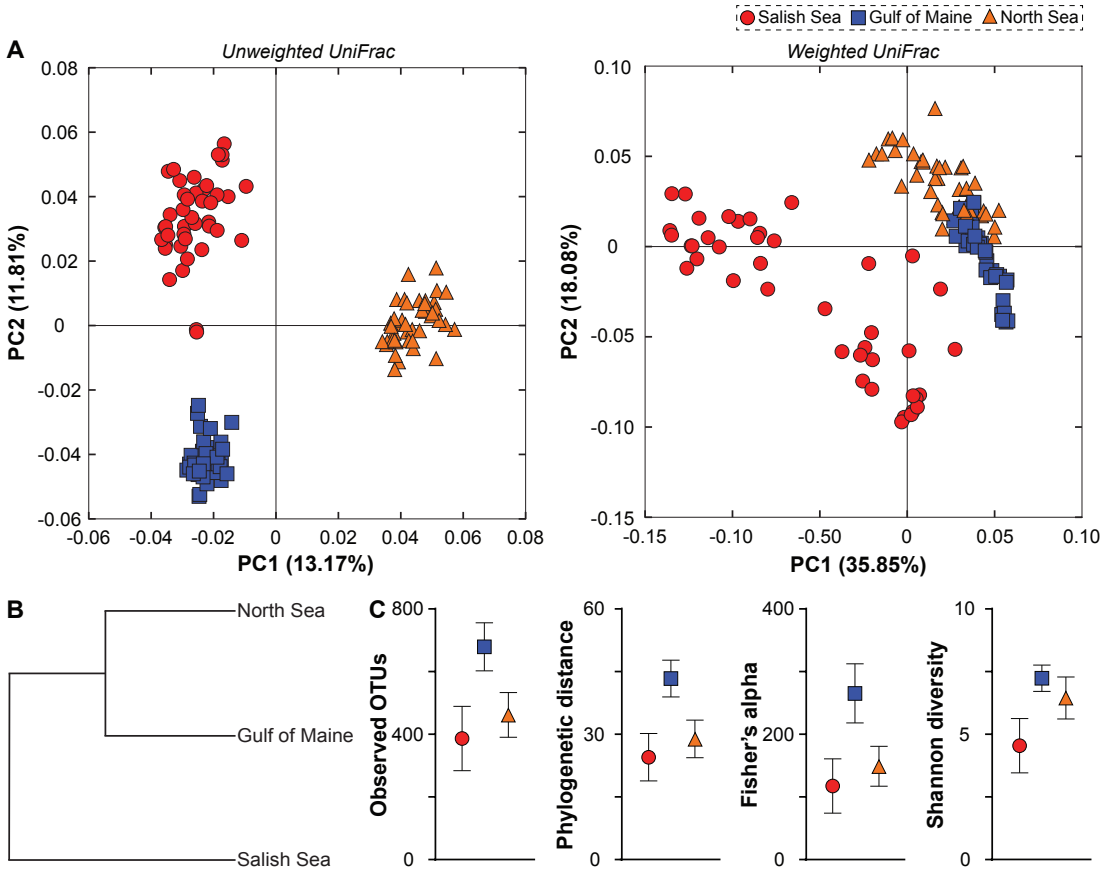


Figure 4.4. Structure of the bacterial communities associated with *Strongylocentrotus droebachiensis* larvae from three populations. Community similarity of *S. droebachiensis* larval-associated bacterial communities between three geographic locations based on (A) unweighted and weighted UniFrac comparisons, with the relatedness of the weighted UniFrac represented by a microbial dendrogram (B). Diversity within each population was estimated using four (C) alpha metrics: observed OTUs, Faith's phylogenetic distance, Fisher's alpha, and Shannon diversity.

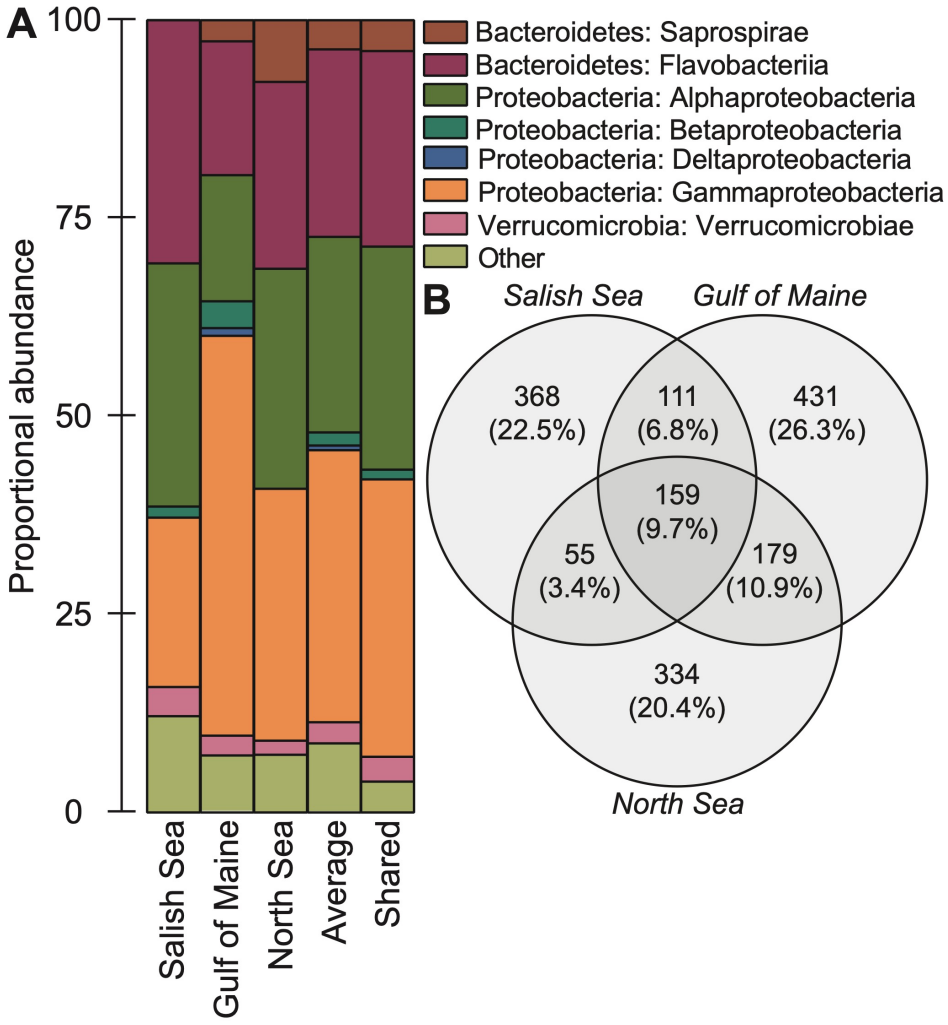


Figure 4.5. Bacterial taxa associated with *Strongylocentrotus droebachiensis* larvae. (A) Class-level profiles of the bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea, Gulf of Maine, and North Sea as well as the mean community across populations and the shared taxa between each population. (B) Distribution of OTUs associated with *S. droebachiensis* larvae uniquely found in each population, common between two populations, and shared between all populations.

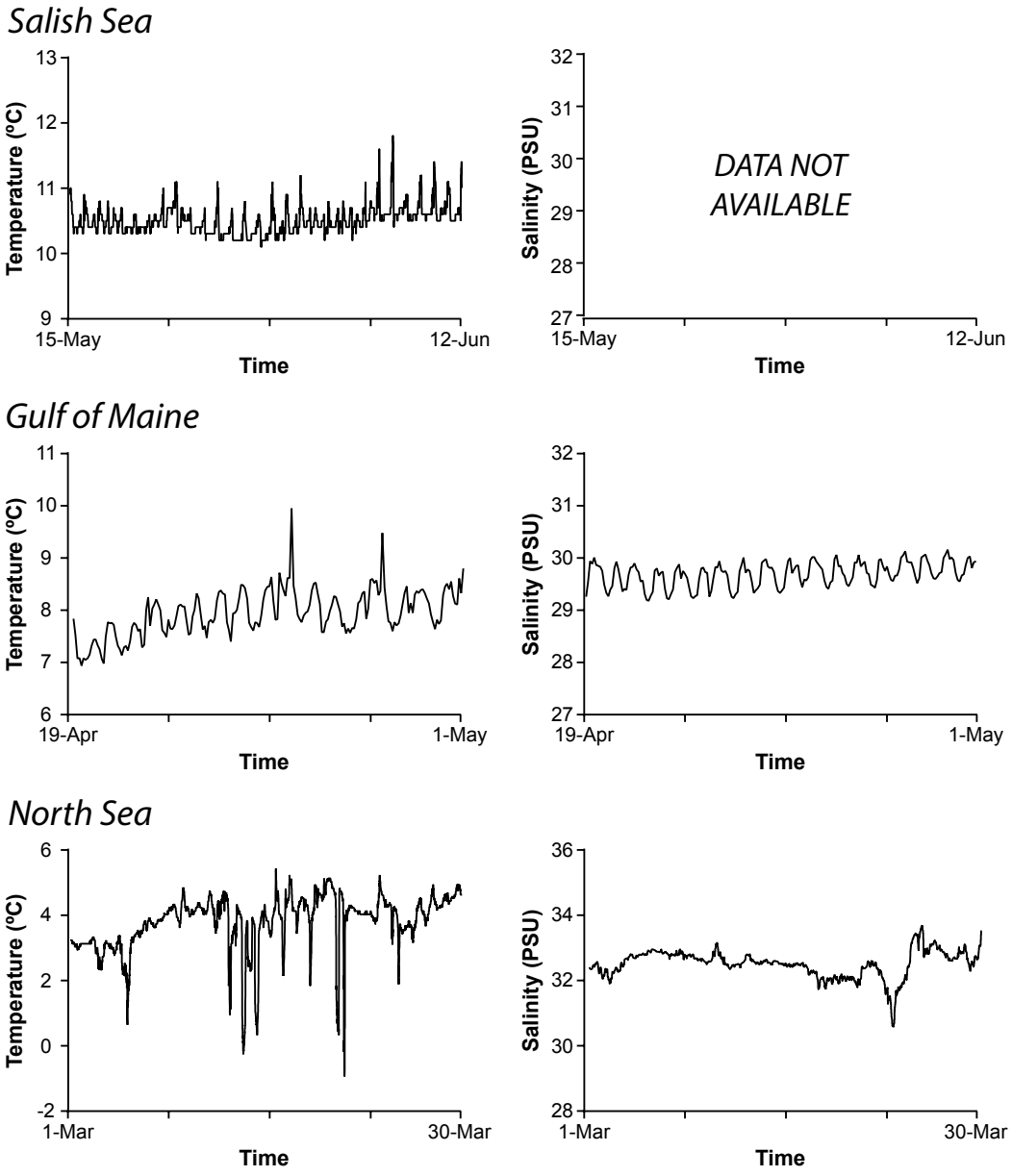


Figure S3.1. *in situ* measurements of temperature and salinity. Temperature and salinity for Friday Harbor, WA (NOAA station, FRDW1), Walpole, ME (NOAA NERACOOS LOBO 2, mid-Damariscotta River), and Kristineberg, Sweden (Sven Lovén Centre for Marine Infrastructure, deep water intake) for larval experimentation.

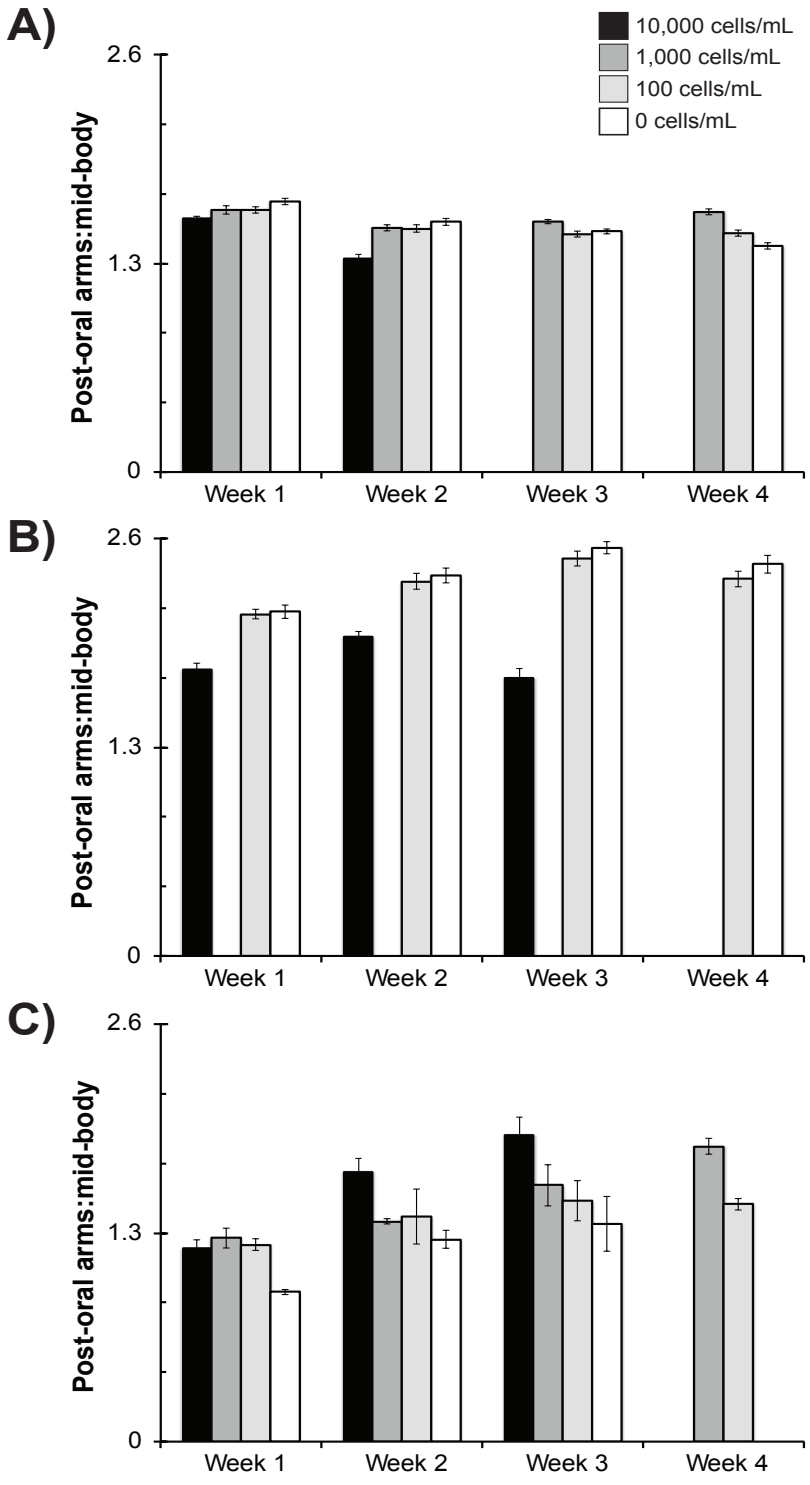


Figure S4.2. *Strongylocentrotus droebachiensis* larvae alter phenotype to feeding environment. Ratio between the post-oral arm and mid body line (mean ± standard error; n=20) for *S. droebachiensis* from the Salish Sea (A), Gulf of Maine (B), and North Sea (C) larvae having been fed either 10,000 (black), 1,000 (dark grey), 100 (grey), and 0 cells•mL$^{-1}$ (white).

