Sea Urchin Larvae (*Strongylocentrotus purpuratus*) Select and Maintain a Unique Microbiome Compared to Environmental Sources

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Abstract

Many organisms may rely on microbes that seed the host body and are typically maintained as a consortial symbiosis. Marine invertebrates have highly diverse microbiomes and offer many different life history traits across which to explore the members and functions of these symbionts but are largely absent from the holobiont and microbiome literature compared to humans and vertebrates. We tracked the microbiome of Strongylocentrotus purpuratus *larvae and examined the role of vertical transmission* via *gametes and the role of horizontal transmission* via *diet and seawater for seeding the developing larvae with microbes potentially critical to holobiont health and fitness. We used* 16S *short-read sequencing to track the composition and relative abundances of bacteria associated with diet (microalgae) and with habitat (filtered seawater), as well as with* S. purpuratus *gametes and larvae under standard lab rearing conditions. The larval microbiome differed across developmental stages and between filtered seawater and algae, and specific bacterial taxa were associated with those differences. In this experiment, developing larvae selected and maintained a unique microbiome compared to their diet and habitat. Eggs were a potentially significant source of vertical transmission during embryonic development (genus* Psychromonas*), while horizontal transmission* via *filtered seawater was the main contributor to larval feeding stages, suggesting that filtered seawater is likely the most important source of potential symbionts. Gaining new insights into how marine invertebrate larval microbiomes are seeded and with what taxa is important for endangered-species aquaculture and for ecosystem restoration and management to protect inoculation sources for early–life stage organisms.*

Introduction

Organismal health and fitness are closely intertwined with a vast array of associated microbes, including but not limited to bacteria, viruses, and fungi (Bordenstein and Theis, 2015; Carey and Assadi-Porter, 2017). This close association between host and microbiome dictates and regulates many aspects of the "holobiont"—the relationship and network of host and associated microbes, *via* the "hologenome," including the host's and microbiome's associated genes—ranging from reproduction and development to digestion and behavior (Cussotto *et al.*, 2018; Kang *et al.*, 2019; Sharon *et al.*, 2019). The microbiome can have functional roles through symbioses or can become pathogenic to the host *via* disruptions, resulting in dysbiosis (Sharon *et al.*, 2019). As such, the microbiome of an organism is critical to the study of the larger physiological state of an organism and, through this, the organism's ecology.

Invertebrates offer a useful system for studying the emerging field of animal microbiomes because they make up the majority of extant species (Petersen and Osvatic, 2018). As such, they provide multiple types of life history traits, such as varied body plans, developmental modes, and diets, that

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Abbreviations: ASV, amplicon sequence variant; CoTS, crown-of-thorns sea star; FSW, filtered seawater; ISA, indicator species analyses; NMDS, nonmetric multidimensional scaling; PCoA, principal coordinate analysis; UCI, University of California, Irvine.

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influence the composition, function, and evolution of their respective microbiomes (Petersen and Osvatic, 2018). Furthermore, they could offer insight into what modes of transmission may be conserved across phyla for successfully seeding the microbiomes of offspring and maintaining specific symbionts throughout adulthood. Marine invertebrates, in particular, are ideal for studying how organisms select for and maintain stable consortia of bacteria, given that they are surrounded by and take in millions of bacterial cells simply by living, reproducing, and consuming food in a seawater habitat (Raina, 2018).

Echinoderms offer a particularly interesting system for studying animal microbiomes because of their complex life cycles and their phylogeny as deuterostomes, making them one of the marine invertebrate groups most closely related to vertebrates. Many are planktotrophic and then settle on the ocean floor, inhabiting different ecosystems as they develop and therefore encountering and interacting with different microbiota *via* diet and habitat. Furthermore, using echinoderms as study systems for holobiont research provides an opportunity to better understand deuterostome holobionts, particularly regarding development.

Marine invertebrates and, more specifically, echinoderms could help shed light on what marine symbiont members and/or functions are potentially beneficial for the holobiont and how those symbionts and the broader microbiome are maintained or changed throughout development to ensure symbiosis between host and bacteria. Importantly, understanding how echinoderm microbiomes are seeded during early life stages could shed more light on how microbiomes develop in marine invertebrates.

Three decades of investigations show that echinoderm larvae associate with microbes, and these microbes may impact the development and ecology of the larvae (Walker and Lesser, 1989; De Ridder and Foret, 2001; Galac *et al.*, 2016; Carrier and Reitzel, 2019a, b, 2020; Carrier *et al.*, 2019; Schuh *et al.*, 2020). These findings are supported by literature on marine invertebrate symbioses showing the potential host benefits conferred by microbial associations, including but not limited to the coral and zooxanthellae pairing (Schwarz *et al.*, 1999; Weis *et al.*, 2001; Rodriguez-Lanetty *et al.*, 2006; Weis, 2019), the sulfide-oxidizing bacteria in polychaete tube worms inhabiting hydrothermal vents (Cavanaugh *et al.*, 1981; McFall-Ngai *et al.*, 2013), and nitrogen-fixing chemosynthetic bacteria in bivalves and nematodes (Petersen *et al.*, 2017). While there is a growing body of literature on symbiont transmission in marine invertebrates (Carrier and Bosch, 2022), certain aspects of how microbiomes are seeded during early life stages remain unclear. The specific sources of microbes, seeding mechanisms, functions of acquired microbes, and how this all may vary across species and developmental stages are not yet fully understood.

In echinoderm larvae, the establishment and persistence of these microbial associations are likely affected by environmental variation such as nutrient availability (Carrier *et al.*, 2019), host species, and host morphology (Galac *et al.*, 2016; Carrier and Reitzel, 2020; Carrier *et al.*, 2021). Both adult and larval echinoderm microbiomes are species and host specific and are markedly different from the seawater microbiota (Høj *et al.*, 2018; Carrier and Reitzel, 2020). For instance, the microbiomes of *Strongylocentrotus purpuratus* larvae, both before and after feeding, have been shown to be distinct from environmental microbes, a pattern consistent across echinoderm microbiome studies thus far on adults and larvae (Nakagawa *et al.*, 2017; Høj *et al.*, 2018; Carrier and Reitzel, 2019a, 2020; Carrier *et al.*, 2019). Additionally, it is thought that the microbiota in larval echinoderms is concentrated in the digestive system (Schuh *et al.*, 2020), and it is hypothesized that the initiation of feeding may be a significant developmental event mediating establishment of host-microbial relationships, similar to the onset of symbioses in coral larvae (Weis, 2019; Carrier and Reitzel, 2020). However, it is currently unknown when and how the onset of symbioses is established in echinoderm larvae.

Diet may be an important source of horizontal transmission for inoculation by symbiotic bacteria that are capable of aiding in macromolecule breakdown within the larval host (Weis *et al.*, 2001; Unzueta-Martínez *et al.*, 2022). Previous studies on larval *S. purpuratus* feeding on single-celled algae in the field and in the lab environment show host microbiomes dominated by bacterial taxa associated with nitrogen-fixing capabilities (De Ridder and Foret, 2001; Høj *et al.*, 2018), such as Gammaproteobacteria of the *Vibrio* genus (De Ridder and Foret, 2001; Webster and Taylor, 2012). *In vitro* studies on bacteria isolated from the gut of adult *S. purpuratus* showed that these bacteria could digest components of the algae consumed by the urchins (De Ridder and Foret, 2001). The bacterial taxa in *S. purpuratus* larvae, such as those of the *Vibrio* genus that are associated with brown, green, and red microalgae and perform algal polysaccharide metabolism (Takemura *et al.*, 2014), may therefore be sourced *via* the algal diet and provide the larvae with readily assimilable metabolites as the larvae optimize for feeding to eventually undergo metamorphosis (De Ridder and Foret, 2001; Galac *et al.*, 2016; Høj *et al.*, 2018).

While some bacterial symbionts may be horizontally transmitted *via* diet, the bacterial biomass associated with single-celled phytoplankton is much lower than that of seawater (Whitman *et al.*, 1998; Seymour *et al.*, 2017; Raina, 2018). Additionally, oligotrophic environments are the norm for many echinoderm larvae developing in the plankton (Vaughn and Allen, 2010; Galac *et al.*, 2016; Carrier *et al.*, 2018a). Crown-of-thorns sea star (CoTS; *Acanthaster* spp.) larvae, which characteristically develop in oligotrophic waters, have a bacterial community that seems to harbor phototrophic bacteria that may contribute to CoTS larval resilience in food-limited waters (Carrier *et al.*, 2018b). In the larvae of the sea star *Mithrodia clavigera* (family Oreasteridae) (Galac *et al.*, 2016), the sponge *Plakina cyanorosea* (Oliveira

et al., 2020), and the sea urchins *Strongylocentrotus purpuratus*, *Mesocentrotus franciscanus*, and *Strongylocentrotus droebachiensis* (Carrier *et al.*, 2018a)—all of which typically experience oligotrophic conditions—the dominant bacteria are Gammaproteobacteria and Alphaproteobacteria (Galac *et al.*, 2016; Carrier *et al.*, 2018a; Oliveira *et al.*, 2020). The microbial symbionts in these systems are more likely to occur in high enough abundances to successfully seed the larval host in seawater rather than in the phytoplanktonic diet (Cole, 1982; Thompson *et al.*, 2004; Jiao *et al.*, 2007; Seymour *et al.*, 2017). Once horizontally transmitted, the microbes may aid in providing nutrients for the host (Carrier *et al.*, 2018a). Coupling of nutrient availability between larval hosts and a high diversity of potential symbionts may be more readily achieved through horizontal transmission from seawater rather than the microalgal diet, which may be present in low abundances in the water column and may or may not harbor a bacterial load high enough or diverse enough to successfully inoculate the host (Whitman *et al.*, 1998; Crenn *et al.*, 2018). The seawater cleared through the larval gut during feeding may be the more critical source of horizontal transmission compared to diet.

The majority of studies documenting echinoderm ontogeny and ecology have lacked consideration of the microbiome of the developing larvae (Vaughn and Allen, 2010; Jackson *et al.*, 2018; Petersen and Osvatic, 2018; Carrier and Reitzel, 2020). Not only does this limit our current knowledge on echinoderm larval physiology, which likely covaries with and is influenced by the host microbiome, but also, importantly, it has left unanswered how echinoderm hosts are seeded with their consortia of associated microbes. Here, we followed the development of the microbiome of *S. purpuratus* as the larvae transition among different stages. Using an ecological approach with a focus on the physiological implications, we investigate the bacterial components of the microalgal diet fed to the developing larvae—*Rhodomonas lens* and *Isochrysis galbana* (Brocco French and Allen, 2021)—as well as the microbiota associated with the filtered seawater (FSW) used to house the larvae.

To estimate what the most significant sources of microbial transmission are to the larval *S. purpuratus* microbiome, we monitored the timing and characteristics of changes in microbial membership and relative abundances in an ontogenetic series of the developing larval microbiome while tracking the microbiota associated with the larval seawater and diet. In doing so, we provide an assessment of total bacterial diversity in lab-reared *S. purpuratus* larvae undergoing development in the plankton, and we test the hypothesis that seawater is the most significant source of horizontal transmission of bacteria for the developing larval host.

Materials and Methods

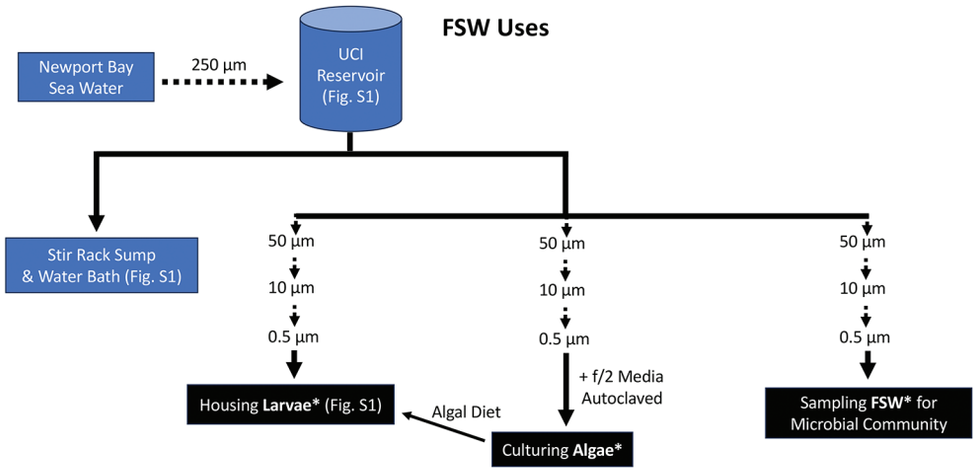
Twenty adults of *Strongylocentrotus purpuratus* (Stimpson, 1857) were collected in June of 2021 *via* snorkel off of the coast of San Pedro, California (33.71° N, 118.28° W). Adults showed no signs of distress (limp spines and falling spines, pale in color, shedding of soft tissue; Strathmann, 1987). Adults were transported to Cabrillo Marine Aquarium (San Pedro), where they were housed in wet table aquaria with flow-through, FSW pumped in from Cabrillo Beach (salinity, 33–34 ppt; 13 °C). Adults were fed *Macrocystis pyrifera ad libitum* and acclimated in aquarium tanks for 2 weeks. Adult *S. purpuratus* were transported in fresh FSW in individual containers to the University of California, Irvine (UCI), where the experiment was conducted.

