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Larvae of Deep-Sea Invertebrates Harbor Low-Diversity Bacterial Communities

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Abstract. Microbial symbionts are a common life-history character of marine invertebrates and their developmental stages. Communities of bacteria that associate with the eggs, embryos, and larvae of coastal marine invertebrates tend to be species specific and correlate with aspects of host biology and ecology. The richness of bacteria associated with the developmental stages of coastal marine invertebrates spans four orders of magnitude, from single mutualists to thousands of unique taxa. This understanding stems predominately from the developmental stages of coastal species. If they are broadly representative of marine invertebrates, then we may expect deep-sea species to associate with bacterial communities that are similar in diversity. To test this, we used amplicon sequencing to profile the bacterial communities of invertebrate larvae from multiple taxonomic groups (annelids, molluscs, crustaceans) collected from 2500 to 3670 m in depth in near-bottom waters near hydrothermal vents in 3 different regions of the Pacific Ocean (the East Pacific Rise, the Mariana Back-Arc, and the Pescadero Basin). We find that larvae of deep-sea invertebrates associate with low-diversity bacterial communities (~30 bacterial taxa) that lack specificity between taxonomic groups. The diversity of these communities is estimated to be ~7.9 times lower than that of coastal invertebrate larvae, but this result depends on the taxonomic group. Associating with a low-diversity community may imply that deep-sea invertebrate larvae do not have a strong reliance on a microbiome and that the hypothesized lack of symbiotic contributions would differ from expectations for larvae of coastal marine invertebrates.

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Abbreviations: ASV, amplicon sequence variant; OTU, operational taxonomic unit; PCR, polymerase chain reaction.

Online enhancements: supplemental tables and data code.

Introduction

Microbial symbioses are a widespread and functionally important life-history character of marine invertebrates and their developmental stages. The eggs, embryos, and larvae of annelids (Giere and Langheld, 1987; Vijayan *et al.*, 2019), bivalves (Sipe *et al.*, 2000; Salerno *et al.*, 2005), bryozoans (Woollacott, 1981; Lopanik *et al.*, 2004), cnidarians (Apprill *et al.*, 2012; Sharp *et al.*, 2012), crustaceans (Gil-Turnes *et al.*, 1989; Guri *et al.*, 2012), echinoderms (Carrier and Reitzel, 2019a, 2020), gastropods (Klussmann-Kolb and Brodie, 1999), and poriferans (Maldonado, 2009; Björk *et al.*, 2019) all associate with microbial symbionts. These partnerships range from single mutualists, such as the nutritional endosymbiont of *Amphipholis squamata* embryos (Walker and Lesser, 1989; Lesser and Walker, 1992), to diverse prokaryotic communities composed of hundreds of unique taxa, as is observed for poriferan larvae (Björk *et al.*, 2019).

The developmental stages of marine invertebrates tend to associate with species-specific bacterial communities that are distinct from the environmental microbiota and correlate with aspects of host biology and ecology (Carrier and Reitzel, 2018; Vijayan *et al.*, 2019). These communities, for example, can undergo a developmental succession and may exhibit community-level shifts in response to abiotic factors (Carrier and Reitzel, 2018, 2019a; Vijayan *et al.*, 2019). Specifically, asteroid and echinoid larvae exposed to different degrees of food availability (Carrier *et al.*, 2018, 2019) and poriferan larvae facing elevated temperatures (Webster *et al.*, 2011) both experience taxonomic and compositional changes in their associated bacterial

communities. These responses are hypothesized to buffer the host from environmental stressors (Zilber-Rosenberg and Rosenberg, 2008; Kohl and Carey, 2016). The current understanding of the properties of symbiont communities associated with the developmental stages of marine invertebrates stems predominately from coastal species, which may not be broadly representative of species beyond the continental shelf.

Studies on the partnerships between the developmental stages of deep-sea invertebrates and microbes focus on the transmission of chemoautotrophic bacteria by species endemic to hydrothermal vents and cold seeps. To maintain these associations between generations, symbionts are either packaged with the egg for vertical transmission or acquired from free-living environmental populations (McFall-Ngai, 2002; Bright and Bulgheresi, 2010; Funkhouser and Bordenstein, 2013; Nyholm, 2020). Vesicomyid clams (*e.g.*, *Calyptogena* spp.), for example, transmit symbionts within follicle cells surrounding the primary oocyte, while the annelid *Riftia pachyptila* is aposymbiotic until recently settled juveniles are colonized by free-living sulfide-oxidizing endosymbionts (Cary and Giovannoni, 1993; Nussbaumer *et al.*, 2006). Similar to vesicomyid clams and to coastal invertebrates, the deep-sea crab *Kiwa puravida* (Goffredi *et al.*, 2014), shrimp *Rimicaris exoculata* (Guri *et al.*, 2012; Methou *et al.*, 2019), and sponges *Craniella* spp. (Busch *et al.*, 2020) inherit bacterial communities that exhibit ontogenetic shifts in community composition and associate with microbial taxa not suspected to be chemoautotrophic mutualists.