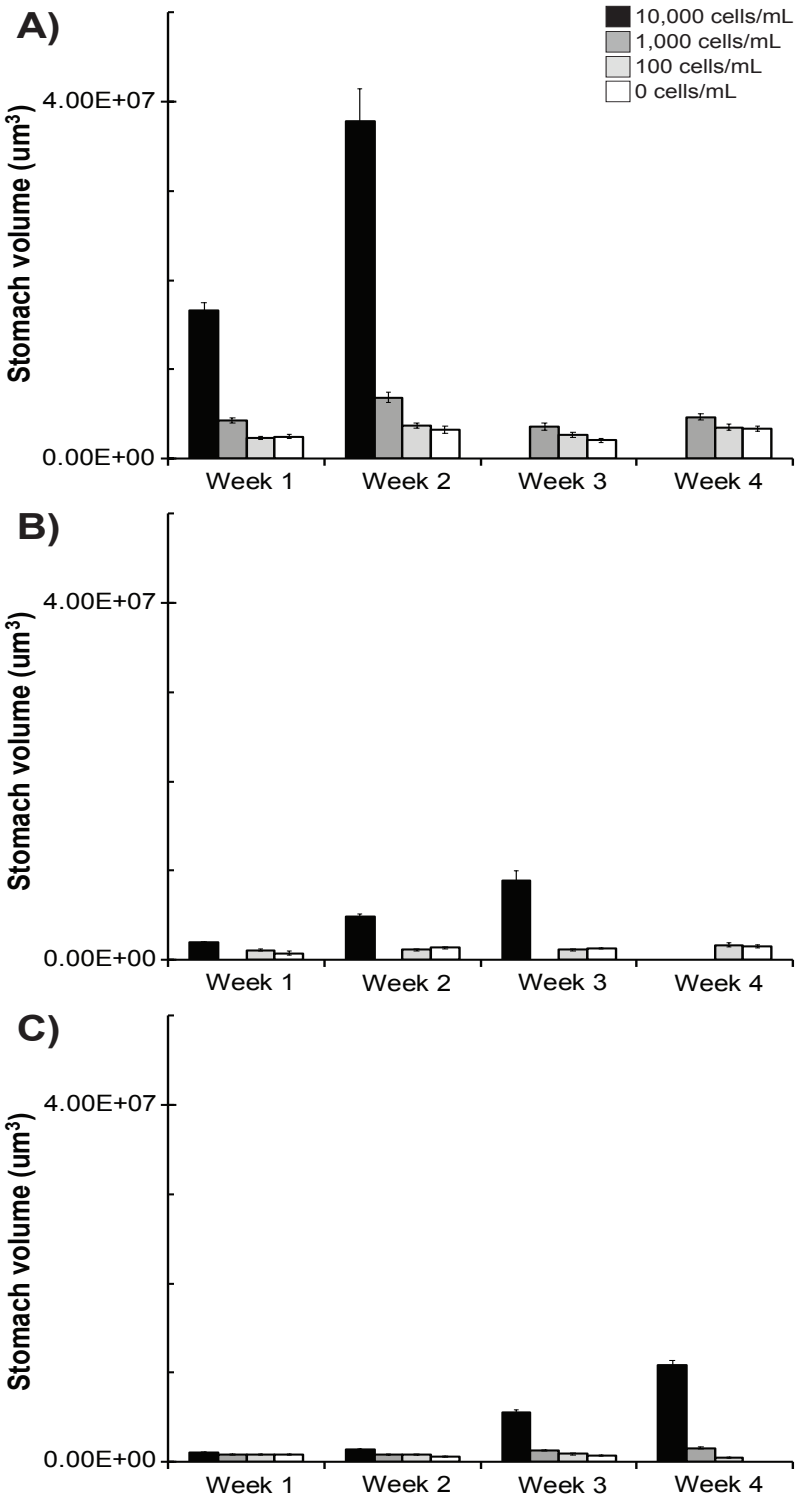


Figure S4.3. *Strongylocentrotus droebachiensis* larvae alter gut volume to feeding environment. Gut volume (mean ± standard error; n=20) for larval *S. droebachiensis* from the Salish Sea (A), Gulf of Maine (B), and North Sea (C) having been fed either 10,000 (black), 1,000 (dark grey), 100 (grey), and 0 cells•mL$^{-1}$ (white).

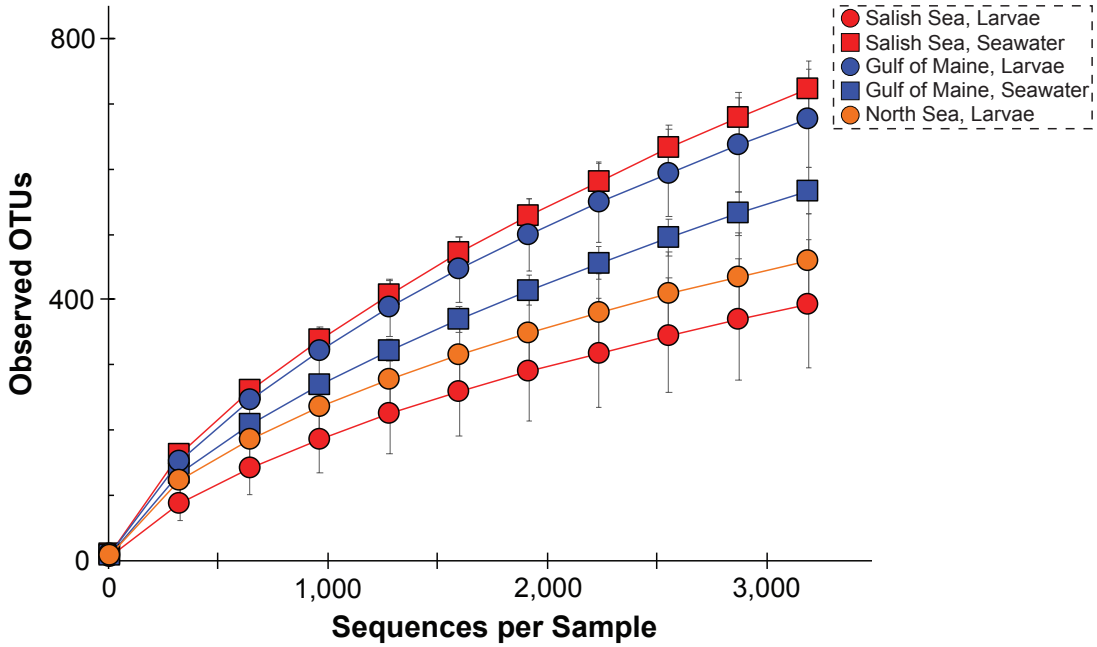


Figure S4.4. Alpha rarefaction curves for *Strongylocentrotus droebachiensis* larvae from three geographic locations and of the seawater. Alpha rarefaction curves for the associated microbiota for *S. droebachiensis* larvae (circles) from the Salish Sea (blue), Gulf of Maine (red), and North Sea (yellow) and seawater (squares) based on the rarefaction depth (3,193 sequences) used for all analyses.

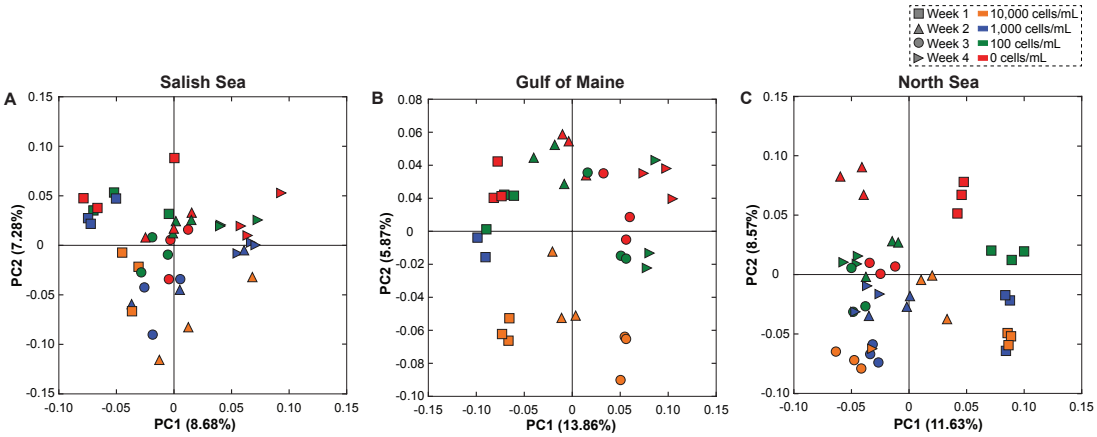


Figure S4.5. Dietary and temporal shifts in the bacterial communities associated with *Strongylocentrotus droebachiensis* larvae. Community similarity based on *S. droebachiensis* larval-associated bacterial communities based on food availability (10,000, 1,000, 100, and 0 cells•mL$^{-1}$ represented by orange, blue, green, and red, respectively) over a multi-week exposure (Week 1, 2, 3, and 4 represented by a square, triangle, circle, and sideways-triangle, respectively) for populations from the Salish Sea (A), Gulf of Maine (B), and North Sea (C). Comparisons between food availability and over time are based on unweighted UniFrac comparisons. (Note: this is the unweighted complement to Figure 3.)

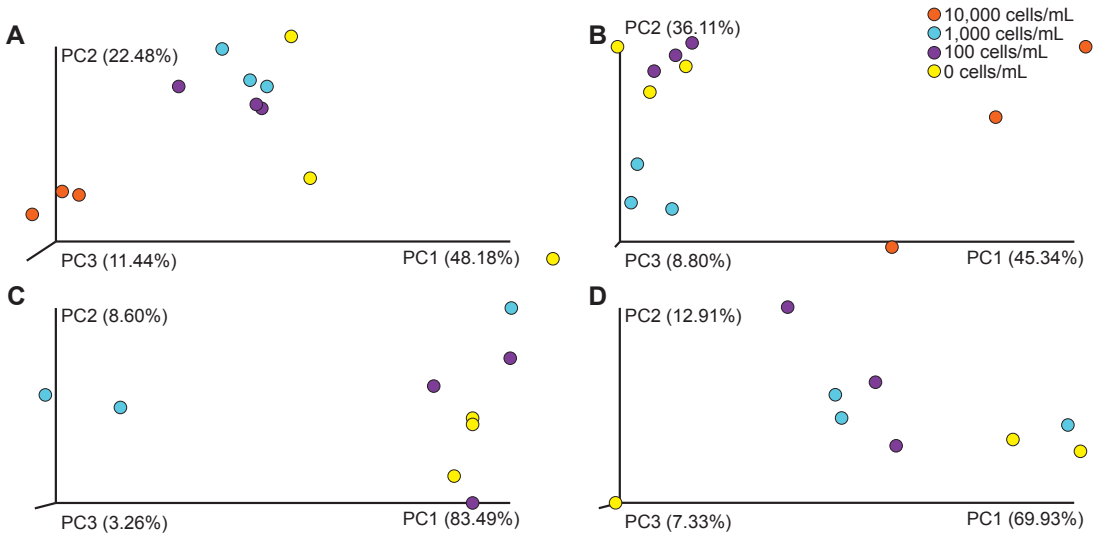


Figure S4.6. Community similarity of *Strongylocentrotus droebachiensis* larvae from the Salish Sea across time. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea for one (A; $p < 0.002$), two (B; $p < 0.001$), three (C; $p < 0.001$), or four (D; $p < 0.001$) weeks and having been fed 10,000 (orange), 1,000 (blue), 100 (purple), or 0 (yellow) cells•mL$^{-1}$.

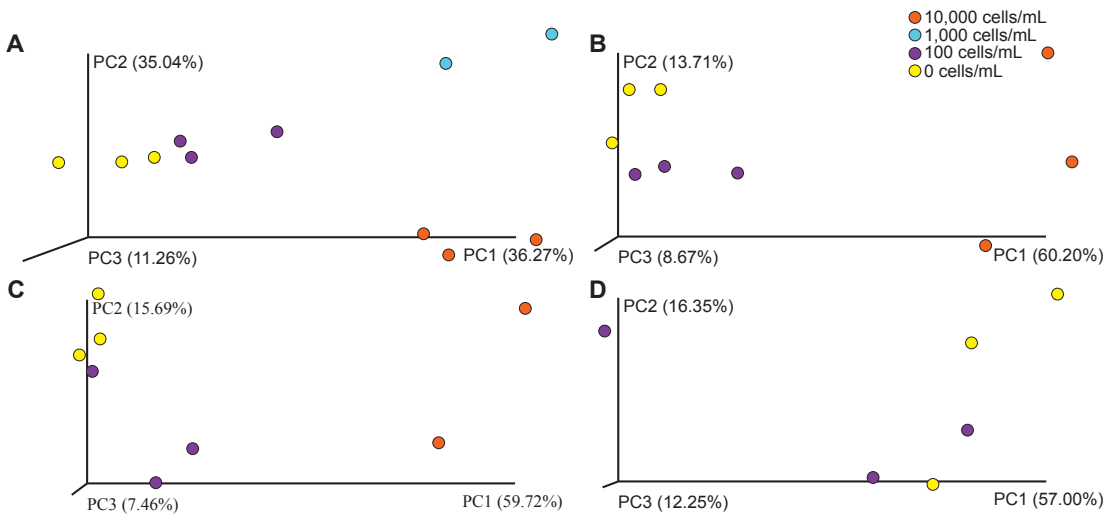


Figure S4.7. Community similarity of *Strongylocentrotus droebachiensis* larvae from the Gulf of Maine across time. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Gulf of Maine for one (A; $p < 0.001$), two (B; $p < 0.005$), three (C; $p < 0.007$), or four (D; $p = 0.430$) weeks and having been fed 10,000 (orange), 1,000 (blue), 100 (purple), or 0 (yellow) cells•$mL^{-1}$.

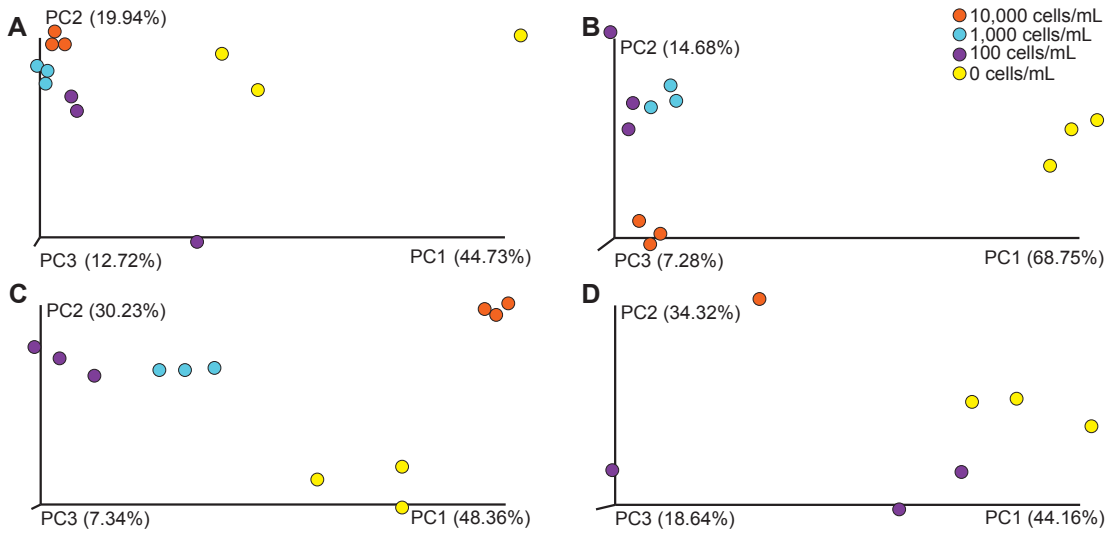


Figure S4.8. Community similarity of *Strongylocentrotus droebachiensis* larvae from the North Sea across time. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the North Sea for one (A; $p < 0.002$), two (B; $p < 0.001$), three (C; $p < 0.001$), or four (D; $p < 0.020$) weeks and having been fed 10,000 (orange), 1,000 (blue), 100 (purple), or 0 (yellow) cells•mL$^{-1}$.

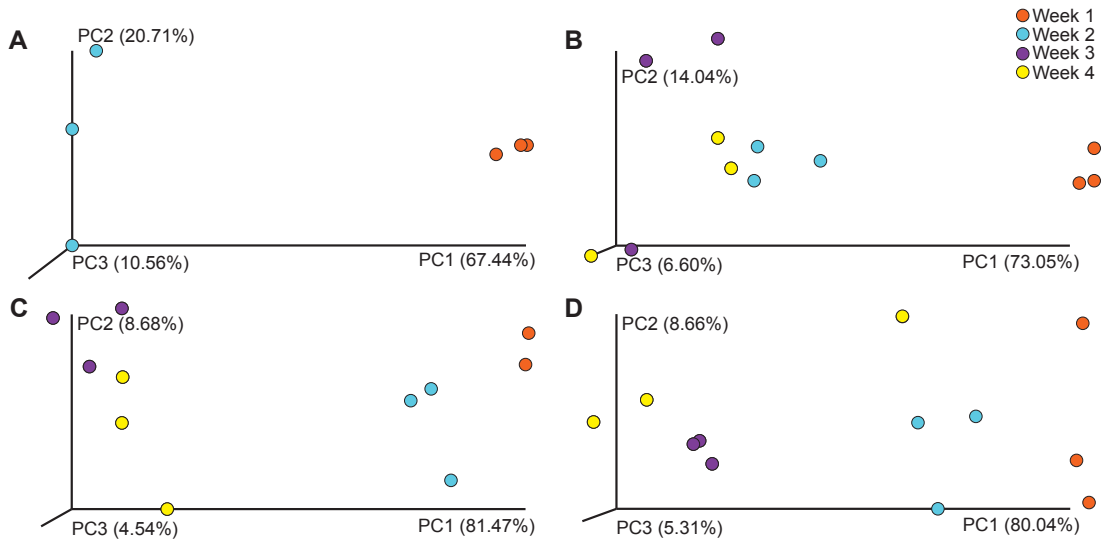


Figure S4.9. Community similarity of *Strongylocentrotus droebachiensis* larvae from the Salish Sea across diets. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea having been fed 10,000 (A; $p < 0.001$), 1,000 (B; $p < 0.001$), 100 (C; $p < 0.001$), or 0 (D; $p < 0.001$) cells•mL$^{-1}$ for one (orange), two (blue), three (purple), or four (yellow) weeks.