Raw seawater for the experiment was obtained from Newport Bay, California (33.35° N, 117.52° W), and transported to UCI, where it was filtered before being used for larval rearing, algal culturing, and seawater microbial community tracking (Fig. 1). Seawater from Newport Bay was initially filtered through 250 μm and then held in a reservoir tank at UCI. The reservoir seawater was used to maintain the temperature of the larval beakers, house the larvae, culture the algae to feed the larvae, and sample for background microbial communities. The seawater was treated differently based on each purpose (Fig. 1).

The first use of seawater was for a chilled water bath surrounding the beakers housing the larvae, and that seawater was not filtered further. The second use of seawater was to house larvae in the beakers in the stir rack. That seawater was filtered further in series from 50 to 10 to 0.5 μm to filter out planktonic organisms that would prey on larvae or foul larval beakers but would keep many microorganisms for microbial sampling. The FSW that was eliminated from larval cultures during larval water changes was discarded from the larval rearing system, so seawater that came into contact with larvae was never returned to the sump or reservoir.

The third use of seawater from the reservoir was to culture the two different species of algae used to feed the larvae (Fig. 1). The seawater for algal cultures was filtered further in series from 50 to 10 to 0.5 μm separately from the seawater that was filtered to house the larvae (Fig. 1). The FSW for the algal cultures was then combined with f/2 media (Pentair Aquatic Ecosystems, Apopka, FL) and autoclaved for sterilization before being added to algal cultures. All algal samples used for microbial tracking were taken from the algal cultures growing in sterilized FSW that did not come into contact with larvae. All algal culturing and sampling was conducted in a separate room of the lab apart from the seawater reservoir and larval stir rack (Fig. 1).

The final use of seawater from the reservoir was to track seawater microbial community (Fig. 1). This seawater was sourced from the reservoir and filtered in series from 50 to 10 to 0.5 μm. The sampled FSW therefore mimicked the seawater that would otherwise be used to house the larvae. The FSW was then filtered through a 0.2-μm filter to capture bacterial cells on the filter to sample the seawater microbial communities (Fig. 1). The FSW sampled for microbial community did not come into contact with



Sequenced Sample Type*	Sampling Frequency for 16S sequencing (Fig. 2)	Sample Type Details
Larvae	1/week	Housed in FSW (filtered down through 0.5 µm) with water changes every other day and live algal feedings. Samples in this category are separate from the samples used to sequence algal microbial community and FSW microbial community.
Algae	1/week	Algae were cultured in FSW. f/2 media was added to the FSW (filtered down through 0.5 µm). FSW with f/2 media was then autoclaved. Algae were then cultured in that autoclaved FSW with the f/2 media. Sequenced samples in this category did not come into contact with larvae.
Filtered Sea Water (FSW)	3 times	Filtered down through 0.5 µm. Sequenced FSW samples did not come into contact with sequenced larval or algal sample types.

Figure 1. Raw seawater was brought into the lab from Newport Bay in Newport Beach, California, and filtered through 250 μm before being stored in a reservoir that fed into a sump to maintain the water bath. Seawater used for larval rearing, algal culturing, and microbial community sampling was filtered separately for each use in series through 50, 10, and 0.5 μm (filtered seawater [FSW]).

larvae, algae, or sequenced samples of larvae or algae. We recognize that filtering to the 0.5-μm level can exclude some microorganisms that would otherwise engage with the larvae in the wild, but this filtering method is important for larval rearing in captivity (Strathmann, 1987) and still allows a rich microbial community to bathe and develop symbioses with the larvae in the laboratory.

Larval spawning and rearing

Spawning was induced in 14 urchins immediately upon arrival at UCI by 1-mL intracoelomic injection of KCl solution (0.5 mol L^{-1}). Adults were spawned dry, not while submerged in seawater. Sperm and eggs were collected dry by directly pipetting from the gonopores on the aboral surface of the adults. Eggs and sperm sampled for microbiome analysis were not in contact with a significant amount of seawater other than any seawater left on the spawned adult sea urchin bodies. Eggs and sperm were examined under a compound microscope for competency (sperm: free swimming and active; eggs: round, uniform in shape and color, no dark spots that would indicate incompetence) (Strathmann, 1987). Eggs were fertilized with diluted sperm to produce embryos from one parental pair. Embryos were scored for fertilization after about 1 min to ensure successful fertilization. Successful fertilization was determined by visualization of the fertilization envelope under a compound microscope and calculated by counting the number of successfully fertilized eggs out of 100 eggs in three replicates. Out of 300 eggs (three batches of 100 eggs each), successful fertilization was determined with fertilization success >90% (Strathmann, 1987).

Three samples each of eggs, sperm, and embryos were collected into Zymo Research (Irvine, CA) bead beating tubes (ZymoBIOMICS DNA miniprep kit D4300) with 1 mL of DNA/RNA Shield (R1100-250; Zymo) and stored in a −80 °C freezer for DNA extraction and microbiome analysis. Embryos were examined to determine successful hatching 24 h after fertilization (rotating blastula that has shed the fertilization envelope). About 1000 nonsampled blastula were then distributed into embryo-safe (materials never before used with chemicals and lightly scrubbed with Alconox soap (White Plains, NY) before being rinsed thoroughly with deionized-water) plastic containers with 500 mL of FSW (250-μm FSW further filtered in series through 50-, 10-, and 0.5-μm filters, which will henceforth be referred to as FSW for the purposes of this experiment) and incubated in a recirculating water bath maintained at 16 °C with a chiller and salinity of 32–34 ppt (Fig. S1 [Figs. S1–S5 are available online]).

Forty-eight hours after fertilization, containers were examined for viable larvae (free-swimming gastrulae suspended in the water column were determined to be viable, while

those at the bottom of the container were determined not to be viable). Three replicates of about 20 viable gastrulae and three replicates of about 20 not viable gastrulae were sampled randomly from the containers. Each replicate was about 250 μL of FSW containing the gastrulae, and this volume (with the gastrulae) was added to 1 mL of DNA/RNA Shield in Zymo bead beating tubes (ZR BashingBead lysis tubes, 0.1 and 0.5 mm; S6012-50) and stored in a −80 °C freezer for DNA extraction and microbiome analysis. About 150 viable larvae were then transferred from the original embryonic containers into each of 20 250-mL glass rearing beakers containing 200 mL of FSW. These beakers had been previously acid washed and autoclaved to become sterilized and embryo safe (Strathmann, 1987; Brocco French and Allen, 2021). The 20 250-mL beakers each containing about 150 viable gastrulae were placed into a water bath at 16 °C and stirred with plexiglass paddles previously washed with Alconox to make them embryo safe (Fig. S1). Containers were rotated throughout the water bath every other day to avoid batch effects, and subsequent sampling was conducted across the beakers randomly.

Gastrulae were monitored beginning 48 h after fertilization for development of the anus (deutorostomes) and for development into the prism pluteus larval stage. The first feeding and water change were performed 3 days after fertilization as the larvae developed their mouths (Strathmann, 1987; Brocco French and Allen, 2021). Larval rearing for the duration of the experiment followed standard larval-rearing techniques (Strathmann, 1987; Trackenberg *et al.*, 2020; Brocco French and Allen, 2021): 50% water changes were performed every other day using FSW, followed by feedings of high concentrations (20,000 cells mL^{-1}) of single-celled *Rhodomonas lens* and *Isochrysis galbana* algae. Algal species were cultured separately in f/2 media and sterilized FSW under a full-spectrum grow lamp and natural light to achieve a 16-h photoperiod (Strathmann, 1987; Brocco French and Allen, 2021).

Sample collection

Larvae were sampled weekly, and morphology and developmental stage were monitored every other day (Fig. 2). Skeletogenesis of skeletal rods in larval arms and of juvenile rudiment spicules was detected using polarized light microscopy on a compound microscope (Heyland and Hodin, 2014; Brocco French and Allen, 2021). Visual inspections of the larval cultures on sampling days confirmed that development was proceeding uniformly, with good quality (no signs of larval distress such as skeletal rods protruding from the ends of postoral arms) and with low mortality. Same–life stage individuals were sampled in batches of seven to nine per replicate because of their size (<200 μm), ensuring sufficient microbial genetic material for DNA analysis that would yield an accurate representation of microbial diversity. Four replicates (seven to nine individual larvae for each replicate) of each life stage were collected into Zymo bead beating tubes with 1 mL of DNA/RNA Shield and stored in a −80 °C freezer for DNA extraction and microbiome analysis.

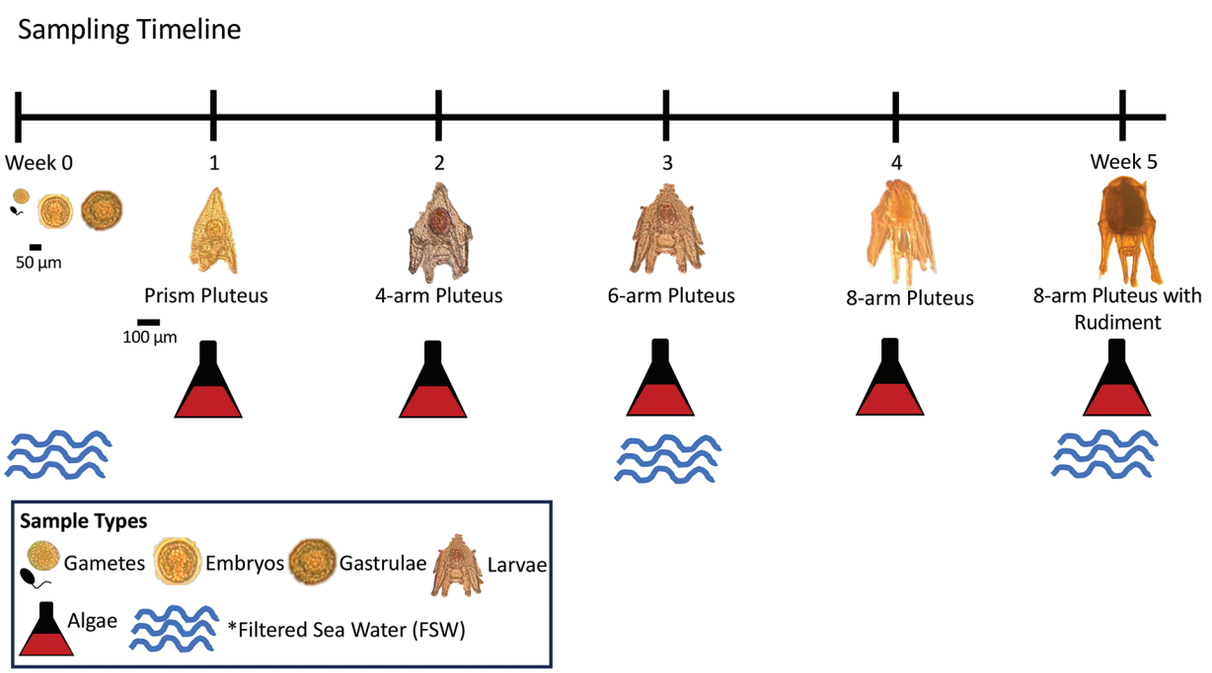


Figure 2. Sampling timeline for all three sample types: *Strongylocentrotus purpuratus* urchin samples, algal samples (*Isochrysis galbana* and *Rhodomonas lens*), and filtered seawater (FSW). FSW was sampled three times for microbial community because all FSW samples and all triplicates across sampling periods did not vary.