Outside of these studies, little is known about the bacterial taxa associated with the developmental stages of deep-sea invertebrates. This is partially because studies to date have focused on the transmission mode of specific mutualists (Cary and Giovannoni, 1993; Peek *et al.*, 1998; Salerno *et al.*, 2005) and the technical limitations of sampling deep-sea larvae. If the developmental stages of coastal species are broadly representative of marine invertebrates, then we would expect deep-sea species to associate with bacterial communities that are similar in diversity. To test this, invertebrate larvae from multiple taxonomic groups were collected at depths ranging from 2500 to 3670 m in near-bottom waters near hydrothermal vents in 3 different regions of the Pacific Ocean: the East Pacific Rise, the Mariana Back-Arc, and the Pescadero Basin (Fig. 1). We then used amplicon sequencing to profile the larval-associated bacterial communities and compared these diversity estimates to larvae of coastal invertebrates that are reported in the literature.

Materials and Methods

Specimen collection

Larvae were collected near hydrothermal vent fields on the East Pacific Rise (9°50′ N; on-axis near the Tica vent) during R/V *Atlantis* cruise AT15-26 in November 2007, on the Mariana Back-Arc Spreading Center (near the Snail and Archaean vent fields; within 300 m of active hydrothermal vents) during R/V *Yokosuka* cruise YK10-11 in September 2010, and in the Pescadero Basin (the Auka vent field) in the Gulf of California during E/V *Nautilus* cruise NA091 in November 2017 (Table S1, available online). The East Pacific Rise and Mariana specimens were collected 3 m above the seafloor, using a McLane WTS-LV50 plankton pump (McLane Labs, Falmouth, MA) pumping seawater for 24 h over a 63-μm mesh at a rate of 30 L min^{-1}. The Pescadero specimens were collected 1 m above the seafloor by using the suction (slurp)

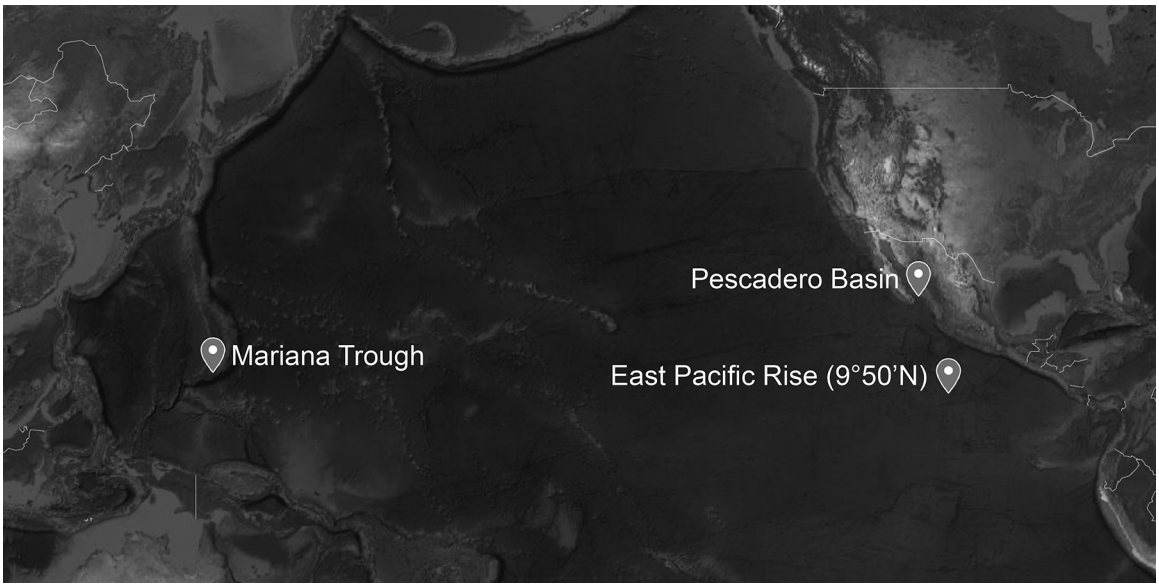

Figure 1. Locations where deep-sea larvae were collected: East Pacific Rise (9°50′ N vent field), Southern Mariana Trough (near Snail vent field), and Pescadero Basin (Auka vent field) in the Gulf of California.