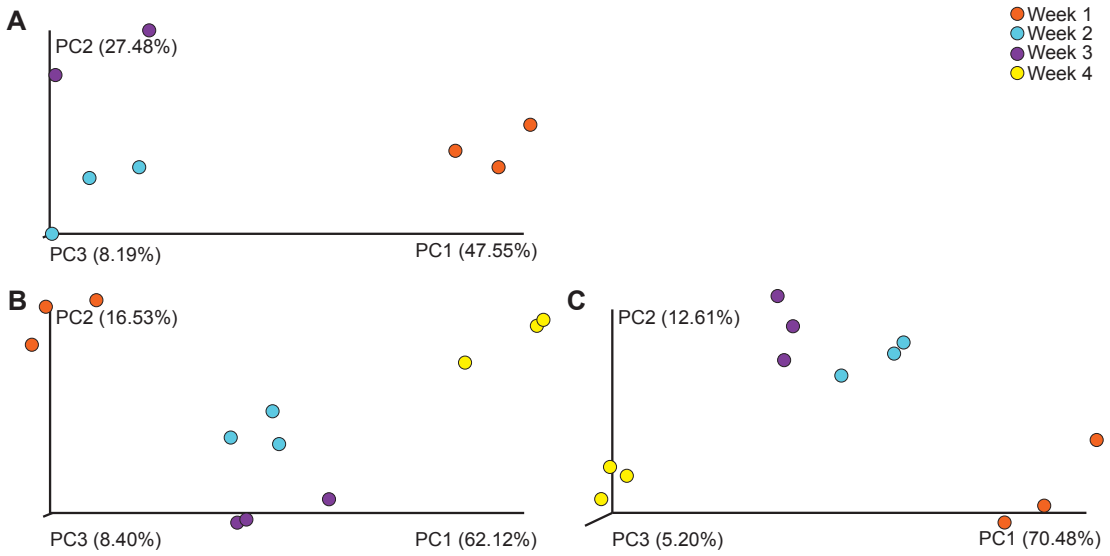


Figure S4.10. Community similarity of *Strongylocentrotus droebachiensis* larvae from the Gulf of Maine across diets. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Gulf of Maine having been fed 10,000 (A; $p < 0.005$), 100 (B; $p < 0.001$), or 0 (C; $p < 0.002$) cells•$mL^{-1}$ for one (orange), two (blue), three (purple), or four (yellow) weeks.

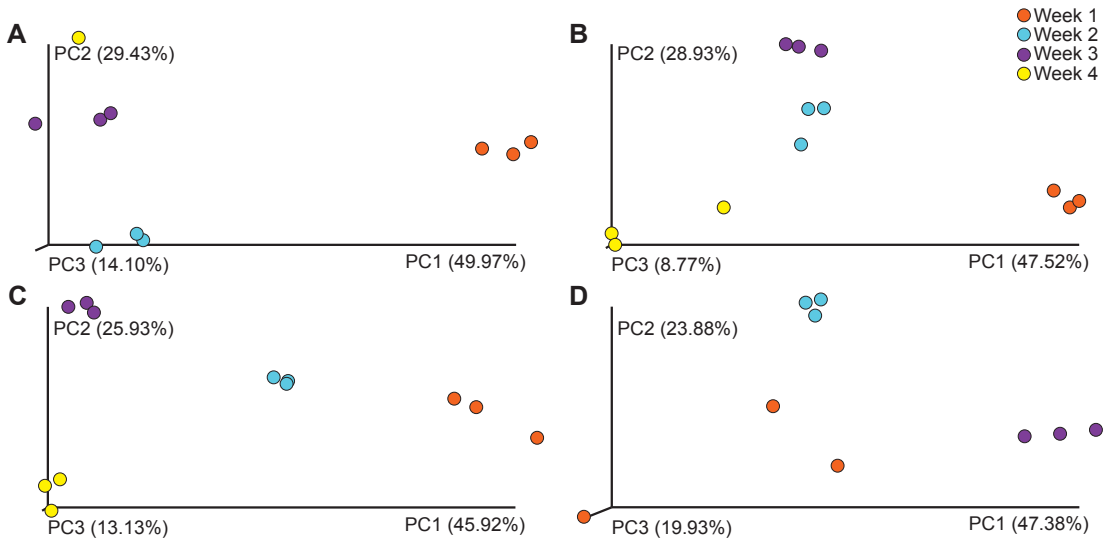


Figure S4.11. Community similarity of *Strongylocentrotus droebachiensis* larvae from the North Sea across diets. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the North Sea having been fed 10,000 (A; $p < 0.002$), 1,000 (B; $p < 0.002$), 100 (C; $p < 0.002$), or 0 (D; $p < 0.006$) cells•mL$^{-1}$ for one (orange), two (blue), three (purple), or four (yellow) weeks.

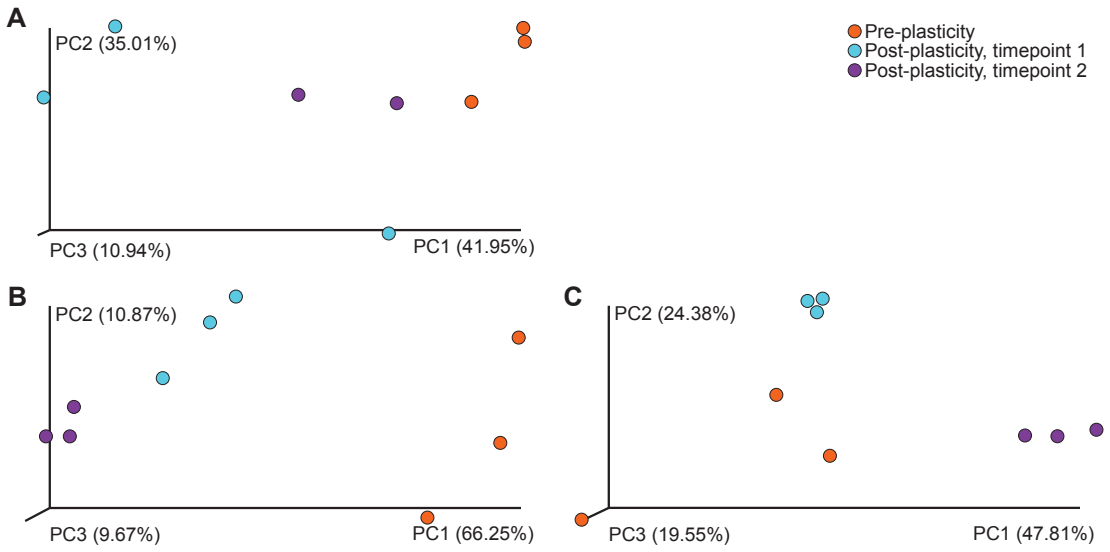


Figure S4.12. Community similarity of the associated microbial community along a phenotypic continuum for *Strongylocentrotus droebachiensis* from three geographic locations. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea (A; $p = 0.046$), Gulf of Maine (B; $p < 0.005$), and North Sea (C; $p < 0.004$) pre (orange) and post (blue and purple) expression of plasticity.

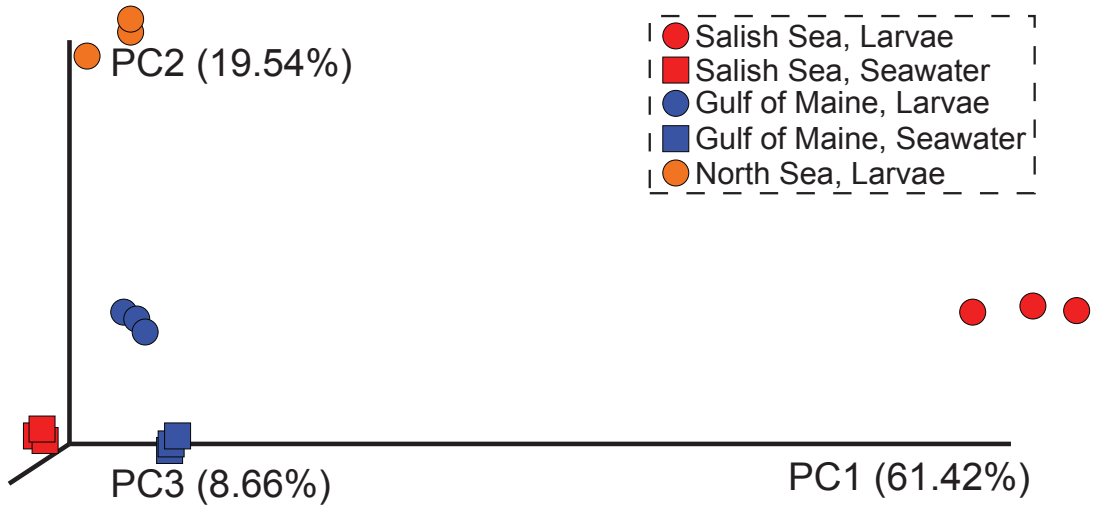


Figure S4.13. Similarity of the associated microbial community for *Strongylocentrotus droebachiensis* larvae from three geographic locations. Community similarity ($p < 0.001$) of the associated microbiota between *S. droebachiensis* larvae (circles) from the Salish Sea (blue), Gulf of Maine (red), and North Sea (yellow) and seawater (squares).

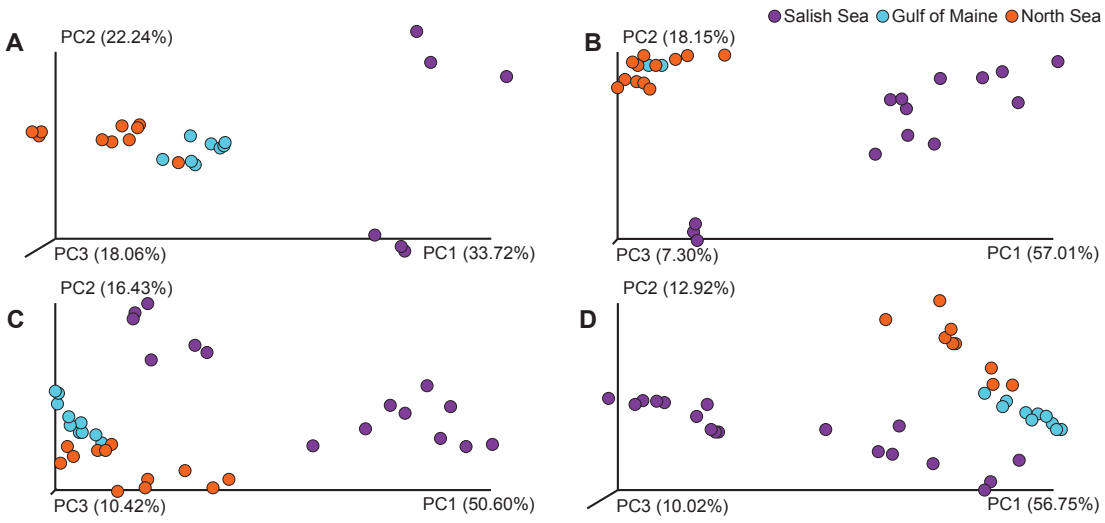


Figure S4.14. Community similarity of *Strongylocentrotus droebachiensis* larvae across diets. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea (purple), Gulf of Maine (blue), or North Sea (orange) and having been fed 10,000 (A; $p < 0.001$), 1,000 (B; $p < 0.001$), 100 (C; $p < 0.001$), and 0 (D; $p < 0.001$) cells•mL$^{-1}$.

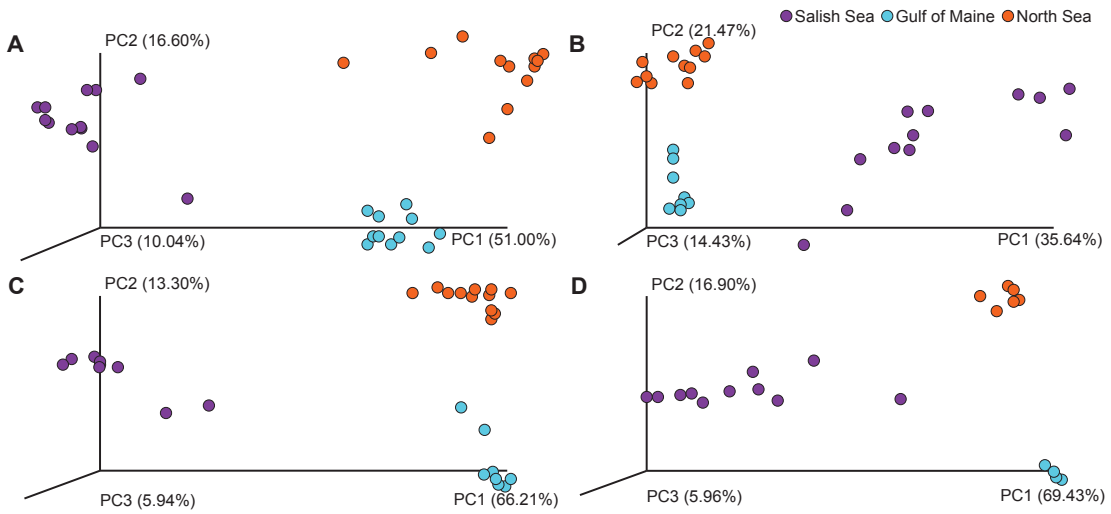


Figure S4.15. Community similarity of *Strongylocentrotus droebachiensis* larvae across time. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea (purple), Gulf of Maine (blue), or North Sea (orange) and having been differentially fed for one (A; $p < 0.001$), two (B; $p < 0.001$), three (C; $p < 0.001$), or four (D; $p < 0.001$) weeks.

# CHAPTER 5

# LOSSES AND GAINS IN AND OF THE MICROBIOME DURING A MAJOR LIFE-HISTORY TRANSITION

Tyler J. Carrier, Brittany A. Leigh, Dione J. Deaker, Seth R. Bordenstein, Maria Byrne, and Adam M. Reitzel

## Abstract

Animal gastrointestinal tracts house diverse microbiota that are integral to host function. Over the course of animal evolution species from various phyla have lost their digestive system, where theory predicts that community diversity and diet-induced shifts in these microbiota should be lost. Here we use the major life-history transition from planktotrophy to lecithotrophy to test for losses in properties of host-associated microbiota. Experimental feeding and profiling of the associated bacteria show that loss of the gut corresponds with a reduction in taxonomic and phylogenetic diversity, microbial load, and the ability to associate with a diet-specific bacterial community. Moreover, eggs of the gutless congener became unicolonized by a novel Rickettsiales lineage that is closely related to *Wolbachia* and that has homologous genes known to manipulate host reproduction and metabolize diacylglycerols. Taken together, this suggests that the microbiome is a dynamic character in animal evolution that may be modified during life-history transitions.

## 5.1 Introduction

The animal gastrointestinal tract houses phylogenetically diverse microbial communities that are integral to host metabolism, immunity, and development (Bates et al., 2006; Fraune & Bosch, 2010; Ley, Lozupone, Hamady, Knight, & Gordon, 2008;

McFall-Ngai et al., 2013; Turnbaugh et al., 2006). Symbioses between the animal host and gut microbiota have deep evolutionary origins (Ley et al., 2008; McFall-Ngai et al., 2013), often co-diverge over evolutionary time (Brooks, Kohl, Brucker, van Opstal, & Bordenstein, 2016; Moeller et al., 2016), and can serve as a physiological buffer to heterogeneous environments (Douglas, 2009; Kohl & Carey, 2016). Despite the utility of the gastrointestinal tract and functional benefits of gut microbiota (Brooks et al., 2016), species in various phyla lack a digestive system due to repeated loss over the course of animal evolution (Strathmann, 1978; Wray, 1996). Loss of the gut should, in theory, result in a reduction in the diversity of host-associated microbiota and the absence of diet-related shifts exhibited by congeners that maintain a functional digestive system.

Major life-history transitions are driven by tradeoffs in reproduction and development that, in turn, impact fitness (Partridge & Harvey, 1988; Stearns, 1989). This is particularly evident in benthic marine invertebrates that support two opposing nutritional and developmental strategies (Mileikovsky, 1971; R. R. Strathmann, 1978; Thorson, 1950; Vance, 1973). The first—planktotrophy—typically includes a high number of small, energy-poor eggs that develop into larvae with a feeding apparatus and tripartite gut used to collect and process suspended particles (*e.g.*, phytoplankton) and other exogenous resources required to reach competency for metamorphosis (Mileikovsky, 1971; Strathmann, 1978; Thorson, 1950; Vance, 1973). The second—lecithotrophy—has fewer large, energy-rich eggs that develop into non-feeding larvae and undergo metamorphosis independently of external nutrients (Mileikovsky, 1971; Strathmann, 1978; Thorson, 1950; Vance, 1973). Species with lecithotrophic development have almost universally evolved from ancestral planktotrophs (Strathmann, 1978, 1985;

Strathmann, 1993; Wray, 1996), during which feeding structures and a functional gastrointestinal tract are lost (Raff, 1992). Life-history transitions between these developmental modes have occurred in several major animal lineages (Strathmann, 1978, 1985) and are readily observed and particularly well-studied in echinoderms (Raff, 1992; Raff & Byrne, 2006; Wray, 1996; Wray & Raff, 1991).