Each larval replicate was about 250 μL of FSW containing the larvae, and this volume (with the larvae) was added to 1 mL of DNA/RNA Shield; 250 μL of FSW diluted fivefold in DNA/RNA Shield is too small of a volume and too dilute to provide a significant addition of microbial genetic material from the seawater itself, which did not appear to alter the microbial signal in the larval samples contained therein (DeLong, 2009; Gilbert *et al.*, 2009; Krabberød *et al.*, 2022; Deutschmann *et al.*, 2024). To accurately capture the microbiota of seawater, volumes no less than 100 mL are recommended, with higher volumes closer to 2 L being more effective (Kumar *et al.*, 2020; Patin and Goodwin, 2022). Larval samples were expected to represent both the microbiome associated with the external surfaces of the larvae and the internal microbiome, particularly within the gut lumen, where the majority of microbial biomass in *S. purpuratus* larvae is concentrated (Schuh *et al.*, 2020). Samples of prism plutei, four-arm plutei, six-arm plutei, eight-arm plutei, and eight-arm plutei at the beginning and late stages of rudiment development were obtained using the above sampling procedure.

Seawater microbial community was tracked in triplicate by sampling three replicates of 1.5 L of FSW at the start, middle, and end of the 5 weeks of the experiment (Figs. 1, 2). Because of financial constraints, FSW was sampled three times for microbial community: week 0, week 3, and week 5 (Fig. 2). All FSW samples across all triplicates and across sampling periods were not significantly different from one another in alpha or beta diversity, confirming that the sampling frequency was sufficient (Figs. 2, S3; Tables S1, S2 [Tables S1–S5 are available online]). The FSW sampled for background microbial community did not come into contact with sampled larvae and was conducted in a separate room of the lab apart from sampled algae (Figs. 1, 2).

The seawater sampled for ambient microbial communities was the FSW (seawater filtered in series from 50 to 10 to 0.5 μm). Although this does not capture the microbes in the raw, unfiltered seawater, it accurately samples the microbes in the seawater that the larvae were housed in. The FSW was sampled using Sterivex filters (0.2-μm pore size; Sterivex filter units C3235, Millipore Sigma, Burlington, MA) and running 1.5 L of FSW through each before extracting the filter and clipping it into pieces using sterilized dissection tools in a biosafety cabinet. Filters were stored in Zymo bead beating tubes with 1 mL of DNA/RNA Shield in a −80 °C freezer until DNA extraction (within 3 months). The filters themselves were processed during DNA extractions to capture the FSW microbial communities.

Algal samples were also obtained to track the microbial composition of the algal diet (Figs. 1, 2). Three replicates of each algal species (*R. lens* and *I. galbana*) were sampled at the same frequency as the larvae were sampled (one per week). Both algal species were cultured in sea water from the reservoir at UCI after the seawater was further filtered in series and autoclaved with f/2 media (Figs. 1, 2). Algal cultures were located in a separate room of the lab compared to the reservoir, larval stir rack, and sump.

Algal samples were obtained by preparing the volume of algae from the original algal culture (not from larval rearing beakers) to obtain a density of 20,000 cells mL^{-1}, as if they were being fed to the larvae. This density was kept constant for all larval feedings and for all algal samples. The volume of algal culture used to obtain that algal cell density varied because the densities of each algal culture and of each algal species varied throughout the course of the experiment (algal volumes to obtain that density varied between about 200 and 1200 μL). Algal samples were then pelleted using a microcentrifuge and stored as a pellet without the supernatant in Zymo bead beating tubes with 1 mL of DNA/RNA Shield in a −80 °C freezer until DNA extraction (within 3 months). Algal samples for microbial community did not come into contact with sampled larvae or with sampled FSW (Figs. 1, 2).

Sample processing

The DNA extractions were performed in a biosafety cabinet on all samples using the Zymo Biomics DNA mini prep kit (D4300). A subset of algal samples, FSW samples, and *S. purpuratus* samples across larval stages from this experiment were extracted and amplified using polymerase chain reaction (PCR) and universal *16S* primers (Chase *et al.*, 2018). Gel electrophoresis was then performed to confirm successful extraction of *16S* rRNA gene on the subset of samples. The PCR targeting the V4–V5 region using the EMP primers (515F [barcoded] and 926R) was followed by targeted short-read deep sequencing of the *16S* rRNA gene amplicon (V4–V5, 2 × 250 bp, paired-end reads) using the Illumina MiSeq platform at UCI's Genomics Research and Technology Hub (Caporaso *et al.*, 2012). A mock bacterial community was sequenced along with experimental samples as a positive control to ensure sequencing accuracy (Leigh *et al.*, 2022).

Computational and Statistical Analyses

Analysis of the *16S* amplicon sequences was performed using QIIME2 (Quantitative Insights into Microbial Ecology ver. 2017.6.0; Caporaso *et al.*, 2012), R (ver. 4.0.3; R Foundation for Stastical Computing, Vienna), and RStudio (Posit, Boston, MA; Caporaso *et al.*, 2012; Carrier and Reitzel, 2019a). Raw reads along with quality scores (Q-scores) were imported to QIIME2, where QIIME2 scripts were used to filter out low-quality reads. Reads were denoised using DADA2, which accomplished the filtration, dereplication, sample inferring, chimera identification, and paired-end read merging for all reads (Callahan *et al.*, 2016); 287 base pairs were trimmed from the forward reads, and 202 base pairs were trimmed from the reverse reads where median quality score started to decline (Phred score < 37) (Hall and Beiko, 2018). Reads were normalized to a rarefaction depth of 3839 reads per sample, which included all samples

while maintaining the highest sequencing depth possible (Hall and Beiko, 2018; Odom *et al.*, 2023). The resulting QIIME2 artifact of "features" was grouped into amplicon sequence variants (ASVs) based on a 100% similarity and were assigned taxonomy using the SILVA database (silva-138-99-515-806-nb-classifier) (Balvočiūtė and Huson, 2017; Carrier and Reitzel, 2019b; Carrier and McAlister, 2022). Nontarget sequences aligning to mitochondria or chloroplasts were discarded (Carrier and Reitzel, 2019b). The filtered biom table was then rarefied to 5721 reads (Hall and Beiko, 2018; Carrier and Reitzel, 2019b).

Alpha diversity was estimated using the Shannon diversity index and visualized using box and whisker plots (Carrier and McAlister, 2022). A Kruskal-Wallis test was performed with subsequent *post hoc* Tukey's tests for pairwise comparisons between sample types (Carrier and McAlister, 2022; Table S1). Species richness was calculated using the Chao1 index, and evenness was calculated using the Vegan package in R Studio (Posit; Hall and Beiko, 2018; Willis, 2019) to determine which measure of diversity was a bigger contributing factor to the diversity indexes calculated above.

To determine whether membership and composition of the bacterial community changed with time and/or across larval developmental stages, unweighted and weighted UniFrac values as well as Bray-Curtis values were calculated using QIIME2 and visualized using principal coordinate analyses (Caporaso *et al.*, 2012; Carey and Assadi-Porter, 2017; Carrier and Reitzel, 2019b). Subsequent PERMANOVA (999 permutations) was performed (Table S2).

To test for differences in structure of the microbiome community composition and membership with ontogeny and across samples types (urchin sample type in addition to two different algal species and FSW; beta diversity), we generated a nonmetric multidimensional scaling (NMDS) plot using a Bray-Curtis dissimilarity matrix (Carrier and Reitzel, 2019b; Carrier and McAlister, 2022). To test whether there were statically significant differences observed in microbial community and membership across larval developmental stages, FSW samples, and algal samples, we used a PERMANOVA global test and subsequent pairwise Adonis comparisons with a Benjamini-Hochberg correction (Carrier and Reitzel, 2019b; Table S2). Additionally, PERMDISP was performed to assess dispersion between sample types on the NMDS, and ANOSIM was performed to assess similarities among sample types based on microbial taxa.

Two indicator species analyses (ISA) were performed using R Studio version 2022.07.0 to determine taxa that were abundant in specific sample types (Posit; DufrêNe and Legendre, 1997; De Cáceres *et al.*, 2012). The first ISA used Spearman's correlation–based vectors using an estimated *P*-value of 0.75, which produced vectors on the NMDS plot showing which taxa were likely driving the clustering of similar samples on the plot. The second ISA was performed using the R package indicspecies and 9999 permutations across 13 sample types to understand which microbial taxa in the samples were likely to be driving the differences in beta diversity (Antharam *et al.*, 2016; Table S3).

The SourceTracker package in QIIME2 using a Gibbs sampler (Markov chain Monte Carlo algorithm) was performed to estimate the relative proportional contribution of each predetermined "source" of vertical or horizontal transmission to each predetermined "sink," or *Strongylocentrotus purpuratus* developmental stage. There were six potential sources of transmission: eggs (gametes), sperm (gametes), FSW, *Rhodomonas lens* larval microalgal diet, *Isochrysis galbana* larval microalgal diet, and unknown (airborne, human, unknown). The sinks were the *S. purpuratus* developmental stages: nonfeeding stages (embryos and gastrulae) and feeding stages (prism pluteus, four-arm pluteus, six-arm pluteus, the first week of eight-arm pluteus stage—no juvenile rudiment, the second week of eight-arm pluteus stage—beginning juvenile rudiment development , and the third week of eight-arm pluteus stage—well-developed juvenile rudiment). SourceTracker results were then transferred to Excel, where proportional contributions of sources to sinks were graphed and standard deviations were calculated. The raw reads of *16S* rRNA gene isolated from *S. purpuratus*, FSW, and both algal species are available at National Center for Biotechnology Information BioProject code PRJNA1023617.

Results

A total of 2,671,124 reads averaging 436 bp in length were obtained. The sequences obtained resulted in 13,939 ASVs, of which chloroplasts and mitochondria accounted for 3270, which were subsequently filtered out. Of the remaining 10,669 ASVs, the Bacteria domain accounted for 96.4% (10,285 sequences), while the remaining 3.6% were categorized under the Archaea domain (Table S5). Thirty-one bacterial phyla were identified, where Pseudomonodota was the dominating phylum, followed by Bacteriodota and Actinobacteriodota. The class level was dominated by Gammaproteobacteria, followed by Alphaproteobacteria. All FSW samples were not significantly different from one another in alpha or beta diversity (Figs. 4, S3, S4; Tables S1, S2).

Microbial diversity

The bacterial families in the taxa bar plot showing the highest relative frequency across 13 sample types including *Strongylocentrotus purpuratus* gametes and larvae, two algal species, and FSW, in order of decreasing relative frequency, were the families Flavobacteriaceae, Rhodobacteraceae, Saccharospirillaceae, Pseudoalteromonadaceae, Microbacteriaceae, and Hyphomonadaceae, an unidentified taxon within family of class Gammaproteobacteria, Celvibrionaceae, Colwelliaceae, and Alteromonadaceae (Figs. 3, S2; Table S4).