sampler on ROV *Hercules* with a 10-min filtration over a 63-μm mesh at a rate of ~100 L min^{-1}. Samples were processed within an hour upon recovery on deck, with many specimens still alive. All samples were washed off the mesh, using 95% non-denatured ethanol, into a 250-mL ethanol-rinsed jar.

Identification of larvae

Portions of the sample were sorted at the Woods Hole Oceanographic Institution by using a wide-mouthed pipette in a petri dish under a dissecting microscope. Specimens were sorted into ethanol-rinsed vials by major taxa (*i.e.*, gastropods, polychaetes, bivalves, and crustaceans). The East Pacific Rise and Pescadero samples were then stored at room temperature, while those from Mariana were stored at 4 °C. Specimens were later identified to morphotypes at the lowest taxonomic level (Table S1, available online; Mills *et al.*, 2009). Samples were then moved into separate 1.5-mL vials with 95% ethanol.

Total DNA was extracted from 25, 25, and 10 larvae from the East Pacific Rise, Mariana Back-Arc, and Pescadero Basin, respectively, using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA) (Table S1, available online). DNA was quantified using a Qubit Fluorometer (Thermo Fisher Scientific). Using 10 ng of DNA per reaction, universal polymerase chain reaction (PCR) primers amplified the *28S rRNA* gene that was then visualized by gel electrophoresis (Table S2, available online; Machida and Knowlton, 2012). These products were extracted from the gel, using the GeneJET Gel Extraction and DNA Clean-Up Micro Kit (Thermo Fisher Scientific) and sequenced directly using Sanger sequencing. Sequences were then compared to those in GenBank by using BLAST (Altschul *et al.*, 1990). These 28S rDNA sequences are accessible in the Dryad Digital Repository (Carrier *et al.*, 2021a).

Profiling bacterial communities

Using the total DNA extracted from individual larvae, the V3/V4 regions of the *16S rRNA* gene of bacterial DNA were amplified using universal primers (Table S2, available online; Klindworth *et al.*, 2013). Additional PCRs to identify potential bacterial contaminants from the DNA extraction kit ($n = 3$) were also run in parallel using the elute from samples where only water was used as the input. Products were purified using the Axygen AxyPrep MAG PCR Clean-Up Kit (Axygen Scientific, Union City, CA), indexed using the Nextera XT Index Kit V2 (Illumina, San Diego, CA), and then purified again. At each cleanup step fluorometric quantitation was performed using a Qubit, and libraries were validated using a Bioanalyzer High-Sensitivity DNA Chip (Agilent Technologies, Santa Clara, CA). Illumina MiSeq sequencing (ver. 3; 2 × 300-bp paired-end reads) was performed in the Department of Bioinformatics and Genomics at the University of North Carolina at Charlotte.

Computational analysis

Raw reads along with quality information were imported into QIIME 2 (ver. 2019.1; Bolyen *et al.*, 2019), where adapters were removed and forward and reverse reads were paired using VSEARCH (Rognes *et al.*, 2016), filtered by quality score, and denoised using Deblur (Amir *et al.*, 2017). The QIIME 2-generated features were analyzed as amplicon sequence variants (ASVs; Callahan *et al.*, 2017) and were assigned taxonomy using SILVA (ver. 132; Quast *et al.*, 2013). All Archaeal ASVs as well as the ASVs observed in the DNA kit (based on reagent blanks) were removed from all samples in the data table. The filtered table was then rarified to 392 sequences per sample (*i.e.*, the read count for the sample with the least remaining reads).

To test whether community membership and composition differed between taxonomic groups of larvae and geography, we calculated unweighted and weighted UniFrac values (Lozupone and Knight, 2005) and compared them by using principal coordinate analyses. Results from these analyses were then re-created in QIIME 1 (ver. 1.9.1; Caporaso *et al.*, 2010) and stylized using Adobe Illustrator. We then used a permutational multivariate analysis of variance (PERMANOVA) to test for differences in membership and composition and performed pairwise comparisons. We also calculated four measures of alpha diversity: total ASVs, Faith's phylogenetic diversity, McIntosh dominance, and McIntosh evenness. Due to replication limitations, we treated each taxonomic group-geography combination as an individual group and compared these values using a one-way analysis of variance (ANOVA), with a *P*-value of less than 0.05 representing significance. Lastly, we summarized the bacterial classes associated with larvae from these taxonomic groups.