One of the most comprehensively studied life-history transitions is the sea urchin *Heliocidaris*, where a speciation event ~4 million years ago (Zigler, Raff, Popodi, Raff, & Lessios, 2003) gave rise to *H. tuberculata* with the ancestral planktotroph and *H. erythogramma* with the derived lecithotroph. Typical of planktotrophs, *H. tuberculata* develops from small (~95 μm) eggs into feeding larvae that offset food limitations by elongating their feeding arms relative to the body (Soars, Prowse, & Byrne, 2009). Environmental elicitation of alternate phenotypes by planktotrophic sea urchins is widely correlated with compositional shifts in the associated microbiota (Carrier, Dupont, & Reitzel, 2019; Carrier & Reitzel, 2018; Carrier & Reitzel, 2019; Carrier et al., 2018) that is presumed to aid in coping with reduced exogenous nutrients. *H. erythogramma*, on the other hand, develops directly from eggs ~100x the volume (~430 μm) of *H. tuberculata* and lack the morphological characters for feeding and a functional digestive track (Raff, 1992). This heterochronic shift in development (Raff, 1992) and life-history mediated a rewiring of the gene regulatory network (Israel et al., 2016), reorganization of cell fates (Wray & Raff, 1989), and mechanisms for gameteogensis (Byrne et al., 1999).

Using *Heliocidaris* as an experimental system, we may test whether the loss of a functional gut coincides with a reduction in symbiont diversity as well as the potential for this community to exhibit diet-related shifts. To test this, we differentially fed both

*Heliocidaris* species and used amplicon sequencing to profile the bacterial communities across development and dietary treatments. In this, we find that *H. erythogramma* eggs are dominated by a novel Rickettsiales lineage, for which we describe the genomic and morphological properties of.

## 5.2 Materials and Methods

### 5.2.1 Specimen collection and larval rearing

Adult *H. tuberculata* and *H. erythrogramma* were collected from populations throughout Sydney Harbor in March-April 2018. Adult urchins were transferred to the Sydney Institute of Marine Science within one hour, where they were maintained in flow-through aquaria.

Within two days of collections, adult urchins were spawned by intracoelomic injection of 0.10 M KCl. To include the environmental microbiota, gametes from three males and females were spawned separately into 5.0 μm filtered seawater (FSW) and pooled by sex. Fertilization of eggs and larval rearing followed Byrne et al. (2001). For *H. tuberculata*, cultures were set to a density of 1 larva•$mL^{-1}$, stirred gently in beakers, and the FSW was changed once every 2.5 days (Byrne et al., 2001). *H. erythrogramma* cultures, on the other hand, were set to a density of 5 larvae•$mL^{-1}$, remained static in shallow dishes, and the FSW was changed twice daily (Byrne et al., 2001).

### 5.2.2 Experimental feeding and sample collection

Larvae of both *Heliocidaris* species were divided into twelve replicate jars and provided an experimental feeding treatment of either 12,000, 1,200, or 0 cells•$mL^{-1}$ of

*Isochrysis galbana* (n=4, per diet). Prism-stage *H. tuberculata* larvae were treated 48 hours post-fertilization (hpf) for twenty days (*i.e.*, to developmental stasis) while *H. erythrogramma* embryos were treated two hpf for five days (*i.e.*, to metamorphosis). *H. tuberculata* (n=100) and *H. erythrogramma* larvae (n=50) from all treatments and replicates were sampled every five days and daily, respectively (Table S13). Immediately after sampling, larval tissues were concentrated using a microcentrifuge, the seawater was removed with a sterile pipette, and frozen at -80°C before being preserved in RNAlater for long-term storage at -20°C.

Complementary to sampling urchin larvae, the environmental microbiota from the seawater was also sampled at each time point for both species. When cultures were sampled, ~0.5-L of seawater was filtering onto a 0.22-µm Millipore filter to retain the environmental microbiota (n=3). Full filter disks were frozen at -80°C before being preserved in RNAlater for long-term storage at -20°C.

### 5.2.3 Larval morphology

To validate that *H. tuberculata* larvae responded to the feeding environment (Soars et al., 2009), twenty larvae (n=20) from a single replicate of each dietary treatment were sampled for morphometric analysis. Larvae were imaged using a compound microscope (Leica DM5500) and morphometrics (length of larval body, post-oral arms, and stomach area; Fig. S5.2) were performed using ImageJ (Schneider, Rasband, & Eliceiri, 2012) (v. 1.9.2). We used a two-way analysis of variance (ANOVA; JMP Pro v. 13) to test whether larval morphology differed across time and between diets. We then performed a Tukey's post hoc test for pairwise comparisons for where statistical differences were detected.

### 5.2.4 Profiling bacterial communities

Total DNA was extracted from larval and seawater samples, as well as DNA kit blanks (n=4), using the GeneJet Genomic DNA Purification Kit (Thermo Scientific). DNA was quantified using a Qubit (Life Technologies) and diluted to 5 $ng \bullet \mu L^{-1}$ using RNase/DNase-free water. Bacterial sequences were then amplified using primers for the V3/V4 regions of the 16S rRNA gene (Klindworth et al., 2013) (Table S14). Products were purified using the Axygen AxyPrep Mag PCR Clean-up Kit (Axygen Scientific), indexed using the Nextera XT Index Kit V2 (Illumina Inc.), and then purified again. At each clean up step, fluorometric quantitation was performed using a Qubit and libraries were validated using a Bioanalyzer High Sensitivity DNA chip (Agilent Technologies). Illumina MiSeq sequencing (v3, 2x300 bp paired-end reads) was performed at the University of North Carolina at Charlotte.

Raw reads along with quality information were imported into QIIME 2 (Bolyen et al., 2019) (v.2019.1), where forward and reverse reads were paired using VSEARCH (Rognes, Flouri, Nichols, Quince, & Mahé, 2016), filtered by quality score, and denoised using Deblur (Amir et al., 2017). QIIME 2-generated 'features' were grouped into operational taxonomic units (ASVs) based on a minimum 99% similarity and were assigned taxonomy using SILVA (Quast et al., 2013) (v.132). Sequences matching to Archaea, present in the DNA kit blanks, or samples with less than 1,000 reads were discarded. The filtered biom table was then rarified to 4,091 sequences (Fig. S5.12).

To test whether community membership and composition were species-specific, shift over time, and in response to food availability, we calculated unweighted and

weighted UniFrac (Lozupone & Knight, 2005) values and compared them using principal coordinate analyses (PCoA). Results from these analyses were then recreated in QIIME 1 (Caporaso et al., 2010) (v.1.9.1) and stylized using Adobe Illustrator. We then used a two-way PERMANOVA to test for differences in membership and composition over the course of the differential feeding experiment and, subsequently, performed pairwise comparisons. To complement UniFrac values, we also calculated several measures of alpha diversity (*i.e.*, total ASVs, phylogenetic distance, McIntosh evenness, and McIntosh dominance) between species, over time, and across diets. We compared these values using a two-way analysis of variance (ANOVA) and, subsequently, performed by a Tukey's post-hoc test for pairwise comparisons between diets and times. Lastly, we summarized the bacterial groups associated with both *Heliocidaris* species and the number of shared and specific ASVs.

Our QIIME-based pipeline used to convert raw reads to ASVs for visualization is presented in detail in Note S1. Moreover, these reads 16S rRNA reads are publicly accessibly on Dryad under the DIO that is yet to be determined.

### 5.2.5 Estimating bacterial load

Total DNA extracted from *Heliocidaris* samples (n = 40 per species), as described above, were standardized to 5 ng•μL$^{-1}$ and used to estimate bacterial load. The relative abundance of 16S copies was quantified using 'universal' primers for the 16S rRNA gene (Baker, Smith, & Cowan, 2003; Alm, Oerther, Larsen, Stahl, & Raskin, 1996) and the Luna Universal qPCR Master Mix (New England BioLabs) (See, Table S14). Relative

abundance was calculated using the delta Ct method, with *H. erythrogramma* as a reference for normalization. Lastly, Ct values for both species were then compared using a Student's t-test and where $p<0.05$ was regarded as statistically significant.

### 5.2.6 Metagenomics

Total DNA extracted from two *H. erythrogramma* egg samples, as described above, were chosen for metagenomic sequencing of the bacterial symbiont. Libraries were made using the Nextera DNA FLEX Kit and labeled via the Nextera CD Index Kit. Libraries were fluorometrically quantified using Qubit and validated using a Bioanalyzer High Sensitivity DNA chip (Agilent Technologies). Illumina MiSeq sequencing (v3, 2x300 bp paired-end reads) was performed at the University of North Carolina at Charlotte.

Raw reads were trimmed and quality checked using Trimmomatic 0.33 (Bolger, Lohse, & Usadel, 2014) and FastQC, respectively. Reads with scores above Q30 were then mapped to the draft *H. erythrogramma* genome (Wray, unpublished data) to remove host reads. All unmapped reads from both samples (1.1 million paired-end reads in total) were co-assembled using SPAdes 3.13.0 (Bankevich et al., 2012) and the quality of the resulting five assembled contigs was assessed using QUAST (Gurevich, Saveliev, Vyahhi, & Tesler, 2013). Assembly statistics are outlined in Table S9. Genome completeness was determined using CheckM (Parks, Imelfort, Skennerton, Hugenholtz, & Tyson, 2015) against the Rickettsiales marker gene database and all contigs were then visualized using Circos (Krzywinski et al., 2009). Coding regions were determined and annotated using the RAST SEED database (Aziz et al., 2008), and each coding region was run through

HMMscan (Finn, Clements, & Eddy, 2011) to identify conserved domains and through EffectiveDB (Eichinger et al., 2015) to determine secreted effectors. KEGG modules were reconstructed in RAST to assess metabolic pathways.

The full length 16S rRNA gene was extracted from the metagenome to determine closest relatives in the NCBI database. Using the top 20 BLAST hits and other members of the Rickettsiales order, a RAxML maximum likelihood (Stamatakis, 2006) gene tree with 1,000 bootstrap replicates was generated in Geneious v11.0.3 (Kearse et al., 2012) with the JC69 substitution model as determined by the jModelTest (Darriba, Taboada, Doallo, & Posada, 2012). As described above for the 16S rRNA gene tree, the position within Rickettsiales was verified using 86 marker genes from known genomes (Table S10). This genome is publicly accessibly on NCBI under accession number that is yet to be determined.

### 5.2.7 Bacterial localization

The relative abundance of *E. raffii* declined following one day of development (Fig. S5.5) during which *H. erythrogramma* embryos shed their jelly coat (Williams & Anderson, 1975), suggesting the cellular location of *E. raffii*. To localize *E. raffii* relative to *H. erythrogramma* eggs (*i.e.*, jelly coat vs. the egg), we mechanically removed according to Edgar *et al.*(2019). Dejellied eggs were then rinsed with sterile ambient seawater, preserved in RNAlater, and stored long-term at -20°C. Total DNA extracted from dejellied *H. erythrogramma* egg, as described above. PCR primers for the *E. raffii* 16S rDNA were then designed using our draft genome (Table S14). The presence of this gene was compared between unfertilized *H. erythrogramma* eggs with and without the

jelly coat using PCR and these products were visualized by gel electrophoresis.

## 5.3 Results

### 5.3.1 Reduction in community diversity

Consistent with other larval echinoderms (Carrier et al., 2019; Carrier & Reitzel, 2018; Carrier & Reitzel, 2019; Carrier et al., 2018), the bacterial communities of *H. tuberculata* and *H. erythogramma* are species-specific and distinct from the environmental microbiota in both membership and composition (PERMANOVA, unweighted UniFrac, $p<0.001$; PERMANOVA, weighted UniFrac, $p<0.001$; Fig. 5.1A; Table S1). The bacterial community of *H. tuberculata* was significantly different from and, on average, more than three times as diverse as (total bacterial ASVs: ANOVA, $p<0.0001$; phylogenetic diversity: ANOVA, $p<0.0001$; Fig. 5.1B; Table S2) and had ~13 times more 16S copies (t-test, $p<0.0001$) than *H. erythogramma*. The planktotrophic *H. tuberculata* were also more taxonomically dominant (ANOVA, $p<0.0001$) and less even (ANOVA, $p=0.0002$) than *H. erythogramma* (Fig. 5.1B; Table S2). Moreover, the environmental microbiota was significantly more diverse than both *Heliocidaris* species (ANOVA, total ASVs, $p<0.0001$; ANOVA, phylogenetic distance, $p<0.0001$; Fig. 5.1B; Table S2).

Despite differences in community composition (Fig. 5.1A) and structure (Fig. 5.1B), *H. tuberculata* and *H. erythogramma* shared 38 ASVs (10.7%), with 263 and 55 ASVs being specific to *H. tuberculata* and *H. erythogramma*, respectively (Fig. 5.1C). A majority of this community was composed of α- and γ-proteobacteria as well as the Bacteroidia. The α-proteobacteria (t-test, 0.014; Fig. 5.1C, S1; Table S3) and γ-

proteobacteria (t-test, 0.051; Fig. 5.1C, S5.1; Table S3), in particular, were significantly different and inversely abundant between *Heliocidaris* species. Bacteroidia, on the other hand, was similar in abundance (t-test, 0.522; Fig. 5.1C, S5.1; Table S3).

### 5.3.2 Loss of a diet specific microbiota

As previously reported (Soars et al., 2009), *H. tuberculata* exhibits morphological plasticity in response to food availability (ANOVA, $p<0.0001$), whereby diet-restricted larvae increase the length of their post-oral (feeding) arms relative to the larval body (Fig. S5.2; Table S4). Morphological plasticity for *H. tuberculata* correlated with shifts in the bacterial community (Figs. 5.2, S5.2), such that food availability induced and correlates with diet-specific microbiota (PERMANOVA, unweighted UniFrac, $p>0.05$; PERMANOVA, weighted UniFrac, $p<0.05$; Fig. 5.2, Tables S5-6). This response, however, was not observed for *H. erythogramma*, as these developmental stages associated with bacterial communities that were consistent across diets (PERMANOVA, unweighted UniFrac, $p>0.05$; PERMANOVA, weighted UniFrac, $p>0.05$; Fig. 5.2; Tables S5-6).

Over the course of differential feeding, community structure did not differ between dietary treatments but did over time for both *H. tuberculata* (ANOVA, total ASVs: diet: $p>0.05$, time: $p<0.001$; ANOVA, phylogenetic distance: diet: $p>0.05$, time: $p=0.005$) and *H. erythogramma* (ANOVA, total ASVs: diet: $p>0.05$, time: $p<0.001$; ANOVA, phylogenetic distance: diet: $p>0.05$, time: $p<0.001$) (Fig. S5.3; Table 7). Each *Heliocidaris* species, however, underwent opposing temporal successions: the bacterial communities of *H. tuberculata* were taxonomically and phylogenetically rich in early

stages and stably fluctuated over developmental time while *H. erythogramma* eggs were dominated by a single bacterial ASVs and diversified throughout development (Fig. S5.3; Table 7).

The taxonomic and phylogenetic diversity of early-stage *H. tuberculata* was typical of planktotrophs (Carrier & Reitzel, 2018) (Fig. S5.4). Taxonomic dominance by a single ASV (representing 95.1% of the community) for *H. erythogramma* eggs, on the other hand, is uncommon for echinoids (Fig. S5.4). This ASV associated with *H. erythogramma* eggs was within the Rickettsiales and was barely detectable (<0.27%) in *H. tuberculata* larvae and the seawater. Moreover, the abundance of this ASV declined abruptly (to ~8.0%) following one day of development and slightly thereafter through to metamorphosis (ANOVA, $p<0.0001$; Fig. S5.5; Table 8). Classification of this ASV suggests that it is most similar to *Wolbachia* spp. and belongs to the Rickettsiales (Fig. S5.6; Note S2), a bacterial order rich with parasitic symbionts that infect animals across the tree of life (Engelstädter & Hurst, 2009; Werren, Baldo, & Clark, 2008).

### 5.3.3 Identification of a novel Rickettsiales lineage

Provided the functional relevance of the Rickettsiales and *Wolbachia* (Engelstädter & Hurst, 2009; Werren et al., 2008) to animal biology, we sequenced the metagenome of *H. erythogramma* eggs. Following trimming and filtering of host sequences based on a *H. erythogramma* draft genome (Wray, unpublished data), a total of ~1.13 million paired-end reads remained across both samples (Fig. S5.7). These co-assembled into five scaffolds that totaled ~1.11 million bp with 460x read coverage. This

draft genome was nearly complete at 97.2% (Fig. S5.8) and had a GC content (33.3%) typical of endosymbionts (see, Table S9 for full assembly statistics) (McCutcheon, Boyd, & Dale, 2019).

One full length 16S rRNA gene was identified within these scaffolds and was compared to other Rickettsiales as well as the top 20 known nucleotide sequences in the NCBI database. This bacterium fell within the Anaplasmataceae family and had an ~85% sequence similarity to various *Wolbachia* endosymbionts from a wide range of nematode and insect hosts (Fig. 5.3A). A phylogenetic analysis using 86 marker genes from Rickettsiales isolates with available genomes verified this position within the Anaplasmataceae and the novelty of this bacterium (Fig. S5.9; Table S10). Due to the divergence of this lineage, we propose the genus "*Candidatus* Echinorickettsia," with this nearly complete genome named "*Candidatus* E. raffii," after the late Dr. Rudy Raff who was an early pioneer of the *Heliocidaris* system (Moczek, Henry, & Byrne, 2019; Wray & Haag, 2019).