Family Flavobacteriaceae had the highest relative frequency in *S. purpuratus* feeding larvae (four-arm pluteus, six-arm pluteus, and eight-arm pluteus), while the prism pluteus larval stage was dominated by Saccharospirillaceae. The gastrulae of *S. purpuratus* showed high dominance by

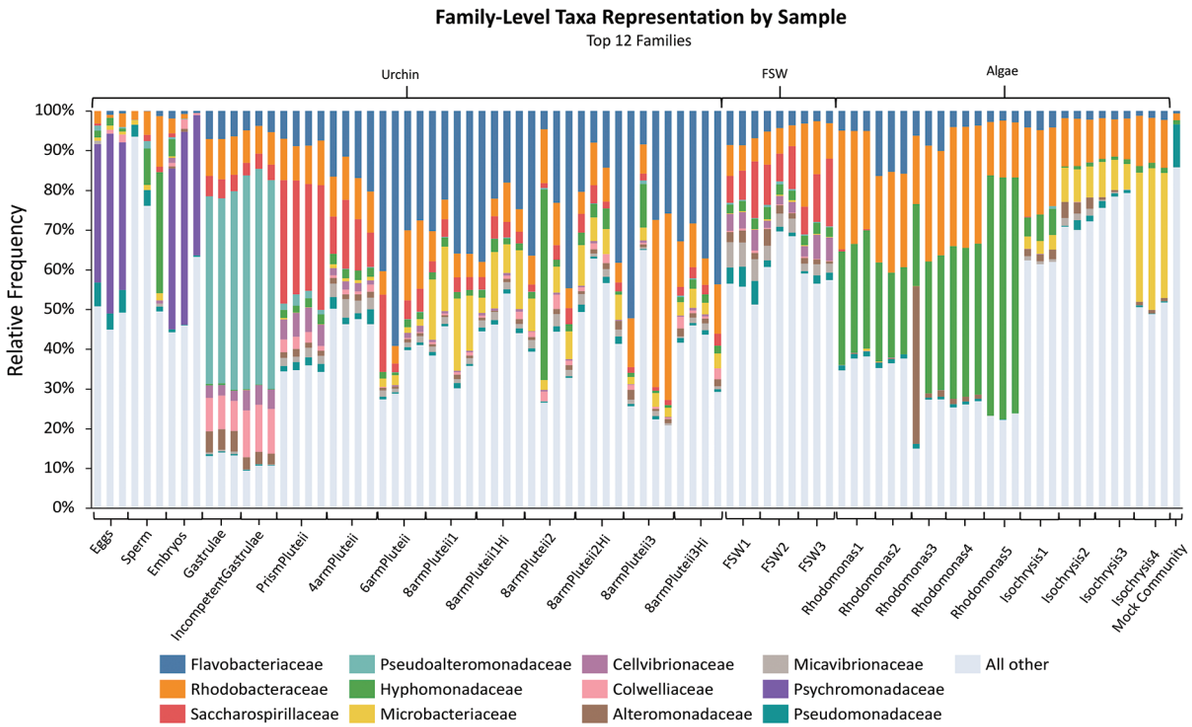


Figure 3. Bacterial taxa bar plot showing relative frequency of top 12 family-level amplicon sequence variants (ASVs) in each sample type. The *x*-axis is ordered by sample date corresponding with sample developmental stage. The *x*-axis labels with numbers represent consecutive weeks of the same sample type (*e.g.*, Isochrysis1 and Isochrysis2 are two consecutive weeks of *Isochrysis* sampling). The mock community was used as a positive control during sequencing. The six most abundant families present across all sample types were Flavobacteriaceae, Rhodobacteraceae, Saccharospirillaceae, Pseudoalteromonadaceae, Hyphomonadaceae, and Psychromonadaceae. Psychromonadaceae was enriched in the eggs and embryos. Gastrulae and incompetent gastrulae were enriched in Pseudoalteromonadaceae. Early stages of feeding larvae—prism plutei and four-arm plutei—were enriched in Pseudoalteromonadaceae. Later stages of feeding larvae (six-arm plutei through eight-arm plutei) were enriched in Flavobacteriaceae and Rhodobacteraceae. The last 2 weeks of eight-arm plutei development were enriched in Hyphomonadaceae, as were the *Rhodomonas lens* algae. A family-level taxa bar plot showing all family-level ASVs can be found in Figure S2.

family Pseudoalteromonadaceae. The eggs and embryos showed high relative frequency of Pseudoalteromonadaceae and Hyphomonadaceae, while the sperm showed high variability at the family level, with relatively high frequencies of Hyphomonadaceae and Rhodobacteraceae families (Fig. 3). The FSW showed high relative frequencies of families Flavobacteriaceae, Rhodobacteraceae, and Saccharospirillaceae. Of the two algal species, *Rhodomonas lens* showed high relative frequencies both of Rhodobacteraceae and of Hyphomonadaceae, while *Isochrysis galbana* showed high relative frequencies of Rhodobacteraceae and Microbacteriaceae (Fig. 3).

Alpha diversity

Alpha diversity varied by sample type (Fig. 4: Kruskal-Wallis, $P < 0.001$, $F = 44.54$; Table S1). The Shannon diversity index was highest in the FSW samples, followed by the prism plutei and the four-arm plutei. Generally, alpha diversity increased with the onset of feeding in the larvae, beginning with the prism pluteus stage and increasing in the four-arm through eight-arm stages (Fig. 4). Variance in diversity was highest in later-stage feeding larvae, particularly in six-arm and eight-arm plutei, compared to most other sample types, with the exception of the variance in the four-arm pluteus (Fig. 4). Variance in diversity was lowest in *R. lens* samples and in early stages of prefeeding and feeding larvae (gastrulae and prism plutei) (Fig. 4). Taxonomic evenness was significantly different across sample types (ANOVA, $P < 0.001$; Fig. 4).

Beta diversity

The NMDS showing beta diversity revealed distinct clustering between larval developmental stages and between *S. purpuratus* sample types, FSW, and algae based on community similarity (Fig. 5: Bray-Curtis distances, PERMANOVA, $P = 0.001$, $R^2 = 0.58849$; PERMDISP, $P = 0.001$; ANOSIM, $P < 0.001$, stress $= 0.13017$; Table S2). The FSW and algae of both species were also significantly different from each other in community similarity (Table S2). *Strongylocentrotus purpuratus* gametes (sperm and eggs) clustered away from *S. purpuratus* larvae, with sperm showing high dispersion (Fig. 5). Embryos clustered near the sperm and eggs and showed higher dispersion than eggs but clustered apart from the rest of the *S. purpuratus* larval stages, including the gastrulae stage. Gastrulae—both viable and not viable—and

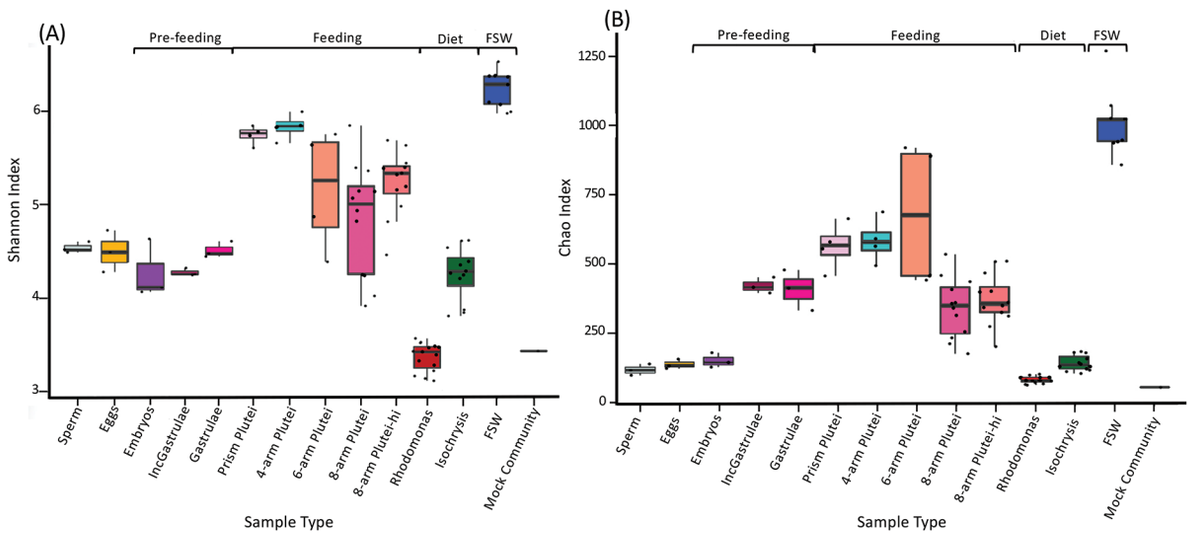


Figure 4. (A) Shannon diversity index estimating alpha diversity across all sample types. Alpha diversity varied by sample type (one-way ANOVA, Kruskal-Wallis, $P < 0.001$, $F = 44.54$). The mock community was used as a positive control during sequencing at the University of California, Irvine, Genomics Research and Technology Hub to ensure successful sequencing. Subsequent *post hoc* Tukey's tests for pairwise comparisons between sample types were performed (Table S1). The highest phylogenetic diversity was observed in the filtered seawater (FSW) samples, prism plutei, and four-arm plutei. *Strongylocentrotus purpuratus* showed significant changes in alpha diversity between nonfeeding and feeding stages. (B) Chao1 richness index estimating alpha diversity across all sample types using a 95% confidence interval. *Post hoc* Tukey's multiple comparisons test was conducted for pairwise comparisons between sample types (Table S1). The highest bacterial species richness was observed in early feeding stages of larvae and in the FSW, while the lowest bacterial species richness was observed in the prefeeding larval stages and in the algal diet.

prism plutei clustered closely but showed distinct clustering among themselves and clustered most closely to the FSW samples compared to the gametes and the feeding larval stages (Fig. 5; Table S2). Prism plutei showed low dispersion and clustered apart from all feeding plutei stages. Six-arm and eight-arm plutei showed community similarity to each other and to four-arm plutei, but the four-arm plutei showed less dispersion than the later larval stages (six-arm

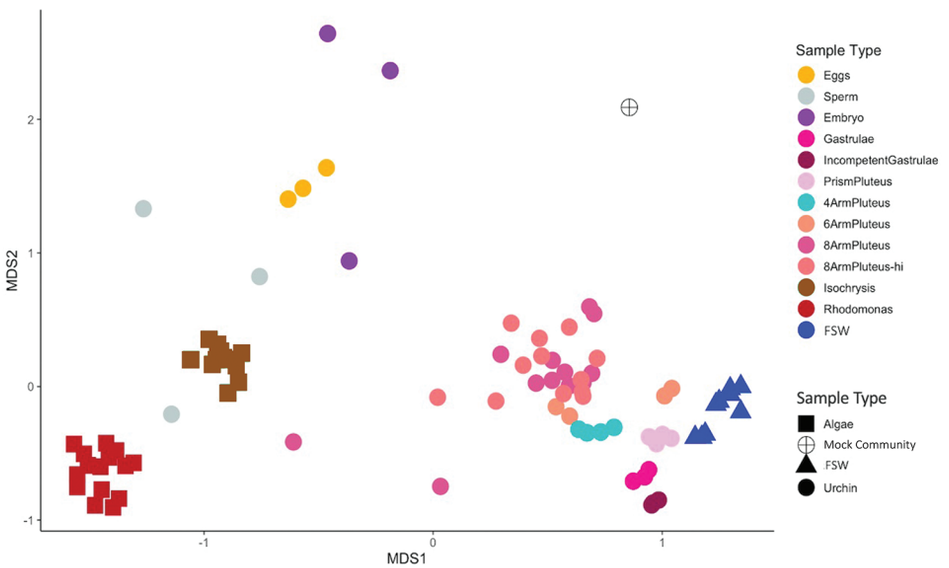


Figure 5. Nonmetric multidimensional scaling analysis (NM-MDS) showing community similarity across all sample types based on a Bray-Curtis dissimilarity matrix (PERMANOVA, $P = 0.001$, $R^2 = 0.58849$; PERMDISP, $P = 0.001$; ANOSIM, $P < 0.001$, stress = 0.13017). Samples labeled "8-Arm Plutei-hi" designate beakers with higher larval densities (lower mortality) observed. The mock community was used as a positive control during sequencing. Gametes and nonfeeding *Strongylocentrotus purpuratus* stages cluster away from all feeding stages and filtered seawater (FSW). The FSW samples cluster closely together and near all feeding stages of *S. purpuratus*. Both algal species (*Rhodomonas lens* and *Isochrysis galbana*) cluster away from all feeding stages, from each other, and from FSW. Significant differences in community similarity are observed across all sample types (Table S2).

and eight-arm plutei) (Fig. 5; Table S2). The FSW samples showed high community similarity to each other with low dispersion and clustered separately from all other sample types. The two algal species clustered separately from the feeding stages of larvae, from each other, and from the FSW (Fig. 5).

A Bray-Curtis principal coordinate analysis (PCoA) showed distinct clustering by sample type (Fig. S3: PERMANOVA, $P = 0.001$). Unweighted and weighted UniFrac PCoAs showed distinct clustering of samples according to sample type as well (urchin gametes, larvae, FSW, and algal species; Fig. S5: unweighted UniFrac, PERMANOVA, $P = 0.001$; weighted UniFrac, PERMANOVA: $P = 0.001$). Different distance matrices showed variations in community similarity among *S. purpuratus* larval developmental stages but were relatively consistent in showing distinctions in community similarity between *S. purpuratus* samples, FSW, and each of the two algal species (*R. lens* and *I. galbana*).