Our QIIME-based pipeline used to convert raw reads to ASVs for visualization is presented in detail in Appendix Note A1, available online. The *16S rRNA* gene reads are accessible in the Dryad Digital Repository (Carrier *et al.*, 2021a).

Diversity comparison of invertebrate larvae

Data for our meta-analysis that compared the bacterial communities associated with coastal and deep-sea invertebrate larvae were sourced from the literature (Table 1). Specifically, we were able to find data for the developmental stages of 33 species of coastal invertebrates (1 annelid, 2 arthropods, 9 cnidarians, 14 echinoderms, 2 molluscs, and 5 poriferans) as well as 4 deep-sea species (2 arthropods and 2 poriferans) (Goffredi *et al.*, 2014; Methou *et al.*, 2019; Busch *et al.*, 2020).

Due to the many confounding variables of microbiome meta-analyses (*e.g.*, sampling technique, molecular methods, sequencing platform, and bioinformatic pipeline) (Knight *et al.*, 2018; Pollock *et al.*, 2018) and no common pipeline to analyze diverse microbiome datasets, we used richness estimates

Table 1

Estimated number of bacterial taxa observed to associate with the developmental stages of coastal invertebrates

Taxonomy	Estimated taxa	Reference
Annelida		
Hydroides elegans	1800	Vijayan *et al.*, 2019
Arthropoda		
Litopenaeus vannamei	284	Xue *et al.*, 2018
Semibalanus balanoides	150	Aldred and Nelson, 2019
Cnidaria		
Acropora digitifera	475	Bernasconi *et al.*, 2019
Acropora millepora	88	Lema *et al.*, 2014
Acropora tenuis	135	Damjanovic *et al.*, 2019
Chrysaora hysoscella	37	Hao *et al.*, 2019
Cyanea lamarckii	35	Hao *et al.*, 2019
Nematostella vectensis	90	Mortzfeld *et al.*, 2015
Pocillopora acuta	742	Damjanovic *et al.*, 2020
Pocillopora meandrina	28	Apprill *et al.*, 2009
Porites astreoides	111	Sharp *et al.*, 2012
Echinodermata		
Acanthaster sp.	1170	Carrier *et al.*, 2018
Diadema antillarum	62	Carrier *et al.*, 2020
Diadema mexicanum	109	Carrier *et al.*, 2020
Echinometra lucunter	190	Carrier *et al.*, 2020
Echinometra vanbrunti	143	Carrier *et al.*, 2020
Echinometra viridis	165	Carrier *et al.*, 2020
Heliocidaris erythrogramma	93	Carrier *et al.*, 2021b
Heliocidaris tuberculata	301	Carrier *et al.*, 2021b
Lytechinus variegatus	218	Carrier and Reitzel, 2019b
Mesocentrotus franciscanus	1710	Carrier and Reitzel, 2018; Carrier and Reitzel, 2019a
Mithrodia clavigera	12	Galac *et al.*, 2016
Strongylocentrotus droebachiensis	1615	Carrier and Reitzel, 2018, 2019a; Carrier *et al.*, 2019
Strongylocentrotus purpuratus	1963	Carrier and Reitzel, 2018, 2019a
"Yellow Oreasteridae"	16	Galac *et al.*, 2016
Mollusca		
Crassostrea virginica	301	Arfkena *et al.*, 2021
Patinopecten yessoensisis	34	Xueying *et al.*, 2016
Porifera		
Amphimedon queenslandica	53	Fieth *et al.*, 2016
Clathria prolifera	582	Sacristán-Soriano *et al.*, 2019
Halichondria bowerbanki	535	Sacristán-Soriano *et al.*, 2019
Rhopaloeides odorabile	37	Webster *et al.*, 2011
Tedania sp.	1502	Wu *et al.*, 2018

for the average number of bacterial taxa (*i.e.*, operational taxonomic units [OTUs] or ASVs) presented by the authors in each study (Table 1). From the data presented in this study, we used our morphological identification to calculate the average number of ASVs for larvae from each taxonomic group (see Table S1, available online, for our specific groupings). We then used a Mann-Whitney *U* test to compare the bacterial diversity of coastal and deep-sea larvae, with a Bonferroni-corrected *P*-value of 0.013 representing significance. Due to limited replication, we compared the taxonomic groups with two or more samples in each habitat and did so using individual Mann-Whitney *U* tests, with a Bonferroni-corrected *P*-value of 0.013 representing significance.