To assess the genetic content of *E. raffii*, we identified 1,206 protein coding sequences (CDS) using RAST (Aziz et al., 2008) and, of these, 324 CDS regions (26.9%) were classified as hypothetical proteins, further supporting the novelty of this bacterium. The *E. raffii* genome included several genes to encode for a flagellum as well as two abundant gene groups (Fig 5.4). First, we observed numerous transport protein complexes, such as a Type IV secretion system (TIVSS) and ABC transporters (Fig 5.4), that are indicative of crosstalk between *E. raffii* and *H. erythogramma*. Second, we observed 65 effector proteins that were predicted to be secreted from these transporters, including three that maintained eukaryotic-like domains (Table S11-12). Two of these eukaryotic-like

effectors were ankyrins, a diverse protein family known to manipulate eukaryotic ligands (Caturegli et al., 2000; Hryniewicz-Jankowska, Czogalla, Bok, & Sikorsk, 2002). The third was a hypothetical protein with a synaptobrevin domain, a protein necessary in the all-or-nothing acrosomal reaction during sea urchin fertilization (Conner, Leaf, & Wessel, 1998).

Nearly one third of the remaining CDS for *E. raffii* had KEGG orthologs (KO) that were related to metabolism. Notably, many of the metabolic modules necessary for carbon metabolism were incomplete (Fig. S5.10), a feature characteristic of parasitic Rickettsiales that scavenge essential resources from the host (Driscoll et al., 2017). *E. raffii* does, however, encode numerous enzymes, for example, within the glycerolphospholipid metabolism pathway (Fig. 5.4). This includes enzymes that are required for the metabolism of diacylglycerols ethers (Fig. 5.4), a primary lipid group acquired during the transition from planktotrophy to lecithotrophy and, specifically, in the eggs and embryos of *H. erythogramma* (Byrne & Sewell, 2019; Davidson et al., 2019; Villinski, Villinski, Byrne, & Raff, 2002).

### 5.3.4 Localization of *Echinorickettsia raffii*

Within hours of the embryonic jelly coat being shed (Williams & Anderson, 1975), we observed that the number of *E. raffii* sequences declined from 95.1% to 8.0% of the community (ANOVA, $p<0.0001$; Fig. S5). To determine if *E. raffii* localized to the jelly coat or within the egg, we compared whether the 16S rRNA sequence for *E. raffii* could be detected in *H. erythogramma* eggs with and without the jelly coat. Following mechanical removal of the jelly coat (Raff et al., 1999), the 16S rRNA sequence of *E.*

*raffii* was detected in dejellied *H. erythogramma* egg (Fig. S5.11), suggesting that *E. raffii* is intracellular.

## 5.4 Discussion

The gastrointestinal tract is a principle animal organ system for microbial colonization and is primarily responsible for the taxonomic and functional diversity of animal-microbe symbioses. Co-evolution of host and microbiota has shaped metabolic codependency and mediated niche expansion of the host (Annunziata, Andrikou, Perillo, Cuomo, & Arnone, In Press; Brooks et al., 2016; Douglas, 2009; Fraune & Bosch, 2010; Ley et al., 2008; McFall-Ngai et al., 2013; Moeller et al., 2016; Shapira, 2016). The evolutionary significance of symbioses between the animal host and the associated microbiota is primarily derived from systems that depend on this organ for survival (Ley et al., 2008; Moeller et al., 2016; Shapira, 2016). This is not always the case, as many gutless invertebrates in coastal sediments (*e.g.*, *Solemya velum*; Stewart & Cavanaugh, 2006)) and the deep-sea (*e.g.*, *Riftia pachyptila*; Stewart & Cavanaugh, 2005) utilize chemosymbionts as the primary vector for nutrition (Dubilier, Bergin, & Lott, 2008). However, we lack an understanding of the consequences that coincide with the evolutionary loss of the gastrointestinal tract that occur, for example, during the transition from planktotrophy to lecithotrophy (Strathmann, 1978, 1985; Vance, 1973; Wray & Raff, 1991).

Differential feeding of the planktotrophic *H. tuberculata* and lecithotrophic *H. erythogramma* supports three primary findings. First, the planktotroph is able to respond to and associate with a bacterial community specific to food availability while the

lecithotroph does not. Second, the bacterial community associated with the lecithotroph is a third as taxonomically and phylogenetically diverse with ~13 times less bacterial cells as the planktotrophic congener. Third, the derived lecithotroph acquired a novel Rickettsiales lineage with homologous genes known to manipulate host reproduction and metabolize diacylglycerol ethers.

Several species of planktotrophic echinoderms associate with bacterial communities that are taxonomically and compositionally specific to food availability (Carrier et al., 2019; Carrier & Reitzel, 2018; Carrier & Reitzel, 2019; Carrier & Reitzel, In review; Carrier et al., 2018). When diet restriction is prolonged and morphological plasticity is expressed, planktotrophs exhibit shifts in the symbiont community that precede and then correlate with the expression of morphological plasticity (Carrier & Reitzel, 2018; Carrier & Reitzel, 2019). Like other planktotrophs, we observed that *H. tuberculata* associated with a diet-specific bacterial community that was induced in response to the feeding environment. Following the loss in the dependency on exogenous resources, we were unable to observe this ecological responsiveness for the derived lecithotroph of *H. erythogramma*. This would suggest that in the transition from planktotrphy to lecithotrophy, larval holobiont may have lost mechanisms to differentially associate with microbiota in different feeding environments

Following a simplification in the morphological architecture, the transition towards lecithotrophy leads to a loss in a functional gastrointestinal tract (Smith, Zigler, & Raff, 2007; Wray & Raff, 1991). For *H. erythogramma*, extension of the archenteron is abbreviated, the blastopore is covered shortly after invagination, and this heavily modified gut remains inactive (Williams & Anderson, 1975; Wray & Raff, 1989; Wray &

Raff, 1991; Wray & Raff, 1991). The taxonomic and phylogenetic diversity of the bacterial community associated with *H. erythogramma* is approximately a third of the ancestral *H. tuberculata* with a bacteria load that is ~13 times less. This suggests that the transition from planktotrophy to lecithotrophy and evolutionary loss of a functional gut (Williams & Anderson, 1975; Wray & Raff, 1989; Wray & Raff, 1991; Wray & Raff, 1991) is correlated with a major reduction in the diversity and bacterial load of host-associated microbiota. This, in principle, is also consistent with the expectation and overall consensus that the evolution of the gut gave rise to dense animal-associated microbial communities that were more taxonomically and phylogenetically diverse (Brooks et al., 2016; Douglas, 2009; Fraune & Bosch, 2010; Ley et al., 2008; McFall-Ngai et al., 2013; Moeller et al., 2016; Shapira, 2016).

Arguably the most notable modification during the transition towards lecithotrophy is to oogenesis (Emlet, McEdward, & Strathmann, 1987; Smith et al., 2007; Strathmann, 1978; Thorson, 1950; Vance, 1973; Wray & Raff, 1991), where maternal investment (*i.e.*, egg size) is altered to provide the offspring with a surplus of diacylglycerol ethers and other nutrients (Byrne & Sewell, 2019; Davidson et al., 2019; Villinski et al., 2002). This biochemical transition in echinoid eggs is correlated with a switch towards taxonomically and phylogenetically shallow bacterial communities and the evolution of vertical transmission (Bright & Bulgheresi, 2010; Carrier & Reitzel, 2019; Carrier & Reitzel, In review; Holland & Nealson, 1978; Manahan, Davis, & Stephens, 1993; McFall-Ngai & Ruby, 2000). For *H. erythogramma*, this includes the potentially unique association with *E. raffii*, a novel Rickettsiales lineage with genes to metabolize diacylglycerol ethers. These lipids are partially utilized during larval

development but primarily reserved for juvenile growth, perhaps suggesting that this plentiful resource may also be used by *E. raffii* to sustain an effective population between life stages.

Metabolism of diacylglycerol ethers was one of several primary genomic features of *E. raffii*. Specifically, *E. raffii* had a number of ankyrin domains, a type IV secretion system, and sperm modulators (Conner et al., 1998; Epel, 1978; Vacquier, Epel, & Douglas, 1972). In *Wolbachia* and other Rickettsiales, these gene groups are known to influence host reproduction through cytoplasmic incompatibility, feminization, and make killing (Engelstädter & Hurst, 2009; Werren et al., 2008). It is unknown whether *E. raffii* is functionally equivalent, but we suspect *Heliocidaris* reproduction may be manipulated for three reasons. First, *Heliocidaris* is sympatric in Sydney Harbor (Mortensen, 1943; Wray & Raff, 1991) and the onset of spawning by *H. erythrogramma* coincides with gamete release for *H. tuberculata* (Laegdsgaard, Byrne, & Anderson, 1991; Williams & Anderson, 1975). Second, *H. tuberculata* sperm bind to but cannot fertilize *H. erythrogramma* eggs unless the jelly coat is removed (Raff et al., 1999; Zigler et al., 2003), allowing for a fertilization efficiency (~85%) equal to the reciprocal cross (Zigler et al., 2003). Third, the adult population of *H. erythrogramma* is disproportionately female (Dix, 1977), which is an unlikely occurrence for benthic marine invertebrates (Levitan, 2005). We, therefore, suspect that *E. raffii* may have similar functions as species of *Wolbachia* known to induce cytoplasmic incompatibility (Bordenstein, O'Hara, & Werren, 2001) and, perhaps, mediate speciation (Brucker & Bordenstein, 2012; Brucker & Bordenstein, 2013).

While the influence on *Heliocidaris* reproduction is unknown, we find evidence

suggesting that *E. raffii* is also an endosymbiont and, due to the sharp decline after hatching, we suspect that this bacterium is located on the periphery of the egg and may enter the jelly coat. For echinoids, the jelly coat encases the egg, in part, to promote conspecific fertilization and is shed during late embryogenesis. This, however, isn't the case for *H. erythrogramma*, as a small piece of the jelly coat remains attached to the vegetal pole, is internalized as the archenteron invaginates, and is then guarded by anti-microbial amoebocytes (Williams & Anderson, 1975). This developmental modification may account for the small portion of *E. raffii* that remained associated with *H. erythrogramma* after hatching (*i.e.*, one day of development) and throughout development. This inoculum may then utilize the high abundance of diacylglycerol ethers allocated for juvenile growth to regain an effective population size in the benthic life stage for *H. erythrogramma* (Byrne & Sewell, 2019; Davidson et al., 2019).

What remains uncertain is when during this life history transition may a bacterium similar to *E. raffii* be acquired. For sea urchins, the transition from planktotrophy to lecithotrophy includes two primary intermediate stages (Fig. 5.5) (Allen & Pernet, 2007; Smith et al., 2007; Vance, 1973; Wray, 1996). The first is facultative planktotrophy (*e.g.*, *Clypeaster*; Emlet, 1986), where an increase in maternal investment has relaxed the requirement, but retained the ability, to feed (Allen & Pernet, 2007; Smith et al., 2007; Wray, 1996). The second is facultative lecithotrophy, whereby the ability to feed has been lost and the morphological and digestive structures have been reduced (Fig. 5.5) (Allen & Pernet, 2007; Smith et al., 2007; Wray, 1996). We suspect that community diversity is inversely related to maternal investment and a symbiont akin to *E. raffii* may be acquired once an effective population can be sustained between host generations (Fig. 5.5). Despite

these unknowns, we find it feasible that the microbiome is a dynamic character in animal evolution and this may be modified during major life-history transitions.

Acknowledgements

We thank the Sydney Institute of Marine Science for their endless assistance with experimental logistics; Karen Lopez and Daniel Janies (UNC Charlotte) for sequencing resources and technical assistance with sequencing; and the Wray Laboratory (Duke University) for providing dejellied eggs and access to in-house genomic resources.

TJC was supported by an NSF Graduate Research Fellowship and an NSF Graduate Research Opportunities World award; B.A.L. was supported by a National Institute of Health F32 Ruth Kirschstein Postdoctoral Fellowship; D.D. was funded by the University of Sydney; S.R.B. was funded by the NIH (R01 AI132581), the NSF (IOS 1456778), and the Vanderbilt Microbiome Initiative; M.B. was funded by Australian Research Council; and A.M.R. was supported by a Human Frontier Science Program Award (RGY0079/2016).

References

Allen, J. D., & Pernet, B. (2007). Intermediate modes of larval development: bridging the gap between planktotrophy and lecithotrophy. *Evolution & Development, 9*, 43-653.

Alm, E.W., Oerther, D.B., Larsen, N., Stahl, D.A., & Raskin, L. (1996). The oligonucleotide probe database. *Applied and Environmental Microbiology, 62*, 3557-3559.

Amir, A., McDonald, D., Navas-Molina, J. A., Kopylova, E., Morton, J. T., Xu, Z. Z., . . . Knight, R. (2017). Deblur rapidly resolves single-nucleotide community sequence patterns. *mSystems, 2*, e00191-00116.

Annunziata, R., Andrikou, C., Perillo, M., Cuomo, C., & Arnone, M. I. (In Press). Development and evolution of gut structures: from molecules to function. *Cell and Tissue Research*.

Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., . . . Zagnitko, O. (2008). The RAST Server: rapid annotations using subsystems technology. *BMC Genomics, 9*, 75.

Baker, G. C., Smith, J. J., & Cowan, D.A. (2003). Review and re-analysis of domain-specific 16S primers. *Journal of Microbiology Methods, 55*, 541-555.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., . . . Pyshkin, A. P. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology, 19*, 455-477.

Bates, J. M., Mittge, E., Kuhlman, J., Baden, K. N., Cheesman, S. E., & Guillemin, K. (2006). Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. *Developmental Biology, 297*, 374-386.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics, 30*(15), 2114-2120.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C., Al-Ghalith, G. A., . . . Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*.

Bordenstein, S. R., O'Hara, F. P., & Werren, J. H. (2001). Wolbachia-induced incompatibility precedes other hybrid incompatibilities in Nasonia. *Nature, 409*, 707-710.

Bright, M., & Bulgheresi, S. (2010). A complex journey: transmission of microbial symbionts. *Nature Reviews Microbiology, 8*, 218-230.

Brooks, A. W., Kohl, K. D., Brucker, R. M., van Opstal, E. J., & Bordenstein, S. R. (2016). Phylosymbiosis: relationships and functional effects of microbial communities across host evolutionary history. *Plos Biology, 14*(11), e2000225.

Brucker, R. M., & Bordenstein, S. R. (2012). Speciation by symbiosis. *Trends in Ecology & Evolution, 27*, 443-451.

Brucker, R. M., & Bordenstein, S. R. (2013). The hologenomic basis of speciation: gut bacteria cause hybrid lethality in the genus Nasonia. *Science, 341*, 667-669.

Byrne, M., Emlet, R. B., & Cerra, A. (2001). Ciliated band structure in planktotrophic and lecithotrophic larvae of Heliocidaris species (Echinodermata: Echinoidea): a demonstration of conservation and change. *Acta Zoologica, 82*, 189-199.

Byrne, M., & Sewell, M. A. (2019). Evolution of maternal lipid provisioning strategies in echinoids with non-feeding larvae: selection for high-quality juveniles. *Marine Ecology Progress Series, 616*, 95-106.

Byrne, M., Villinski, J. T., Cisternas, P., Siegel, R. K., Popodi, E., & Raff, R. A. (1999). Maternal factors and the evolution of developmental mode: Evolution of oogenesis in Heliocidaris erythrogramma. *Development Genes and Evolution, 209*, 275-283.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., . . . Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods, 7*(5), 335-336.

Carrier, T. J., Dupont, S., & Reitzel, A. M. (2019). Geographic location and food availability offer differing levels of influence on the bacterial communities associated with larval sea urchins. *Fems Microbiology Ecology, 95*, fiz103.

Carrier, T. J., & Reitzel, A. M. (2018). Convergent shifts in host-associated microbial communities across environmentally elicited phenotypes. *Nature Communications, 9*, 952.

Carrier, T. J., & Reitzel, A. M. (2019). Bacterial community dynamics during embryonic and larval development of three confamilial echinoids. *Marine Ecology Progress Series, 611*, 179-188.

Carrier, T. J., & Reitzel, A. M. (2019). Shift in bacterial taxa precedes morphological plasticity in a larval echinoid 166: 164.

Carrier, T. J., & Reitzel, A. M. (In review). Symbiotic life of echinoderm larvae.

Carrier, T. J., Wolfe, K., Lopez, K., Gall, M., Janies, D. A., Byrne, M., & A.M., R. (2018). Diet-induced shifts in the crown-of-thorns (Acanthaster sp.) larval microbiome. *Marine Biology, 165*, 157.

Caturegli, P., Asanovich, K. M., Walls, J. J., Bakken, J. S., Madigan, J. E., Popov, V. L., & Dumler, J. S. (2000). ankA: an Ehrlichia phagocytophila group gene encoding a cytoplasmic protein antigen with ankyrin repeats. *Infection and Immunity, 68*, 5277-8523.

Conner, S., Leaf, D., & Wessel, G. (1998). Members of the SNARE hypothesis are associated with cortical granule exocytosis in the sea urchin egg. *Molecular Reproduction and Development, 48*, 106-118.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods, 9*, 772.

Davidson, P. L., Thompson, J. W., Foster, M. W., Moseley, M. A., Byrne, M., & Wray, G. A. (2019). A comparative analysis of egg provisioning using mass spectrometry during rapid life history evolution in sea urchins. *Evolution & Development, 21*, 188-204.