Indicator species analyses

The vector analysis identified seven unique bacterial taxa likely driving the distinct clustering observed in the two algal species, the *S. purpuratus* larvae, and the FSW: families Rhodobacteraceae, Flavobactereacea, Saccharospirilaceae; genera *Cadidatus Campbellbacteria*, and *Balneola*; and species *Oceanicaulis alexandrii* and *Pseudopelagicola gijamensis* (Fig. S4). The FSW, gastrulae, and feeding larvae samples were enriched in the bacterial families Rhodobacteraceae, Flavobacteriacea, and Saccharospirillaceae, while the two algal species were enriched in the family Rhodobacteraceae, in the genus *Balneola*, and in the species *O. alexandrii* (Fig. S4).

The second ISA showed associations of specific bacterial taxa to certain developmental stages, as well as to FSW and to the two algal species (Table S3). Eggs were enriched in seven unique taxa, mostly of the genus *Psychromonas* in the class Gammaproteobacteria. Sperm were enriched in six unique taxa, mostly of classes Bacilliales and Gammaproteobacteria. Eighteen unique indicator taxa associated with embryos varied in class, while the 17 indicator taxa for gastrulae consisted mostly of classes Gammaproteobacteria and Bacteroidia. Prism plutei were enriched in 26 unique taxa mostly of the classes Alphaproteobacteria and Gammaproteobacteria. Four-arm plutei were enriched in 26 unique taxa as well, consisting mostly of classes Alphaproteobacteria, Gammaproteobacteria, and Bacteroidia (Table S3). Six-arm plutei were enriched in nine unique taxa of classes Alphaproteobacteria and Gammaproteobacteria. Filtered seawater was enriched in 20 unique taxa dominated by classes Gammaproteobacteria and Bacteroidia. *Rhodomonas lens* was associated with 13 unique taxa mostly of classes Alphaproteobacteria and Bacteroidia, while *Isochrysis galbana* was associated with more unique taxa (24) dominated by class Alphaproteobacteria (Table S3). Specific groupings of multiple sample types were enriched in unique taxa as well (Table S3).

SourceTracker analysis

The SourceTracker analysis showed the proportional contribution of each of five sources to each of eight developmental stages (also correlating with sampling time points). As described in "Materials and Methods," sampled larvae, sampled FSW, and sampled algae did not come into contact with one another (Fig. 1). Additionally, the lack of statistically significant differences in alpha or beta diversity between FSW samples across replicates and sampling time points suggests that our sampling intervals for FSW were sufficient (Figs. 4, 5, S2; Tables S1, S2). For the first sampling time point—the embryos—the source that showed the greatest contribution to the microbiome according to the SourceTracker analysis was the *S. purpuratus* eggs, suggesting a large proportion of vertical transmission (Fig. 6).

The SourceTracker analysis showed that once the embryos progressed to the gastrulae stage (second time point), the eggs as a potential source of vertical transmission were no longer a significant contributor to the *S. purpuratus* microbiome. Rather, the FSW became the largest proportional contributor (Fig. 6). The feeding stages, including the prism pluteus, four-, six-, and early eight-arm stages, were all largely contributed to by FSW and no other sources of vertical or horizontal transmission (Fig. 6). However, as the larvae progressed through the later stages of eight-arm pluteus development toward metamorphosis, the horizontal transmission *via* FSW decreased in proportion to the unknown source of transmission, which was the dominating contributor for the second and third weeks of eight-arm pluteus development (Fig. 6).

The two species of algae fed to the larvae did not result in high contributions to the microbiome of the feeding developmental stages (Fig. 6). *Isochrysis galbana* showed an increase in proportional contribution during the first week of eight-arm pluteus development, which was followed by an increase in contribution from *Rhodomonas lens* during the second week of eight-arm pluteus development. Both sources of horizontal transmission *via* diet were insignificant contributors to the larval microbiome before and after the first 2 weeks of eight-arm pluteus development (Fig. 6). Of note, *R. lens* may be a source of horizontal transmission *via* diet during the second week of eight-arm pluteus development. *Roselvivax halotolerans* was one bacterial taxon enriched in eight-arm plutei and *R. lens* compared to all other sample types during the second week of eight-arm pluteus development (Fig. 6). Additionally, family Hyphomonadaceae was enriched in this stage of eight-arm pluteus development and in *R. lens*.

Discussion

Although the study of marine invertebrate, and particularly echinoderm, larval microbiomes is developing, the bacterial components are known to be species specific, distinct from their seawater environment, and highly variable across ontogeny and different environments (Galac *et al.*,

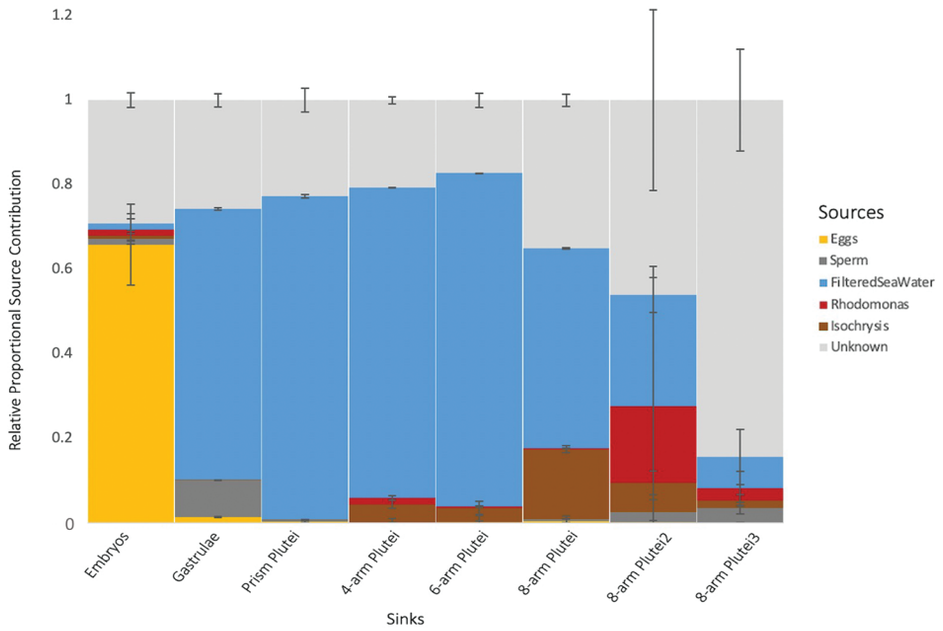


Figure 6. Estimates of relative proportional contributions of bacteria to predetermined sinks from predetermined sources were made using SourceTracker package in QIIME2 with a Gibbs sampler (Markov chain Monte Carlo algorithm). Sinks were *Strongylocentrotus purpuratus* developmental stages: non-feeding stages (embryo and gastrulae) and all feeding stages (prism pluteus, four-arm pluteus, six-arm pluteus, first week of eight-arm pluteus stage—no juvenile rudiment, second week of eight-arm pluteus stage—beginning juvenile rudiment development, and third week of eight-arm pluteus stage—well-developed juvenile rudiment). Sources were vertical (eggs and sperm) and horizontal (filtered seawater [FSW] and the two types of microalgae used, *Rhodomonas lens* and *Isochrysis galbana*). Unknown sources such as airborne, human, or otherwise unknown are included as a source. Eggs were the highest proportional contributors to the embryos. The FSW was the dominant contributor beginning in the gastrulae and in all of the feeding stages of *S. purpuratus*. Algae and unknown sources surpassed FSW in relative proportional contribution to the *S. purpuratus* larval microbiome toward later stages of eight-arm pluteus development.

2016; Nakagawa *et al.*, 2017; Høj *et al.*, 2018; Carrier *et al.*, 2019; Carrier and Reitzel, 2019b, 2020). Here, too, the larval microbiome significantly differed through ontogeny and from environmental microbiota, building on evidence that there is host-mediated selection of associated microbes. Consequently, the microbiome of larval marine invertebrates is correlated with the evolution of specific life history traits (Carrier and Reitzel, 2020; Carrier *et al.*, 2021) and has been hypothesized to benefit host health through metabolic functions (De Ridder and Foret, 2001; Webster and Taylor, 2012; Takemura *et al.*, 2014; Høj *et al.*, 2018). Although there is a growing list of studies on the echinoderm larval microbiome, whether they get those microbes from horizontal or vertical transfer remains unclear.

As the larvae interacted with the surrounding seawater and fed on microalgae, we observed changes in the taxonomic composition of the *Strongylocentrotus purpuratus* larval microbiome that provide evidence for how and when echinoderm larvae are seeded with potentially symbiotic bacteria. Using an ecological framework, our results suggest potential vertical transmission through gametes and a role of horizontal transmission *via* diet and seawater. Notably, eggs may have been the main source of vertical transmission for the embryonic stages (Fig. 6), while FSW appears to be a potential source of horizontal transmission for the feeding larval stages (Fig. 6).

Potential evidence for vertical transmission of Psychromonas *to embryos via the eggs*

As in other echinoid eggs and embryos, those of *S. purpuratus* show *Psychromonas* dominating in relative abundance, particularly compared to the FSW and both algal species (Carrier *et al.*, 2019, 2020; Carrier and McAlister, 2022) (Figs. 3, S2). *Strongylocentrotus purpuratus*, *Mesocentrotus franciscanus*, and *Strongylocentrotus droebachiensis* unfertilized eggs had the highest abundances of *Psychromonas* compared to embryonic and larval stages, with significantly lower proportions beginning at the four-arm pluteus stages once larvae had developed an anus, mouth, and gut and were fully capable of feeding (about 5–7 days after fertilization) (Carrier and McAlister, 2022). Gastrulae diverge from eggs and embryos in richness and abundance of bacterial taxa quickly after embryonic development has completed (about 4 days after fertilization) (Figs. 4, 5, S2), a pattern likely driven by reductions in relative abundance of *Psychromonas*. Our results show that *Psychromonas* is present in the eggs and increases in relative abundance in the embryos and then disappears from the *S. purpuratus*

larval microbiome as soon as the embryos develop into gastrulae (Figs. 3, S2). *Psychromonas* is also not present in any of the environmental samples, including FSW (Figs. 3, S2). The *Psychromonas* observed could have been vertically transmitted *via* the adult coelomic fluid or gonad and not the egg, given that we collected eggs dry from the aboral surface of the adults. However, there is no evidence to suggest that *Psychromonas* is in high abundance within the coelomic fluid or gonads of adult *S. purpuratus*, despite its abundance in the gut digesta (Hakim *et al.*, 2019). Fluorescence *in situ* hybridization or another method to visualize bacterial cells on *S. purpuratus* eggs and gastrulae would be a useful and necessary method to confirm the presence and abundance of *Psychromonas* as a potentially vertically transmitted microbe.

Nevertheless, the enrichment of, and the SourceTracker results on, *Psychromonas* suggest that this bacterial genus may be important for the initial egg-embryo-gastrulae transition in planktotrophic larvae and, additionally, that the egg may be an important source vertically transmitting *Psychromonas*, a potential symbiont, to the embryo. Because echinoderm eggs are associated with high lipid contents, *Psychromonas*—which uses a wide variety of carbon polymers, including lipids—may aid in macromolecule breakdown for energy use by early-stage embryos (Kämpfer *et al.*, 2017; Carrier and McAlister, 2022). Once lipids are depleted leading into later stages of larval development, *Psychromonas* accordingly decrease in abundance (Fig. 3). This drop in relative abundance of *Psychromonas* from eggs and embryos to the gastrula and feeding larval stages corresponds with a switch from potentially vertical (eggs) to horizontal (FSW) transmission as the main source of inoculation (Fig. 6).

Vertical transmission can carry risk to gametes and resulting embryos (Schmitt *et al.*, 2007); therefore, bacteria such as *Psychromonas* potentially associated with vertical transfer to the embryo may have coevolved closely with the host. Upon finding a significant proportion of microbes shared by eggs and early-stage larvae of *Saccostrea glomerata* oysters, Unzueta-Martínez *et al.* (2022) hypothesized that vertical transmission of potential symbionts *via* eggs could be a form of maternal provisioning. Thus, vertical transmission of microbial taxa across various marine invertebrate phyla (Porifera, Mollusca, and Echinodermata)—and, indeed, across terrestrial invertebrate phyla (Arthropoda: aphids, mosquitoes, beetles; Nematoda: beneficial nematodes; Annelida: earthworms) (Haine, 2008; Chaston and Goodrich-Blair, 2010)—could suggest a conserved mechanism for vertical transmission *via* maternal provisioning of symbionts such as *Psychromonas* (Carrier *et al.*, 2023).