Dix, T. G. (1977). Reproduction in Tasmanian populations of Heliocidaris erythrogramma (Echinodermata: Echinometridae). *Australian Journal of Marine and Freshwater Research, 28*, 509-520.

Douglas, A. E. (2009). The microbial dimension in insect nutritional ecology. *Functional Ecology, 23*, 38-47.

Driscoll, T. P., Verhoeve, V. I., Guillotte, M. L., Lehman, S. S., Rennoll, S. A., Beier-Sexton, M., . . . Gillespie, J. J. (2017). Wholly Rickettsia! Reconstructed metabolic

profile of the quintessential bacterial parasite of eukaryotic cells. *mBio, 8*, e00859-00817.

Dubilier, N., Bergin, C., & Lott, C. (2008). Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. *Nature Reviews Microbiology, 6*(10), 725-740.

Edgar, A., Byrne, M., & Wray, G. (2019). Embryo microinjection of the lecithotrophic sea urchin Heliocidaris erythrogramma. *Journal of Biological Methods, 6*(e119).

Eichinger, V., Nussbaumer, T., Platzer, A., Jehl, M. A., Arnold, R., & Rattei, T. (2015). EffectiveDB—updates and novel features for a better annotation of bacterial secreted proteins and Type III, IV, VI secretion systems. *Nucleic Acids Research, 44*, D669-D674.

Emlet, R. B. (1986). Facultative planktotrophy in the tropical echinoid Clypeaster rosaceus (Linnaeus) and a comparison with obligate planktotrophy in Clypeaster subdepressus (Gray) (Clypeasteroida, Echinoidea). *Journal of Experimental Marine Biology and Ecology, 95*, 183-202.

Emlet, R. B., McEdward, L. R., & Strathmann, R. R. (Eds.). (1987). *Echinoderm larval ecology viewed from the egg*: CRC Press.

Engelstädter, J., & Hurst, G. D. D. (2009). The ecology and evolution of microbes that manipulate host reproduction. *Annual Review of Ecology, Evolution, and Systematics, 40*, 127-149.

Epel, D. (1978). Mechanisms of activation of sperm and egg during fertilization of sea urchin gametes. *Current Topics in Developmental Biology, 12*, 185-246.

Finn, R. D., Clements, J., & Eddy, S. R. (2011). HMMER web server: interactive sequence similarity searching. *Nucleic Acids Research, 39*, W29-W37.

Fraune, S., & Bosch, T. C. G. (2010). Why bacteria matter in animal development and evolution. *Bioessays, 32*, 571-580.

Gurevich, A., Saveliev, V., Vyahhi, N., & Tesler, G. (2013). QUAST: quality assessment tool for genome assemblies. *Bioinformatics, 29*, 1072-1075.

Holland, N. D., & Nealson, K. H. (1978). The fine structure pf the echinoderm cuticle and subcuticular bacteria of echinoderms. *Acta Zoologica, 59*, 169-185.

Hryniewicz-Jankowska, A., Czogalla, A., Bok, E., & Sikorsk, A. (2002). Ankyrins, multifunctional proteins involved in many cellular pathways. *Folia Histochemica et Cytobiologica, 40*, 239-249.

Israel, J. W., Martik, M. L., Byrne, M., Raff, E. C., Raff, R. A., McClay, D. R., & Wray, G. A. (2016). Comparative developmental transcriptomics reveals rewiring of a highly conserved gene regulatory network during a major life history switch in the sea urchin genus Heliocidaris. *Plos Biology, 14*(3), e1002391.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., . . . Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics, 28*, 1647-1649.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glockner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research, 41*(1), e1.

Kohl, K. D., & Carey, H. V. (2016). A place for host-microbe symbiosis in the comparative physiologist's toolbox. *Journal of Experimental Biology, 219*, 3496-3504.

Krzywinski, M., Schein, J., Birol, I., Connors, J., Gascoyne, R., Horsman, D., . . . Marra, M. A. (2009). Circos: an information aesthetic for comparative genomics. *Genome Research, 19*, 1639-1645.

Laegdsgaard, P., Byrne, M., & Anderson, D. T. (1991). Reproduction of sympatric populations of Heliocidaris erythrogramma and H. tuberculata (Echinoidea) in New South Whales. *Marine Biology, 110*, 359-374.

Levitan, D. L. (2005). The distribution of male and female reproductive success in a broadcast spawning marine invertebrate. *Integrative and Comparative Biology, 45*, 848-855.

Ley, R. E., Lozupone, C. A., Hamady, M., Knight, R., & Gordon, J. I. (2008). Worlds within worlds: evolution of the vertebrate gut microbiota. *Nature Reviews Microbiology, 6*, 776.

Lozupone, C., & Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology, 71*(12), 8228-8235.

Manahan, D. T., Davis, J. P., & Stephens, G. C. (1993). Bacteria-free sea urchin larvae: selective uptake of neutral amino acids from seawater. *Science, 220*(4593), 204-206.

McCutcheon, J. P., Boyd, B. M., & Dale, C. (2019). The life of an insect endosymbiont from the cradle to the grave. *Current Biology, 29*, R485–R495.

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C. G., Carey, H. V., Domazet-Loso, T., Douglas, A. E., . . . Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences of the United States of America, 110*(9), 3229-3236.

McFall-Ngai, M., & Ruby, E. G. (2000). Developmental biology and marine invertebrate symbioses. *Current Opinion in Microbiology, 3*, 603-607.

Mileikovsky, S. A. (1971). Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evalution. *Marine Biology, 10*, 193-213.

Moczek, A. P., Henry, J. J., & Byrne, M. (2019). Rudolf A. Raff. *Evolution & Development, 21*, 113.

Moeller, A. H., Caro-Quintero, A., Mjungu, D., Georgiev, A. V., Lonsdorf, E. V., Muller, M. N., . . . Ochman, H. (2016). Cospeciation of gut microbiota with hominids. *Science, 353*(6297), 380-382.

Mortensen, T. (1943). *A Monograph of the Echinoidea: Camarodonota* (Vol. III): Reitzel Press.

Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., & Tyson, G. W. (2015). CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Research, 25*, 1043-1055.

Partridge, L., & Harvey, P. H. (1988). The ecological context of life history evolution. *Science, 241*, 1449-1455.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., . . . Glockner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research, 41*, 590-596.

Raff, E. C., Popodi, E. M., Sly, B. J., Turner, F. R., Villinski, J. T., & Raff, R. A. (1999). A novel ontogenetic pathway in hybrid embryos between species with different modes of development. *Development, 126*(9), 1937-1945.

Raff, R. A. (1992). Direct-developing sea urchins and the evolutionary reorganization of early development. *Bioessays, 12*(4), 211-218.

Raff, R. A., & Byrne, M. (2006). The active evolutionary lives of echinoderm larvae. *Heredity, 97*, 244-252.

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ, 4*, e2584.

Schneider, C., Rasband, W., & Eliceiri, K. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, 9*, 671-675.

Shapira, M. (2016). Gut microbiotas and host evolution: scaling up symbiosis. *Trends in Ecology & Evolution, 31*(7), 539-549. doi:10.1016/j.tree.2016.03.006

Smith, S. S., Zigler, K. S., & Raff, R. A. (2007). Evolution of direct-developing larvae: selection vs loss. *Bioessays, 29*, 566-571.

Soars, N. A., Prowse, T. A. A., & Byrne, M. (2009). Overview of phenotypic plasticity in echinoid larvae, 'Echinopluteus transversus' type vs. typical echinoplutei. *Marine Ecology Progress Series, 383*, 113-125.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics, 22*, 2688-2690.

Stearns, S. C. (1989). Trade-offs in life-history evolution. *Functional Ecology, 3*, 259-268.

Stewart, F. J., & Cavanaugh, C. M. (2005). Symbiosis of thioautotrophic bacteria with Riftia pachyptila. In J. Overmann (Ed.), *Molecular Basis of Symbiosis* (pp. 197-225): Springer.

Stewart, F. J., & Cavanaugh, C. M. (2006). Bacterial endosymbioses in Solemya (Mollusca: Bivalvia)—model systems for studies of symbiont–host adaptation. *Antonie Van Leeuwenhoek, 90*, 343-360.

Strathmann, R. R. (1978). The evolution and loss of feeding larval stages of marine invertebrates. *Evolution, 32*(894-906).

Strathmann, R. R. (1985). Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *Annual Review of Ecology and Systematics, 16*, 339-361.

Strathmann, R. R. (1993). Hypotheses on the origins of marine larvae. *Annual Review of Ecology and Systematics, 24*, 89-117.

Thorson, G. (1950). Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews, 25*, 1-45.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature, 444*, 1027-1031.

Vacquier, V. D., Epel, D., & Douglas, L. A. (1972). Sea urchin eggs release protease activity at fertilization. *Nature, 237*, 34-36.

Vance, R. R. (1973). On reproductive strategies in marine benthic invertebrates. *The American Naturalist, 107*, 339-352.

Villinski, J. T., Villinski, J. C., Byrne, M., & Raff, R. A. (2002). Convergent maternal provisioning and life-history evolution in echinoderms. *Evolution, 56*, 1764-1775.

Werren, J. H., Baldo, L., & Clark, M. E. (2008). Wolbachia: master manipulators of invertebrate biology. *Nature Reviews Microbiology, 6*, 741-751.

Williams, D. H. C., & Anderson, D. T. (1975). The reproductive system, embryonic development, larval development, and metamorphosis of the sea urchin Heliocidaris erythrogramma (Val.) (Echinodea: Echinometridae). *Australian Journal of Zoology, 23*, 371-403.

Wray, G. A. (1996). Parallel evolution of nonfeeding larvae in echinoids. *Systematic Biology, 45*, 308-322.

Wray, G. A., & Haag, E. S. (2019). Rudolf A. Raff (1941–2019). *Nature Ecology & Evolution, 3*, 518-519.

Wray, G. A., & Raff, R. A. (1989). Evolutionary modification of cell lineage in the direct-developing sea urchin Heliocidaris erythrogramma. *Developmental Biology, 132*, 458-470.

Wray, G. A., & Raff, R. A. (1991). The evolution of developmental strategy in marine invertebrates. *Trends in Ecology & Evolution, 6*(2), 45-50.

Wray, G. A., & Raff, R. A. (1991). Rapid evolution of gastrulation mechanisms in a sea urchin with lecithotrophic larvae. *Evolution, 45*, 1741-1750.

Zigler, K. S., Raff, E. C., Popodi, E., Raff, R. A., & Lessios, H. A. (2003). Adaptive evolution of bindin in the genus Heliocidaris is correlated with the shift to direct development. *Evolution, 57*(10), 2293-2302.

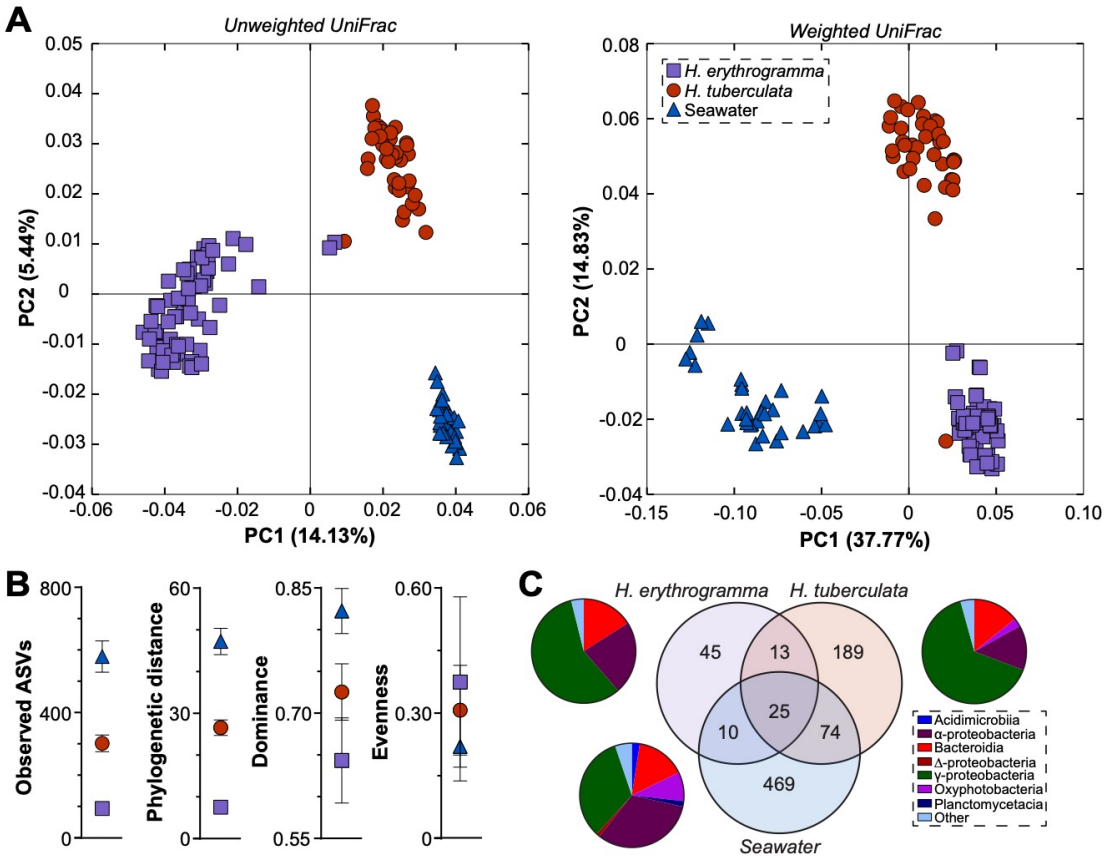


Figure 5.1. Differential structuring of the microbiota between life-histories. (A) Community similarity of the larval-associated microbiota based on membership (unweighted UniFrac; left) and composition (weighted UniFrac; right) for *Heliocidaris tuberculata* (red triangles), *H. erythrogramma* (purple squares), the environmental microbiota (blue circles). (B) Enumeration of operational taxonomic units (ASVs), phylogenetic distance of those ASVs, dominance, and evenness between both *Heliocidaris* species and the seawater. (C) Distribution of total ASVs between both *Heliocidaris* species and the seawater, and class-level summaries of those ASVs.

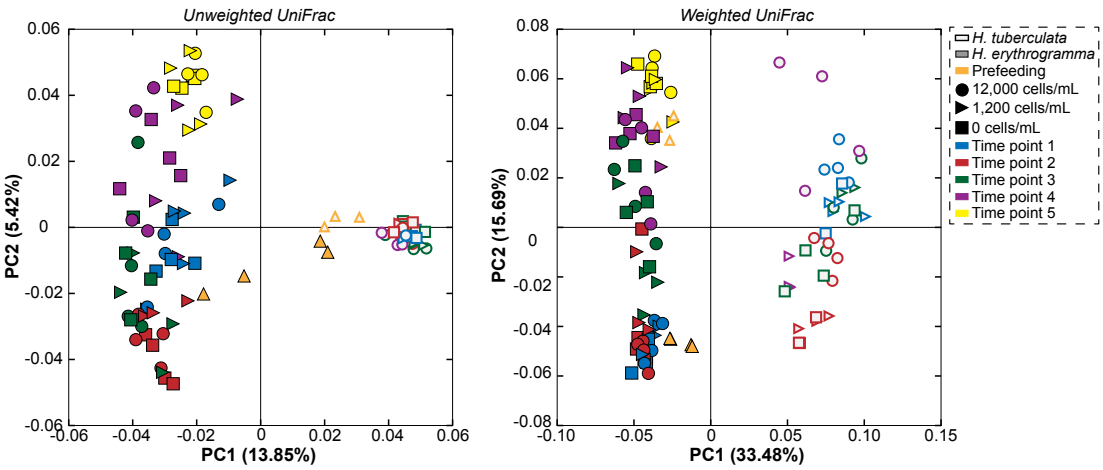


Figure 5.2. Loss of diet-specificity in the larval-associated microbiota. Community similarity (membership/unweighted UniFrac, left; composition/weighted UniFrac, right) of the larval-associated microbiota for *Heliocidaris tuberculata* and *H. erythrogramma* based on food availability (12,000, 1,200, and 0 cells•mL$^{-1}$ of a phytoplankton represented by rightward triangle, circle, and square, respectively) and over several time points (times 1, 2, 3, 4, and 5 were represented by blue, red, green, purple, and yellow colorings).