The importance of *Psychromonas* in prefeeding planktotrophic larvae may be further supported by its lower relative abundance in lecithotrophic larvae, such as abalone (Danckert *et al.*, 2021). In lecithotrophs, a yolk composed mainly of lipids is the only energy source fueling planktonic development (Lindquist and Hay, 1996; Vaughn and Allen, 2010). *Psychromonas*, which uses lipids as substrate, may deplete energy sources (lipids) too quickly if in high abundance in lecithotrophic larvae, leaving lecithotrophic hosts with depleted yolks while still proceeding to metamorphosis (Lindquist and Hay, 1996; Vaughn and Allen, 2010; Danckert *et al.*, 2021). In planktotrophic larvae such as *S. purpuratus*, *Psychromonas* may be enriched to rapidly break down the abundance of lipids in the prefeeding stages (embryo and gastrulae), releasing more energy faster so the host can grow digestive organs and then feed in the plankton once the lipids are depleted (Herrera *et al.*, 1995; Vaughn and Allen, 2010; Carrier and McAlister, 2022). We say this while acknowledging that lecithotrophic larvae can indeed have symbiotic bacteria that are also capable of digesting and metabolizing lipids (Carrier *et al.*, 2021).

Patterns of horizontal transmission may be driven by seawater in Strongylocentrotus purpuratus *larvae during feeding stages*

The onset of feeding during prism pluteus stages of echinoid echinoderms is associated with a drastic change in microbial community compared to prefeeding stages (gastrulae) (Carrier *et al.*, 2018a, 2019, 2020; Schuh *et al.*, 2020), a pattern also evident in our study (Figs. 3–6, S2). Feeding and prefeeding larvae show significant differences in community similarity compared to both algal species, suggesting that diet is not the major route of horizontal transmission for seeding larval bodies with microbes within our experimental design (Figs. 3, 5). Rather, the source of horizontal transmission may be the clearing of seawater, harboring a large bacterial load (Whitman *et al.*, 1998; Raina, 2018), through the larval body, which begins during the prism pluteus stage in echinoid echinoderms (Strathmann, 1975; Figs. 3, 6). This stage may be a transition period for the larval host in selecting and establishing its microbiome *via* horizontal transmission from seawater, thereby allowing successful development through feeding stages and through eventual metamorphosis (Weis *et al.*, 2001; Carrier *et al.*, 2018a).

Although a small volume of FSW (about 250 μL, diluted fivefold by the DNA/RNA Shield) was present in the *S. purpuratus* larval samples, the contribution of a microbial signal directly from that FSW to the larval samples is most likely limited given the statistically significant differences in alpha and beta diversity between the samples of FSW, algae, and *S. purpuratus* at all stages of development (Figs. 4, 5; Tables S1, S2). As stated in "Materials and Methods," volumes less than 100 mL do not provide an accurate profile of microbiota associated with seawater (DeLong, 2009; Gilbert *et al.*, 2009; Kumar *et al.*, 2020; Krabberød *et al.*, 2022; Patin and Goodwin, 2022; Deutschmann *et al.*, 2024). Furthermore, the bacterial communities associated with the *S. purpuratus* samples that were statistically different from those in FSW and algal samples were dominated by

bacterial classes associated with "rinsed" larval samples of *S. purpuratus* and other echinoid larvae in related studies (Gammaproteobacteria, Alphaproteobacteria, and Bacteroidia) (Galac *et al.*, 2016; Carrier *et al.*, 2018a, 2021; Oliveira *et al.*, 2020; Schuh *et al.*, 2020). The FSW in the larval samples would have been no more than 20% of the total volume in each sample and does not appear to have contributed to the larval microbial signal in an appreciable way, particularly given that the majority of the microbial biomass in *S. purpuratus* larvae is located in the gut lumen (Schuh *et al.*, 2020).

The SourceTracker analysis still suggests that the proportional contribution of microbes from FSW is most likely from the FSW sample as a source of bacteria, not from the FSW as a contaminant to the larval sample microbiomes (Figs. 5, 6). Moreover, the algal food in their diet was cultured in sterilized FSW and does not show a high contribution in the SourceTracker analysis (Fig. 6). Therefore, the *S. purpuratus* larvae in this experiment appear to seed their microbiomes, at least in part, through horizontal transmission *via* seawater. However, this may not be true for marine invertebrate larvae in the wild since their food is not growing in sterile seawater and their diet is likely more varied.

It is worth noting that our experimental design did not allow us to disentangle allochthonous (transient microbes from the environment) from autochthonous microbes (those that are established symbionts within the larvae) for this study, as described by Schuh *et al.* (2020). However, there is growing evidence that allochthonous microbiota may play significant roles in modulating host physiology and morphology, particularly during feeding (Costello *et al.*, 2010). Allochthonous microbes, not just autochthonous, may matter in planktotrophic marine invertebrate larvae, such as *S. purpuratus*, that are feeding frequently when algal cells are available.

Specific indicator taxa were associated with *S. purpuratus* developmental stages, FSW, and microalgal food, providing clues as to what taxa might have specific roles in the host and what taxa might be coming from different sources of transmission. Families Psychromonadaceae, Pseudoalteromonadaceae, Saccharospirrilaceae, Flavobactereaceae, and Rhodobactereaceae appeared to seed the embryonic and larval microbiome in sequential order (Figs. 3, S2, S4; Table S4), beginning in *S. purpuratus* eggs, suggesting a pattern of microbial succession starting with potential vertical transmission followed by horizontal transmission from FSW and less so from diet within our experimental design (Fig. 3). Microbial succession is crucial in systems requiring certain microbiota to perform specific functions in sequential order, such as food fermentation producing soy sauce and sauerkraut (Ivey *et al.*, 2013), or for microbiome development in animals such as the human infant (Koenig *et al.*, 2011; Bode, 2012) and oyster larvae/spat (Unzueta-Martínez *et al.*, 2022). Microbial succession, facilitated by a switch from potentially vertical to horizontal transmission in *S. purpuratus* larvae, may be driving the ecology of the larval microbiome, allowing for the establishment of symbioses benefitting host health (Guevarra *et al.*, 2019; Xiong *et al.*, 2020; Deng *et al.*, 2021; Xiao *et al.*, 2021).

Delving into the common families found within the different larval stages, the major players were Pseudoalteromonadaceae, Saccharospirillaceae, Flavobactereaceae, Rhodobactereaceae, and Hyphomonadaceae (Fig. 3; Table S5). Members of Pseudoalteromonadaceae, particularly in the genus *Pseudoalteromonas*, were enriched in the gastrulae and prism plutei in this study (Table S3). These bacteria are important in larval settlement and metamorphosis of marine invertebrates (Bowman, 2007; Tebben *et al.*, 2011; Ivanova *et al.*, 2014; Offret *et al.*, 2016; Sprockett *et al.*, 2018; Peng *et al.*, 2020; Kowallik and Mikheyev, 2021). Members of Saccharospirillaceae produce many metabolites, including digestive enzymes, and thus can be foundational species that help establish microbial communities in invertebrates (Satomi *et al.*, 2002; Sigler and Zeyer, 2004; González and Whitman, 2006; Garcia-Pichel *et al.*, 2013; Storey *et al.*, 2018; Aanderud *et al.*, 2019; Giraud *et al.*, 2022). Taxa in the family Flavobacteriaceae were shared across 75% of *S. purpuratus* samples in this study (Figs. S2, S4; Table S3) and are common in healthy echinoderms (De Ridder and Foret, 2001; Nedashkovskaya *et al.*, 2009; Ivanova *et al.*, 2010; Høj *et al.*, 2018). They are implicated in the production of diverse biomolecules (Hameed *et al.*, 2014; Chen *et al.*, 2022).

Later in development (eight-arm plutei), bacteria from the families Rhodobactereaceae and Hyphomonadaceae, which were more common in the algal feed (*Rhodomonas lens* cultures, in particular), became more common in the larvae (Fig. 3). Members of Rhodobactereaceae were shared across 55% of all samples, and they may modulate host metabolic pathways involved in immune responses during later development (Pujalte *et al.*, 2014; Kanukollu *et al.*, 2016; Sonnenschein *et al.*, 2021). In adult echinoderms, they help increase the uptake of monomeric nutrients like dissolved free amino acids (Walker and Lesser, 1989; Carrier and Reitzel, 2020). Last, family Hyphomonadaceae was enriched in the second week of eight-arm pluteus development and in *R. lens*. One common species of this family, *Algimonas porphyrae*, contains orange carotenoid pigments, which likely contribute to the synthesis of pigment in late-stage *S. purpuratus* larvae and juveniles (Griffiths, 1965; Gibson and Burke, 1985; Bandaranayake, 2006; Smith *et al.*, 2008; Høj *et al.*, 2018).

Habitat continues to emerge as a crucial component driving marine larval microbial compositions (Stephens *et al.*, 2016; Oliveira *et al.*, 2020). Here, horizontal transmission appears to be the most significant contributor of potential bacterial symbionts to planktotrophic echinoid larvae compared to vertical transmission (Weis, 2019; Oliveira *et al.*, 2020; Unzueta-Martínez *et al.*, 2022). Horizontal

transmission may allow marine invertebrates to associate with habitat-specific microbial phenotypes that confer greater advantage by being suited to the variable conditions the offspring encounter compared to the adults (Vaughn and Allen, 2010; Weis, 2019; Oliveira *et al.*, 2020).

The larval phenotypes resulting from environmental variation may be not just a genotype-by-environment interaction but also a genotype-by-environment-by-microbiome interaction where the microbiome significantly impacts the phenotype of the host, namely under abiotically or biotically stressful conditions (Carrier *et al.*, 2018a). Developmental changes in morphology that vary with feeding conditions can be preceded by shifts in the composition of the larval microbiome, suggesting that the microbiome could respond faster to changes in food abundance than the host itself (Carrier *et al.*, 2018b; Carrier and Reitzel, 2020), aiding the larvae during unfavorable conditions. The larval host microbiome dominated by the discussed families and their respective taxa suggests a partnership that establishes the larval microbiome through microbial succession, aids in larval immune responses, protects from abiotic stressors like nutrient availability and UV light, and allows the host to undergo the energetically costly transition to the juvenile form on the benthos, among other potential functions (De Ridder and Foret, 2001; Galac *et al.*, 2016; Høj *et al.*, 2018; Oliveira *et al.*, 2020). The highly specific microbiome selected for by the larvae during critical stages of development indicates that the microbes involved may share a specialized evolutionary relationship with the larvae and may serve important functions in the larval holobiont.

We used an ecological approach to study the microbiome of developing echinoid larvae with a focus on the potential sources of inoculation. Within our experimental design, our investigation suggests that surrounding seawater habitat may indeed be an important contributor of microbiota to the feeding larval stages of *S. purpuratus*, compared to parents, diet, and unknown sources, in a laboratory environment. Studies such as this one further our understanding of what bacterial taxa, including where they are sourced, may be necessary for successful development of marine invertebrate larvae. While specific functions of echinoderm and marine invertebrate larval microbiomes remain largely unknown (Petersen and Osvatic, 2018; Carrier and Reitzel, 2020), this study supports the hypothesis that horizontal rather than vertical transmission is of greater importance in seeding the marine invertebrate larval holobiont (Weis, 2019; Carrier and McAlister, 2022). Using various -omics and physiological approaches, future work must illuminate the specific functions of echinoderm larval microbiomes. Having a planktotrophic larval microbiome seeded by the surrounding environment may be a critical adaptive strategy for successful association with microbial symbionts in the highly variable environment encountered by marine invertebrate offspring.

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Data Accessibility

The raw reads of *16s* rRNA gene isolated from *Strongylocentrotus purpuratus*, filtered seawater, and both algal species are available at National Center for Biotechnology Information BioProject (https://www.ncbi.nlm.nih.gov/bioproject/) under code PRJNA1023617.

Literature Cited

Aanderud, Z. T., J. Bahr, D. M. Robinson, J. Belnap, T. P. Campbell, R. A. Gill, B. McMillian, and S. St. Clair. 2019. The burning of biocrusts facilitates the emergence of a bare soil community of poorly-connected chemoheterotrophic bacteria with depressed ecosystem services. *Front. Ecol. Evol.* **7:** 467.

Antharam, V. C., D. C. McEwen, T. J. Garrett, A. T. Dossey, E. C. Li, A. N. Kozlov, Z. Mesbah, and G. P. Wang. 2016. An integrated metabolomic and microbiome analysis identified specific gut microbiota associated with fecal cholesterol and coprostanol in *Clostridium difficile* infection. *PLoS One* **11:** e0148824.

Balvočiūtė, M., and D. H. Huson. 2017. SILVA, RDP, Greengenes, NCBI and OTT: How do these taxonomies compare? *BMC Genomics* **18:** 114.

Bandaranayake, W. M. 2006. The nature and role of pigments of marine invertebrates. *Nat. Prod. Rep.* **23:** 223.

Bode, L. 2012. Human milk oligosaccharides: Every baby needs a sugar mama. *Glycobiology* **22:** 1147–1162.

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

Bowman, J. 2007. Bioactive compound synthetic capacity and ecological significance of marine bacterial genus *Pseudoalteromonas*. *Mar. Drugs* **5:** 220–241.

Brocco French, K. I., and J. D. Allen. 2021. Cannibalism of newly metamorphosed juvenile sea stars. *Ecology* **102:** e03352.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13:** 581–583.

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S. M. Owens, J. Betley, L. Fraser, M. Bauer *et al.* 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* **6:** 1621–1624.

Carey, H. V., and F. M. Assadi-Porter. 2017. The hibernator microbiome: host-bacterial interactions in an extreme nutritional symbiosis. *Ann. Rev. Nutr.* **37:** 477–500.

Carrier, T. J., and T. C. G. Bosch. 2022. Symbiont transmission in marine invertebrates: from gametes to metamorphosis. *Development* **149:** dev200552.

Carrier, T. J., and J. S. McAlister. 2022. Microbiota associated with echinoid eggs and the implications for maternal provisioning. *Mar. Ecol. Prog. Ser.* **683:** 67–79.

Carrier, T. J., and A. M. Reitzel. 2019a. Bacterial community dynamics during embryonic and larval development of three confamilial echinoids. *Mar. Ecol. Prog. Ser.* **611:** 179–188.

Carrier, T. J., and A. M. Reitzel. 2019b. Shift in bacterial taxa precedes morphological plasticity in a larval echinoid. *Mar. Biol.* **166:** 164.

Carrier, T. J., and A. M. Reitzel. 2020. Symbiotic life of echinoderm larvae. *Front. Ecol. Evol.* **7:** 509.

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

Carrier, T. J., K. Wolfe, K. Lopez, M. Gall, D. A. Janies, M. Byrne, and A. M. Reitzel. 2018b. Diet-induced shifts in the crown-of-thorns (*Acanthaster* sp.) larval microbiome. *Mar. Biol.* **165:** 157.

Carrier, T. J., S. Dupont, and A. M. Reitzel. 2019. Geographic location and food availability offer differing levels of influence on the bacterial communities associated with larval sea urchins. *FEMS Microbiol. Ecol.* **95:** fiz103.

Carrier, T. J., H. A. Lessios, and A. M. Reitzel. 2020. Eggs of echinoids separated by the Isthmus of Panama harbor divergent microbiota. *Mar. Ecol. Prog. Ser.* **648:** 169–177.

Carrier, T. J., B. A. Leigh, D. J. Deaker, H. R. Devens, G. A. Wray, S. R. Bordenstein, M. Byrne, and A. M. Reitzel. 2021. Microbiome reduction and endosymbiont gain from a switch in sea urchin life history. *Proc. Natl. Acad. Sci. USA* **118:** e2022023118.

Carrier, T. J., L. Schmittmann, S. Jung, L. Pita, and U. Hentschel. 2023. Maternal provisioning of an obligate symbiont in a sponge. *Ecol. Evol.* **13:** e10012.

Cavanaugh, C. M., S. L. Gardiner, M. L. Jones, H. W. Jannasch, and J. B. Waterbury. 1981. Prokaryotic cells in the hydrothermal vent tube worm *Riftia pachyptila* Jones: possible chemoautotrophic symbionts. *Science* **213:** 340–342.

Chase, A. B., Z. Gomez-Lunar, A. E. Lopez, J. Li, S. D. Allison, A. C. Martiny, and J. B. H. Martiny. 2018. Emergence of soil bacterial ecotypes along a climate gradient. *Environ. Microbiol.* **20:** 4112–4126.

Chaston, J., and H. Goodrich-Blair. 2010. Common trends in mutualism revealed by model associations between invertebrates and bacteria. *FEMS Microbiol. Rev.* **34:** 41–58.

Chen, J., Y. Zang, Z. Yang, T. Qu, T. Sun, S. Liang, M. Zhu, Y. Wang, and X. Tang. 2022. Composition and functional diversity of epiphytic bacterial and fungal communities on marine macrophytes in an intertidal zone. *Front. Microbiol.* **13:** 839465.

Cole, J. J. 1982. Interactions between bacteria and algae in aquatic ecosystems. *Annu. Rev. Ecol. Syst.* **13:** 291–314.

Costello, E. K., J. I. Gordon, S. M. Secor, and R. Knight. 2010. Postprandial remodeling of the gut microbiota in Burmese pythons. *ISME J.* **4:** 1375–1385.

Crenn, K., D. Duffieux, and C. Jeanthon. 2018. Bacterial epibiotic communities of ubiquitous and abundant marine diatoms are distinct in short- and long-term associations. *Front. Microbiol.* **9:** 2879.

Cussotto, S., K. V. Sandhu, T. G. Dinan, and J. F. Cryan. 2018. The neuroendocrinology of the microbiota-gut-brain axis: a behavioural perspective. *Front. Neuroendocrinol.* **51:** 80–101.

Danckert, N. P., N. Wilson, K.-Y. Phan-Thien, and D. A. J. Stone. 2021. The intestinal microbiome of Australian abalone, *Haliotis laevigata* and *Haliotis laevigata* × *Haliotis rubra*, over a 1-year period in aquaculture. *Aquaculture* **534:** 736245.

De Cáceres, M., P. Legendre, S. K. Wiser, and L. Brotons. 2012. Using species combinations in indicator value analyses. *Methods Ecol. Evol.* **3:** 973–982.

DeLong, E. F. 2009. The microbial ocean from genomes to biomes. *Nature* **459:** 200–206.

Deng, Y., F. Kokou, E. H. Eding, and M. C. J. Verdegem. 2021. Impact of early-life rearing history on gut microbiome succession and performance of Nile tilapia. *Anim. Microbiome* **3:** 81.

De Ridder, C., and T. W. Foret. 2001. Non-parasitic symbiosis between echinoderms and bacteria. Pp. 111–169 in *Echinoderm Studies*, Vol. 6, J. M. Lawrence and M. Jangoux, eds. Balkema, Rotterdam.

Deutschmann, I. M., E. Delage, C. R. Giner, M. Sebastián, J. Poulain, J. Arístegui, C. M. Duarte, S. G. Acinas, R. Massana, J. M. Gasol *et al.* 2024. Disentangling microbial networks across pelagic zones in the tropical and subtropical global ocean. *Nat. Comm.* **15:** 126.

Dufrêne, M., and P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol. Monogr.* **67:** 345–366.

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

Garcia-Pichel, F., V. Loza, Y. Marusenko, P. Mateo, and R. M. Potrafka. 2013. Temperature drives the continental-scale distribution of key microbes in topsoil communities. *Science* **340:** 1574–1577.

Gibson, A. W., and R. D. Burke. 1985. The origin of pigment cells in embryos of the sea urchin *Strongylocentrotus purpuratus*. *Dev. Biol.* **107:** 414–419.

Gilbert, J. A., D. Field, P. Swift, L. Newbold, A. Oliver, T. Smyth, P. J. Somerfield, S. Huse, and I. Joint. 2009. The seasonal structure of microbial communities in the western English Channel. *Environ. Microbiol.* **11:** 3132–3139.

Giraud, C., N. Callac, V. Boulo, J.-S. Lam, D. Pham, N. Selmaoui-Folcher, and N. Wabete. 2022. The active microbiota of the eggs and the nauplii of the Pacific blue shrimp *Litopenaeus stylirostris* partially shaped by a potential vertical transmission. *Front. Microbiol.* **13:** 886752.

González, J. M., and W. B. Whitman. 2006. Oceanospirillum and related genera. Pp. 887–915 in *The Prokaryotes*, M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, and E. Stackebrandt, eds. Springer, New York.

Griffiths, M. 1965. A study of the synthesis of naphthaquinone pigments by the larvae of two species of sea urchins and their reciprocal hybrids. *Dev. Biol.* **11:** 433–447.

Guevarra, R. B., J. H. Lee, S. H. Lee, M.-J. Seok, D. W. Kim, B. N. Kang, T. J. Johnson, R. E. Isaacson, and H. B. Kim. 2019. Piglet gut microbial shifts early in life: causes and effects. *J. Anim. Sci. Biotechnol.* **10:** 1.

Haine, E. R. 2008. Symbiont-mediated protection. *Proc. R. Soc. B* **275:** 353–361.

Hakim, J. A., J. B. Schram, A. W. E. Galloway, C. D. Morrow, M. R. Crowley, S. A. Watts, and A. K. Bej. 2019. The purple sea urchin *Strongylocentrotus purpuratus* demonstrates a compartmentalization of gut bacterial microbiota, predictive functional attributes, and taxonomic co-occurrence. *Microorganisms* **7:** 35.

Hall, M., and R. G. Beiko. 2018. 16S rRNA Gene Analysis with QIIME2. *Methods Mol. Biol.* **1849:** 113–129.

Hameed, A., M. Shahina, S.-Y. Lin, W.-A. Lai, Y.-H. Hsu, Y.-C. Liu, and C.-C. Young. 2014. *Aquibacter zeaxanthinifaciens* gen. nov., sp. nov., a zeaxanthin-producing bacterium of the family Flavobacteriaceae isolated from surface seawater, and emended descriptions of the genera *Aestuariibaculum* and *Gaetbulibacter*. *Int. J. Syst. Evol. Microbiol.* **64:** 138–145.

Herrera, J. C., S. K. McWeeney, and L. R. McEdward. 1995. Diversity of energetic strategies among echinoid larvae and the transition from feeding to nonfeeding development. *Oceanol. Acta* **19:** 313–321.

Heyland, A., and J. Hodin. 2014. A detailed staging scheme for late larval development in *Strongylocentrotus purpuratus* focused on readily-visible juvenile structures within the rudiment. *BMC Dev. Biol.* **14:** 22.

Høj, L., N. Levy, B. K. Baillie, P. L. Clode, R. C. Strohmaier, N. Siboni, N. S. Webster, S. Uthicke, and D. G. Bourne. 2018. Crown-of-thorns sea star *Acanthastercf solaris* has tissue-characteristic microbiomes with potential roles in health and reproduction. *Appl. Environ. Microbiol.* **84:** AEM.00181-18.

Ivanova, E. P., R. Christen, N. M. Gorshkova, N. V. Zhukova, V. V. Kurilenko, R. J. Crawford, and V. V. Mikhailov. 2010. *Winogradskyella exilis* sp. nov., isolated from the starfish *Stellaster equestris*, and emended description of the genus *Winogradskyella*. *Int. J. Syst. Evol. Microbiol.* **60:** 1577–1580.

Ivanova, E. P., H. J. Ng, and H. K. Webb. 2014. The family Pseudoalteromonadaceae. Pp. 575–582 in *The Prokaryotes*, E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, eds. Springer, Berlin.

Ivey, M., M. Massel, and T. G. Phister. 2013. Microbial interactions in food fermentations. *Annu. Rev. Food Sci. Technol.* **4:** 141–162.

Jackson, E. W., C. Pepe-Ranney, S. J. Debenport, D. H. Buckley, and I. Hewson. 2018. The microbial landscape of sea stars and the anatomical and interspecies variability of their microbiome. *Front. Microbiol.* **9:** 1829.

Jiao, N., Y. Zhang, Y. Zeng, N. Hong, R. Liu, F. Chen, and P. Wang. 2007. Distinct distribution pattern of abundance and diversity of aerobic anoxygenic phototrophic bacteria in the global ocean. *Environ. Microbiol.* **9:** 3091–3099.

Kämpfer, P., R. Irgang, M. Poblete-Morales, S. P. Glaeser, M. Cortez-San Martín, and R. Avendaño-Herrera. 2017. *Psychromonas aquatilis* sp. nov., isolated from seawater samples obtained in the Chilean Antarctica. *Int. J. Syst. Evol. Microbiol.* **67:** 1306–1311.