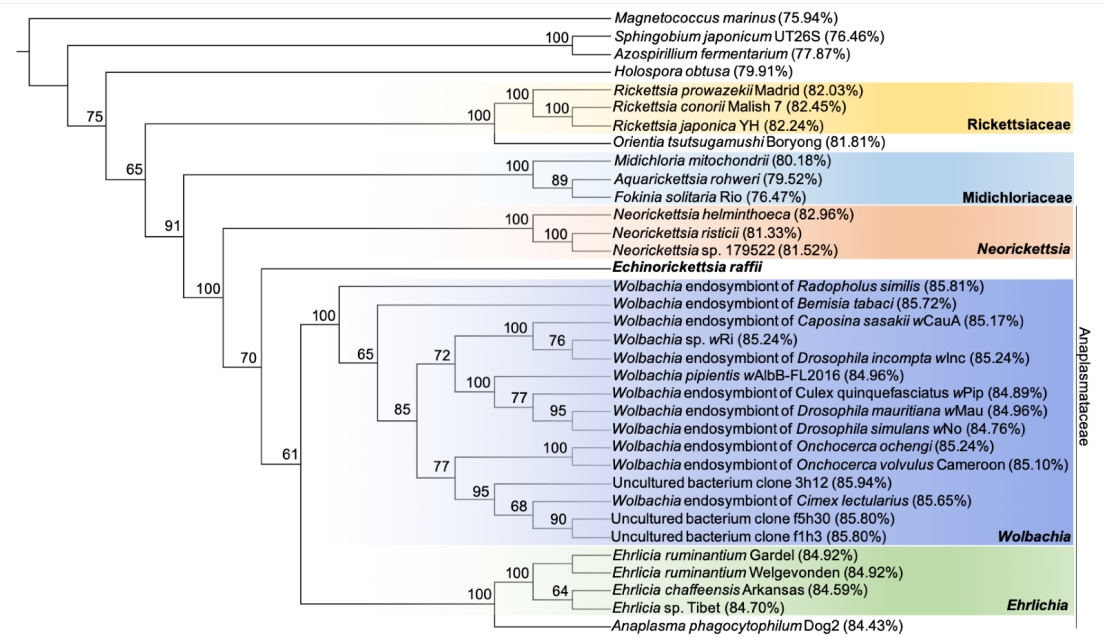


Figure 5.3. Gene tree of Rickettsiales with the novel *Echinorickettsia raffii* lineage. A RAxML maximum likelihood gene tree for the full length 16S rRNA gene of Rickettsiales as well as the top 20 sequences with the highest similarity in the NCBI database. A total of 1,000 bootstrap replicates were utilized, placing *E. raffii* into the Anaplasmataceae family and as a sister genus to the *Wolbachia*.

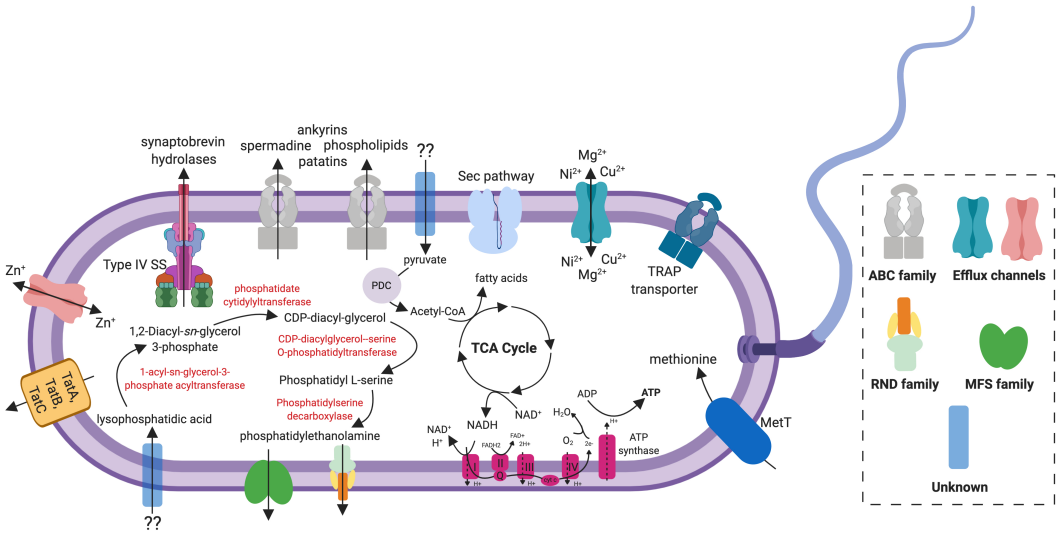


Figure 5.4. Genomic features of *Echinorickettsia raffii* lineage. The major enzymatic pathways (*e.g.*, catabolism of diacylglycerols ethers), transport systems (*e.g.*, efflux channels and ABC transporters), and secretion systems (*e.g.*, a Type IV secretion system) in the genome of *E. raffii* are illustrated in this cartoon. Moreover, enzymes are in red text and predicted effector proteins are illustrated outside of the cell from their predicted secretion system.

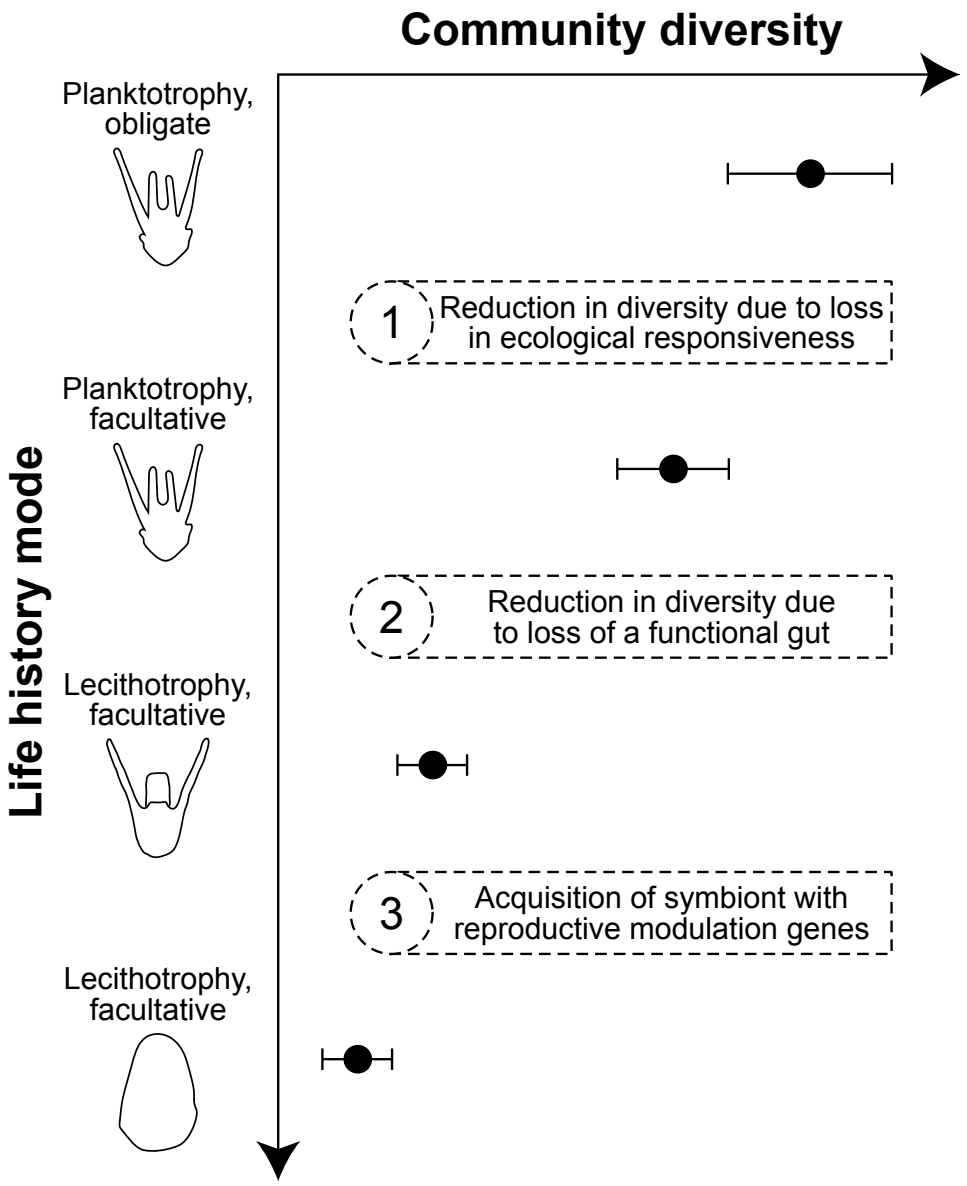


Figure 5.5. Revised model of life-history transitions. A theoretical model for the primary transitional steps during the evolution of lecithotrophy presented by Wray and Raff (1991) and revised by Smith *et al.* (2007) is revised again here. This second revision includes three additional components pertaining to larval-associated bacterial communities. First, a reduction in community diversity during the transition from obligate to facultative planktotrophy as the need to feed is relaxed. Second, a reduction in community diversity during the transition from facultative planktotrophy to lecithotrophy while a functional gut is lost. Third, the acquisition of a novel bacterial lineage with the genomic features to manipulate host reproduction.

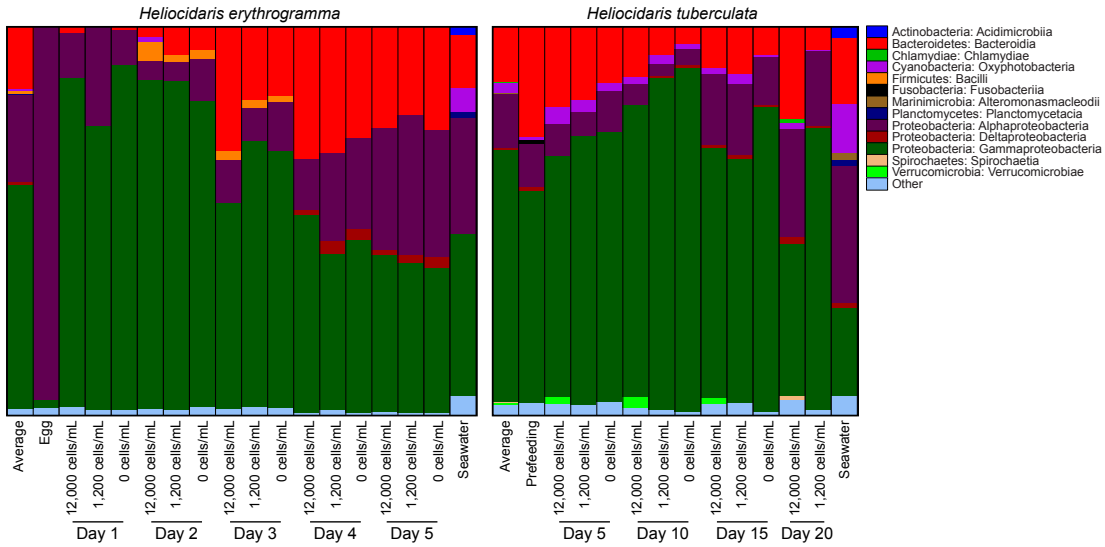


Figure S5.1. Bacterial taxa associated with *Heliocidaris* larvae. Bacterial classes associated with *Heliocidaris* larvae having been provided either 12,000, 1,200, or 0 cells•mL$^{-1}$ of phytoplankton over the course of larval development for *H. erythrogramma* and for twenty days for *H. tuberculata*.

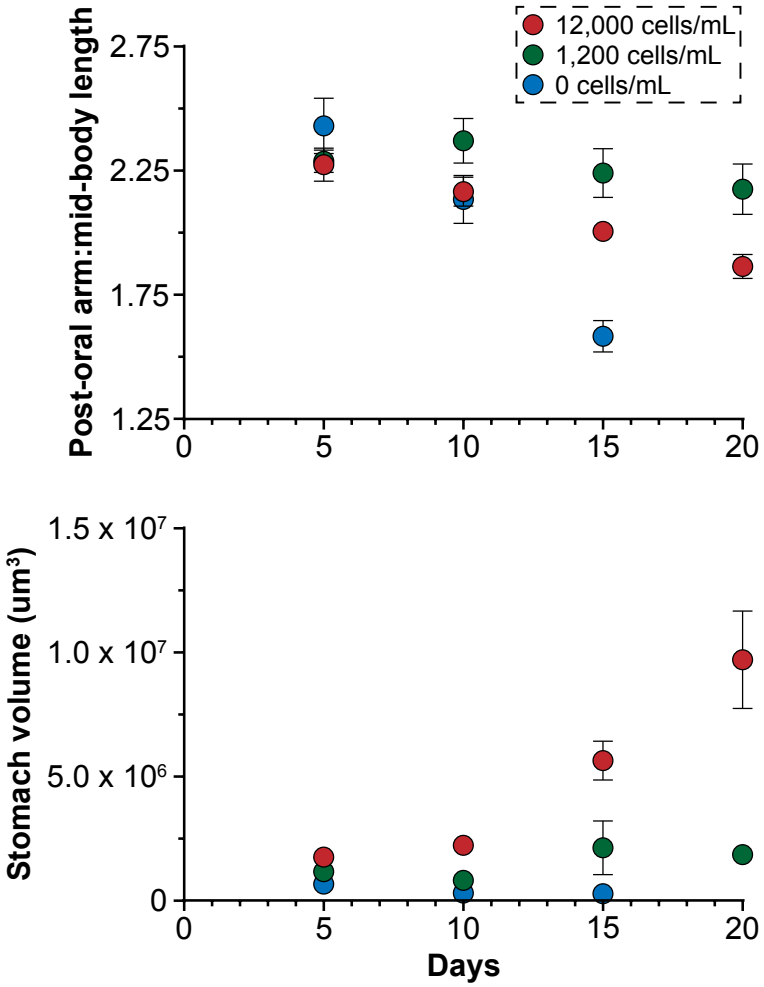


Figure S5.2. Morphometrics of *Heliocidaris tuberculata* larvae. Post-oral arm to mid body line ratio (top) and stomach volume (bottom) (average ± standard error) of *H. tuberculata* larvae having been fed either 12,000 (red), 1,000 (green), or 0 cells•mL$^{-1}$ (blue) over the course of twenty days.

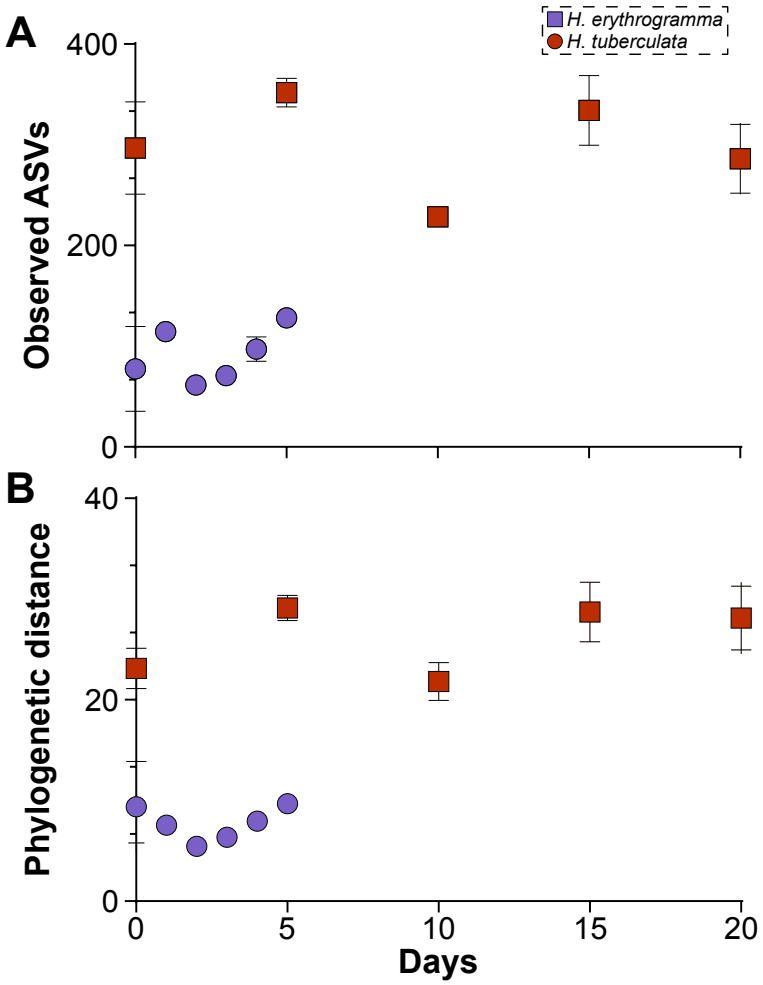


Figure S5.3. Alpha diversity metrics of the bacterial community associated with *Heliocidaris* larvae. Total ASVs (top) and phylogenetic distance (bottom) of bacterial taxa for *H. erythrogramma* and *H. tuberculata* larvae over the course of differential feeding experiments. Larvae of both species were provided either 12,000, 2,000, and 0 cells•mL$^{-1}$ of a phytoplankton (represented by red, green, and red, respectively).