Kang, D.-W., J. B. Adams, D. M. Coleman, E. L. Pollard, J. Maldonado, S. McDonough-Means, J. G. Caporaso, and R. Krajmalnik-Brown. 2019. Long-term benefit of microbiota transfer therapy on autism symptoms and gut microbiota. *Sci. Rep.* **9:** 5821.

Kanukollu, S., S. Voget, M. Pohlner, V. Vandieken, J. Petersen, N. C. Kyrpides, T. Woyke, N. Shapiro, M. Göker, H.-P. Klenk *et al.* 2016. Genome sequence of *Shimia* str. SK013, a representative of the *Roseobacter* group isolated from marine sediment. *Stand. Genomic Sci.* **11:** 25.

Koenig, J. E., A. Spor, N. Scalfone, A. D. Fricker, J. Stombaugh, R. Knight, L. T. Angenent, and R. E. Ley. 2011. Succession of microbial consortia in the developing infant gut microbiome. *Proc. Natl. Acad. Sci. USA* **108:** 4578–4585.

Kowallik, V., and A. S. Mikheyev. 2021. Honey bee larval and adult microbiome life stages are effectively decoupled with vertical transmission overcoming early life perturbations. *mBio* **12:** e02966-21.

Krabberød, A. K., I. M. Deutschmann, M. F. M. Bjorbækmo, V. Balagué, C. R. Giner, I. Ferrera, E. Garcés, R. Massana, J. M. Gasol, and R. Logares. 2022. Long-term patterns of an interconnected core marine microbiota. *Environ. Microbiome* **17:** 22.

Kumar, G., J. E. Eble, and M. R. Gaither. 2020. A practical guide to sample preservation and pre-PCR processing of aquatic environmental DNA. *Mol. Ecol. Resour.* **20:** 29–39.

Leigh, S. C., C. Catabay, and D. P. German. 2022. Sustained changes in digestive physiology and microbiome across sequential generations of zebrafish fed different diets. *Comp. Biochem. Physiol. A* **273:** 111285.

Lindquist, N., and M. E. Hay. 1996. Palatability and chemical defense of marine invertebrate larvae. *Ecol. Monogr.* **66:** 431–450.

McFall-Ngai, M., M. G. Hadfield, T. C. G. Bosch, H. V. Carey, T. Domazet-Lošo, A. E. Douglas, N. Dubilier, G. Eberl, T. Fukami, S. F. Gilbert *et al.* 2013. Animals in a bacterial world, a new imperative for the life sciences. *Proc. Natl. Acad. Sci. USA* **110:** 3229–3236.

Nakagawa, S., H. Saito, A. Tame, M. Hirai, H. Yamaguchi, T. Sunata, M. Aida, H. Muto, S. Sawayama, and Y. Takaki. 2017. Microbiota in the coelomic fluid of two common coastal starfish species and characterization of an abundant *Helicobacter*-related taxon. *Sci. Rep.* **7:** 8764.

Nedashkovskaya, O. I., M. Vancanneyt, S. B. Kim, and N. V. Zhukova. 2009. *Winogradskyella echinorum* sp. nov., a marine bacterium of the family Flavobacteriaceae isolated from the sea urchin *Strongylocentrotus intermedius*. *Int. J. Syst. Evol. Micrbiol.* **59:** 1465–1468.

Odom, A. R., T. Faits, E. Castro-Nallar, K. A. Crandall, and W. E. Johnson. 2023. Metagenomic profiling pipelines improve taxonomic classification for 16S amplicon sequencing data. *Sci. Rep.* **13:** 13957.

Offret, C., F. Desriac, P. Le Chevalier, J. Mounier, C. Jégou, and Y. Fleury. 2016. Spotlight on antimicrobial metabolites from the marine bacteria *Pseudoalteromonas*: chemodiversity and ecological significance. *Mar. Drugs* **14:** 129.

Oliveira, B. F. R., I. R. Lopes, A. L. B. Canellas, G. Muricy, A. D. W. Dobson, and M. S. Laport. 2020. Not that close to mommy: horizontal transmission seeds the microbiome associated with the marine sponge *Plakina cyanorosea*. *Microorganisms* **8:** 1978.

Patin, N. V., and K. D. Goodwin. 2022. Capturing marine microbiomes and environmental DNA: a field sampling guide. *Front. Microbiol.* **13:** 1026596.

Peng, L.-H., X. Liang, J.-K. Xu, S. Dobretsov, and J.-L. Yang. 2020. Monospecific biofilms of *Pseudoalteromonas* promote larval settlement and metamorphosis of *Mytilus coruscus*. *Sci. Rep.* **10:** 2577.

Petersen, J. M., and J. Osvatic. 2018. Microbiomes in *Natura*: importance of invertebrates in understanding the natural variety of animal-microbe interactions. *mSystem* **3:** e00179-17.

Petersen, J. M., A. Kemper, H. Gruber-Vodicka, U. Cardini, M. Van Der Geest, M. Kleiner, S. Bulgheresi, M. Mußmann, C. Herbold, B. K. B. Seah *et al.* 2017. Chemosynthetic symbionts of marine invertebrate animals are capable of nitrogen fixation. *Nat. Microbiol.* **2:** 16195.

Pujalte, M. J., T. Lucena, M. A. Ruvira, D. Ruiz Arahal, and M. C. Macián. 2014. The family Rhodobacteraceae. Pp. 439–512 in *The Prokaryotes*, E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, eds. Springer, Berlin.

Raina, J.-B. 2018. The life aquatic at the microscale. *mSystem* **3:** e00150-17.

Rodriguez-Lanetty, M., E. M. Wood-Charlson, L. L. Hollingsworth, D. A. Krupp, and V. M. Weis. 2006. Temporal and spatial infection dynamics indicate recognition events in the early hours of a dinoflagellate/coral symbiosis. *Mar. Biol.* **149:** 713–719.

Satomi, M., B. Kimura, T. Hamada, S. Harayama, and T. Fujii. 2002. Phylogenetic study of the genus *Oceanospirillum* based on 16S rRNA and gyrB genes: emended description of the genus *Oceanospirillum*, description of *Pseudospirillum* gen. nov., *Oceanobacter* gen. nov. and *Terasakiella* gen. nov. and transfer of *Oceanospirillum*. *Int. J. Syst. Evol. Microbiol.* **52:** 739–747.

Schmitt, S., J. B. Weisz, N. Lindquist, and U. Hentschel. 2007. Vertical transmission of a phylogenetically complex microbial consortium in the viviparous sponge *Ircinia felix*. *Appl. Environ. Microbiol.* **73:** 2067–2078.

Schuh, N. W., T. J. Carrier, C. S. Schrankel, A. M. Reitzel, A. Heyland, and J. P. Rast. 2020. Bacterial exposure mediates developmental plasticity and resistance to lethal *Vibrio lentus* infection in purple sea urchin (*Strongylocentrotus purpuratus*) larvae. *Front. Immunol.* **10:** 3014.

Schwarz, J. A., D. A. Krupp, and V. M. Weis. 1999. Late larval development and onset of symbiosis in the scleractinian coral *Fungia scutaria*. *Biol. Bull.* **196:** 70–79.

Seymour, J. R., S. A. Amin, J.-B. Raina, and R. Stocker. 2017. Zooming in on the phycosphere: the ecological interface for phytoplankton–bacteria relationships. *Nat. Microbiol.* **2:** 17065.

Sharon, G., N. J. Cruz, D.-W. Kang, M. J. Gandal, B. Wang, Y.-M. Kim, E. M. Zink, C. P. Casey, B. C. Taylor, C. J. Lane *et al.* 2019. Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. *Cell* **177:** 1600–1618.

Sigler, W. V., and J. Zeyer. 2004. Colony-forming analysis of bacterial community succession in deglaciated soils indicates pioneer stress-tolerant opportunists. *Microb. Ecol.* **48:** 316–323.

Smith, M. M., L. C. Smith, R. A. Cameron, and L. A. Urry. 2008. The larval stages of the sea urchin, *Strongylocentrotus purpuratus*. *J. Morphol.* **269:** 713–733.

Sonnenschein, E. C., G. Jimenez, M. Castex, and L. Gram. 2021. The *Roseobacter*-group bacterium *Phaeobacter* as a safe probiotic solution for aquaculture. *Appl. Environ. Microbiol.* **87:** e0258120.

Sprockett, D., T. Fukami, and D. A. Relman. 2018. Role of priority effects in the early-life assembly of the gut microbiota. *Nat. Rev. Gastroenterol. Hepatol.* **15:** 197–205.

Stephens, W. Z., A. R. Burns, K. Stagaman, S. Wong, J. F. Rawls, K. Guillemin, and B. J. M. Bohannan. 2016. The composition of the zebrafish intestinal microbial community varies across development. *ISME J.* **10:** 644–654.

Storey, S., M. M. Ashaari, N. Clipson, E. Doyle, and A. B. de Menezes. 2018. Opportunistic bacteria dominate the soil microbiome response to phenanthrene in a microcosm-based study. *Front. Microbiol.* **9:** 2815.

Strathmann, R. R. 1975. Larval feeding in echinoderms. *Am. Zool.* **15:** 717–730.

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, Seattle.

Takemura, A. F., D. M. Chien, and M. F. Polz. 2014. Associations and dynamics of Vibrionaceae in the environment, from the genus to the population level. *Front. Microbiol.* **5:** 38.

Tebben, J., D. M. Tapiolas, C. A. Motti, D. Abrego, A. P. Negri, L. L. Blackall, P. D. Steinberg, and T. Harder. 2011. Induction of larval metamorphosis of the coral *Acropora millepora* by tetrabromopyrrole isolated from a *Pseudoalteromonas* bacterium. *PLoS One* **6:** e19082.

Thompson, F. L., T. Iida, and J. Swings. 2004. Biodiversity of vibrios. *Microbiol. Mol. Biol. Rev.* **68:** 403–431.

Trackenberg, S. N., E. L. Richardson, and J. D. Allen. 2020. Effects of embryo energy, egg size, and larval food supply on the development of asteroid echinoderms. *Ecol. Evol.* **10:** 7839–7850.

Unzueta-Martínez, A., E. Scanes, L. M. Parker, P. M. Ross, W. O'Connor, and J. L. Bowen. 2022. Microbiomes of the Sydney rock oyster are acquired through both vertical and horizontal transmission. *Anim. Microbiome* **4:** 32.

Vaughn, D., and J. D. Allen. 2010. The peril of the plankton. *Integr. Comp. Biol.* **50:** 552–570.

Walker, C. W., and M. P. Lesser. 1989. Nutrition and development of brooded embryos in the brittlestar *Amphipholis squamata*: Do endosymbiotic bacteria play a role? *Mar. Biol.* **103:** 519–530.

Webster, N. S., and M. W. Taylor. 2012. Marine sponges and their microbial symbionts: love and other relationships. *Environ. Microbiol.* **14:** 335–346.

Weis, V. M. 2019. Cell biology of coral symbiosis: foundational study can inform solutions to the coral reef crisis. *Integr. Comp. Biol.* **59:** 845–855.

Weis, V. M., W. S. Reynolds, M. Deboer, and D. Krupp. 2001. Host-symbiont specificity during onset of symbiosis between the dinoflagellates *Symbiodinium* spp. and planula larvae of the scleractinian coral *Fungia scutaria*. *Coral Reefs* **20:** 301–308.

Whitman, W. B., D. C. Coleman, and W. J. Wiebe. 1998. Prokaryotes: the unseen majority. *Proc. Natl. Acad. Sci. USA* **95:** 6578–6583.

Willis, A. D. 2019. Rarefaction, alpha diversity, and statistics. *Front. Microbiol.* **10:** 02407.

Xiao, F., W. Zhu, Y. Yu, Z. He, B. Wu, C. Wang, L. Shu, X. Li, H. Yin, J. Wang *et al.* 2021. Host development overwhelms environmental dispersal in governing the ecological succession of zebrafish gut microbiota. *NPJ Biofilms Microbiomes* **7:** 5.

Xiong, J., X. Li, M. Yan, J. Lu, Q. Qiu, and J. Chen. 2020. Comparable ecological processes govern the temporal succession of gut bacteria and microeukaryotes as shrimp aged. *Microb. Ecol.* **80:** 935–945.