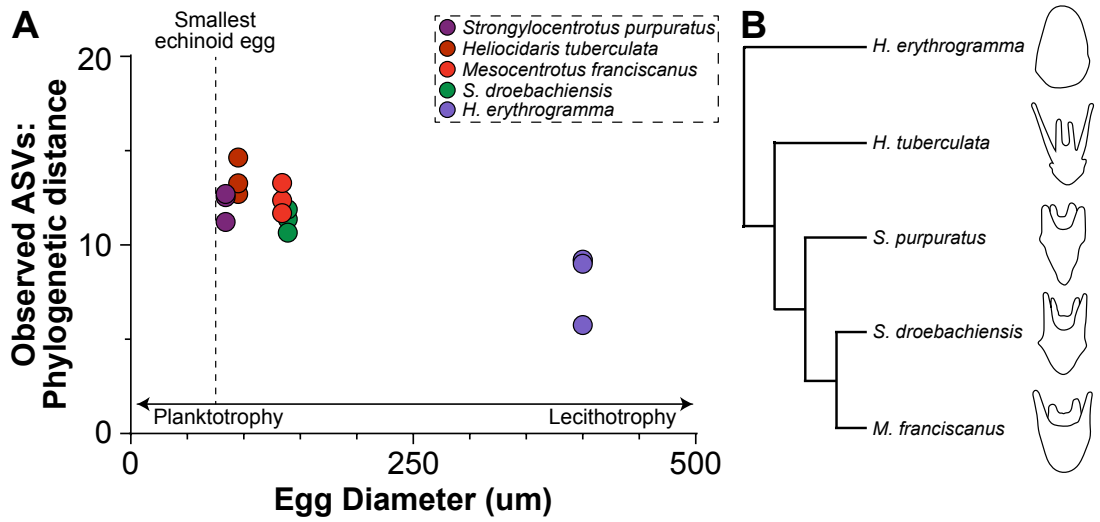


Figure S5.4. Bacterial communities of echinoid eggs. Ratio of observed ASVs to phylogenetic distance of the bacterial community associated with echinoid eggs, and how this relates to egg dimeter, a proxy for maternal investment and life-history mode. Data for *Strongylocentrotus purpuratus*, *Mesocentrotus franciscanus*, and *S. droebachiensis* were originally presented in Carrier and Reitzel (2018; 2019) and reanalyzed using the computational pipeline presented here for comparison to both *Heliocidaris* species.

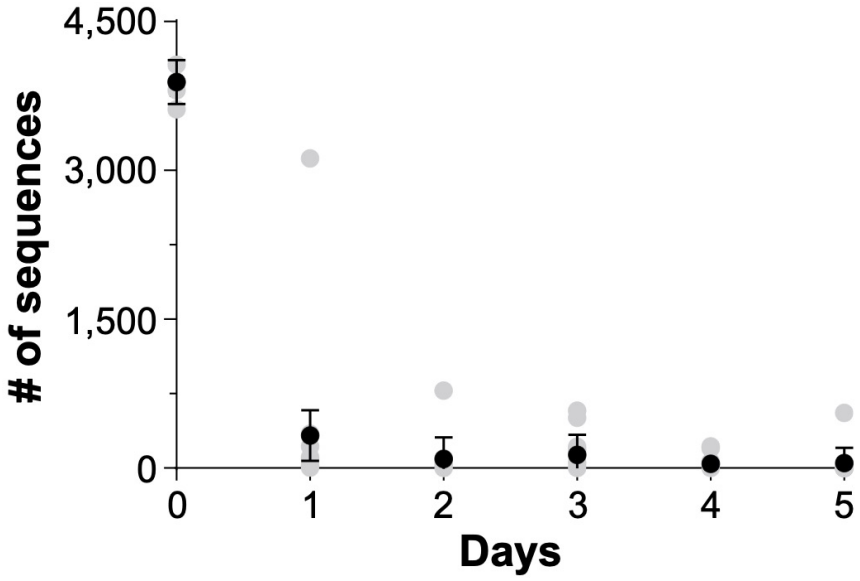


Figure S5.5. Abundance profile of *Echinorickettsia raffii*. The abundance profile of the predominant operational taxonomic unit—that was later characterized as *E. raffii*—associated with unfertilized *Heliocidaris erythrogramma* eggs over the course of development.

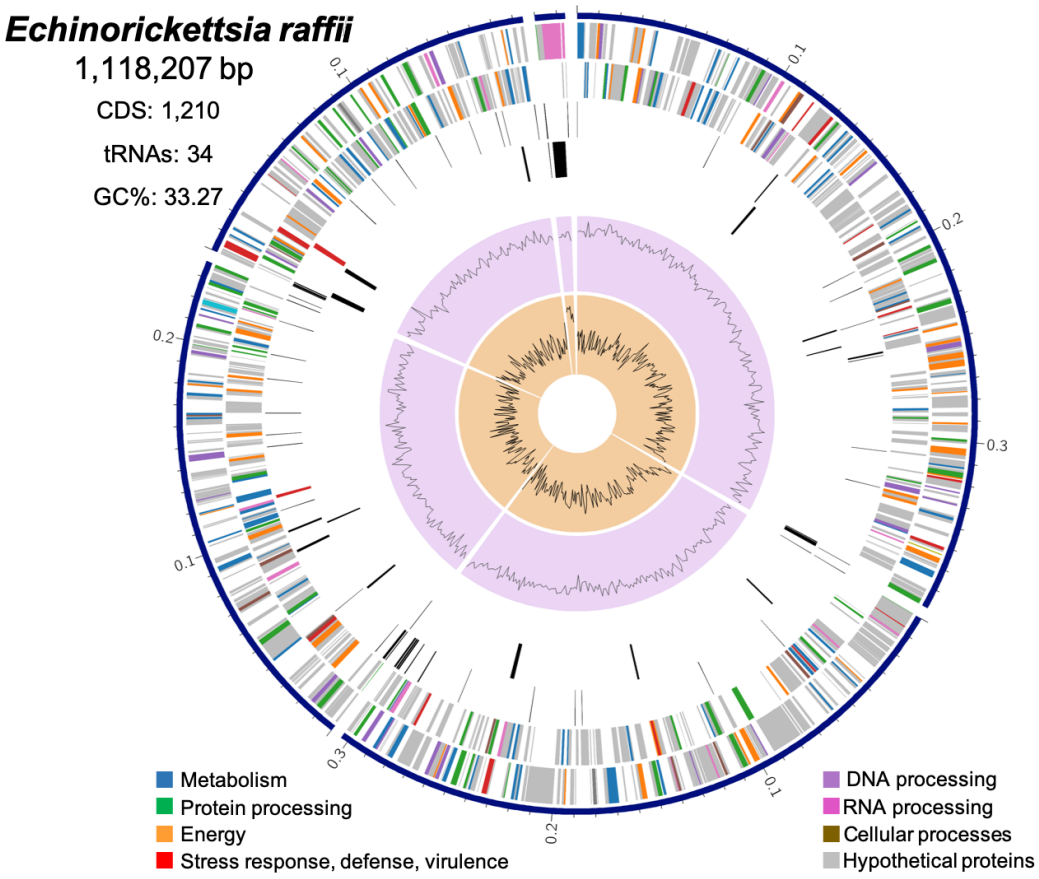


Figure S5.7. Draft genome for *Echinorickettsia raffii*. Circos depiction of the draft genome for *E. raffii* that, from outer to inner rings, includes five scaffolds in blue, predicted CDS on the forward strand, predicted CDS on the reverse strand, RNA genes, predicted CDS with homology to known antimicrobial resistance genes, predicted CDS with homology to known virulence factors, GC content, and GC skew. The colors in the CDS rings portray the subsystem category these genes belong to (see legend).

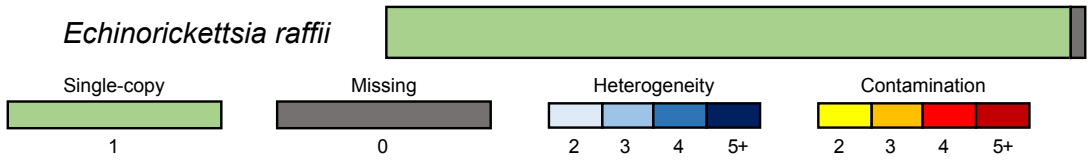


Figure S5.8. Completeness of the *Echinorickettsia raffii* genome. Assessment of the completeness for the *E. raffii* genome using CheckM, as determined through comparison to a total of 324 marker genes from 83 genomes within the Rickettsiales. A total of 317 of these single-copy genes were found in *E. raffii* and, of those, one was in duplicate and six were not recovered. This yielded a completeness of 97.2% as well as 0.5% contamination.

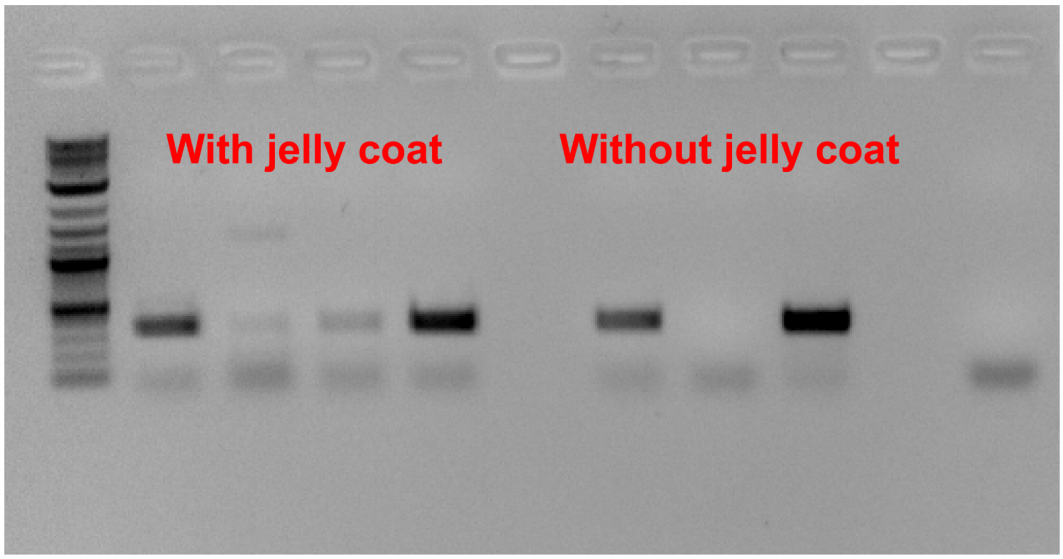


Figure S5.10. Localization of *Echinorickettsia raffii*. Using the 16S rRNA sequence recovered from the draft genome and polymerase chain reaction, *E. raffii* was found to be present in a majority of *Heliocidaris erythrogramma* eggs as well as those that had their (external) jelly coat chemically removed by a brief exposure to glacial acetic acid-acidified seawater[35].

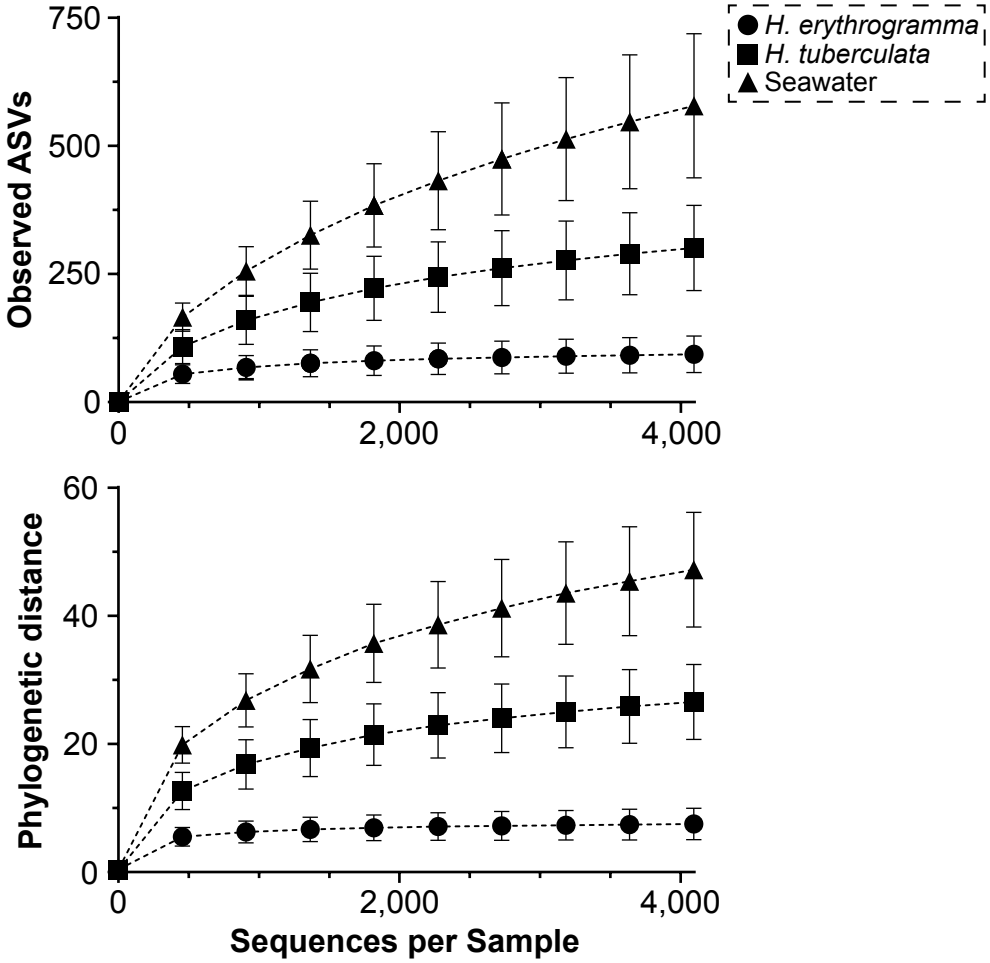


Figure S5.11. Alpha rarefaction curve for *Heliocidaris* larvae and the seawater. Alpha rarefaction curve for the bacterial community associated with *H. erythrogramma* and *H. tuberculata* across differential feeding experiments, and of the seawater. This was based on a rarefaction depth of 1,426 sequences and was used for all analyses.

# CHAPTER 6

# CONCLUDING REMARKS

This dissertation uses a series of field-based experiments to assess the relationship between larval urchins and their bacterial symbionts across space, time, and developmental modes. The first chapter reviews our understanding of symbioses between echinoderm larvae and bacterial symbionts. The second chapter tests whether larval urchins associate with bacterial communities specific to their two diet-induced phenotypes. The third and fourth chapters then assess this relationship across time and space, respectively. Lastly, the fifth chapter attempts to expand upon the role of the environment and developmental stages driving associations with microbiota focused on in the second, third, and fourth chapters by testing the hypothesis that phenotype-specific bacterial community that has been widely observed in feeding larvae is lost when a feeding larva is lost over evolutionary time.

In the first chapter, we review our understanding of echinoderm larvae and the symbiont communities that they associate with. Our synthesis of the literature suggests three primary themes. First, larval echinoderms associate with ‘subcuticle bacteria’ that appear to colonize select tissue types. Second, the bacterial communities associated with larval echinoderms exhibit compositional shifts that are correlated with several fundamental properties of larval biology (*e.g.*, development and morphological plasticity) and ecology (*e.g.*, feeding environment). Third, echinoderm larvae exhibit specific responses to pathogenic bacteria that may aid in maintaining the symbiont community and avoid dysbiosis.

In the second chapter, we test the idea that morphological plasticity by larvae and the differential composition of the microbiome are correlated responses. To do so we induce morphological plasticity in three species of sea urchin larvae and measure changes in the associated bacterial community. While each species of larval sea urchin associated with a unique bacterial community, the expression of morphological plasticity resulted in the convergence on a phenotype-specific microbiota. Moreover, by manipulating diet quantity over time, we also observed that differentially associating with microbiota along a phenotypic continuum is bidirectional. From this chapter, our results support the hypothesis that phenotypic plasticity extends beyond a host genome-by-environment interaction.

In the third chapter, we test whether the association with a phenotype-specific microbiota occurs before, during, or following the expression of morphological plasticity. To do so, compare the temporal pattern of the microbial community and how it relates to the timing of morphological plasticity for larvae of the sea urchin *Lytechinus variegatus*. Daily profiling of these responses shows a four-stage successional pattern and that shifts in the bacterial taxa precede morphological plasticity, suggesting a temporal asynchrony in distinct acclimation responses by larval sea urchins.

In the fourth chapter, we test whether the microbial taxa associated with a phenotype-specific microbiota is consistent across the geographical location of the larval host. To do so, we use the sea urchin *Strongylocentrotus droebachiensis* and perform differential feeding experiments using two populations from the Atlantic Ocean and one from the Pacific. By comparing across populations, we find that inter-population differences in the composition of larval-associated bacterial communities are larger than

intra-population variation due to a heterogeneous feeding environment. Moreover, 20-30% of OTUs associated with larvae were specific to a single location while less than 10% were shared. These results suggest that the geographical location of the host is a better predictor of which microbial taxa sea urchin larvae associate with than the phenotype they express.

Lastly, in the fourth chapter, we test whether phenotype-specific microbiota pattern that we observed across time (*i.e.*, the second and third chapters) and space (*i.e.*, the fourth chapter) are maintained when the ability to feed is lost. To do so, we use the major life-history transition from planktotrophy to lecithotrophy that has been well-studied for the sea urchins *Heliocidaris tuberculata* and *H. erythogramma*. By differentially feeding both sea urchin species, we observed that the non-feeder does not associate with a diet-specific bacterial community and that this transition correlates with a major reduction in taxonomic and phylogenetic diversity as well as microbial load. Moreover, eggs of *H. erythogramma* were unicolonized by a novel Rickettsiales lineage that is closely related to *Wolbachia* that had homologous genes known to manipulate host reproduction and metabolize diacylglycerols. Collectively, these results suggest that phenotype-specific as well as diet-specific patterning of the associated microbiota may be lost during major life-history transitions.