Results

Identification of larvae

Some of the larval specimens from the East Pacific Rise, Mariana Back-Arc, and Pescadero Basin could be identified to the family or genus level but not to species (Tables 2, S1, available online), with particular larvae being assigned to groups known to inhabit deep-sea hydrothermal vents. We compared these classifications with the sFDvent species traits database (Chapman *et al.*, 2019) and do not have confidence that any of the larval specimens were from species known to associate with chemosynthetic symbionts. Moreover, in most cases, 28S rDNA sequences were insufficient

Table 2

Taxonomic summary of deep-sea larvae in this study

	Taxonomy	
Study site and vent	Highest level	Lowest level
East Pacific Rise		
Tica vent (9°50′ N)	Crustacea	Decapod zoea, unidentified
		Leptostracan juvenile, unidentified
	Gastropoda	*Lepetodrilus* sp.
	Polychaeta	Chaetosphaerid
		Ophryotrocha sp.
		Unidentified
Mariana Back-Arc		
Archaean vent	Gastropoda	*Lepetodrilus* sp.
		Unidentified
	Polychaeta	Polynoid-like
Snail vent	Bivalvia	Unidentified
	Gastropoda	*Lepetodrilus* sp.
		Unidentified
	Polychaeta	Chaetosphaerid
		Glycerid
		Nectochaete
		Polynoid
		Polynoid-like
		Unidentified
Pescadero Basin		
Auka vent field	Gastropoda	Unidentified
	Polychaeta	*Ophryotrocha* sp.

See the Biological and Chemical Oceanography Data Management Office (BCO-DMO) dataset (Beaulieu *et al.*, 2021) for more details.

to give the specimens any additional identification, because there was no match in GenBank; thus, the genetic identification served as support for the morphological identification but could not provide additional taxonomic resolution.

We are confident that 21 of the 25 East Pacific Rise specimens are vent species: 20 gastropod larvae in the genus *Lepetodrilus* and 1 polychaete larva in the genus *Ophryotrocha*. We are somewhat confident for one leptostracan. The other three East Pacific Rise specimens were one decapod zoea and two unidentified polychaete larvae (Tables 2, S1, available online). We are confident that 2 of the 25 Mariana Back-Arc specimens are vent species: 2 gastropod larvae in the genus *Lepetodrilus* (species known at these vents). None of the Mariana polychaete larvae could be identified to genus level; however, we are somewhat confident that three polychaete larvae identified to families Iphionidae and Polynoidae may be vent species. Although there is some genetic evidence for genus *Paralvinella*, we had to conservatively assign two polychaete larvae in morphotype "nectochaete, classic morphology" to Terebellomorpha. The other 18 Mariana specimens were 3 unidentified gastropod larvae, 1 unidentified bivalve larva, 2 glycerid polychaete larvae, 5 polychaete larvae in Spioniformia, 2 polychaete larvae in order Phyllodocida, and 5 unidentified polychaete larvae (Tables 2, S1, available online). We are confident that 5 of the 10 Pescadero Basin specimens are vent species: 5 polychaete larvae in genus *Ophryotrocha*. We are somewhat confident for five gastropod larvae with the same morphotype and genetic sequences not yet matched to a species known at these vents (Tables 2, S1).

Larval-associated bacterial communities

We successfully amplified the bacterial communities for 54 of 60 (90%) deep-sea invertebrate larvae. These samples totaled 107,345 high-quality sequences, with 392 and 16,791 sequences representing the lowest and highest read count, respectively (Fig. A1A). This low rarefaction depth included 30.1% (±18.7%) of the high-quality sequences but 85.6% (±9.9%) of the ASVs for each sample. The taxonomic and phylogenetic diversity of the larval-associated bacterial communities at this rarefaction depth had essentially plateaued (Fig. 2; Table S3, available online), supporting that much of the community richness was profiled.

These larvae associated with ~30 (±11) ASVs on average, ranging from 4 ASVs for a gastropod from Pescadero Basin to 73 ASVs for a polychaete from the Mariana Back-Arc (Fig. A1B). The taxonomic and phylogenetic diversity, as well as community dominance and evenness, were consistent

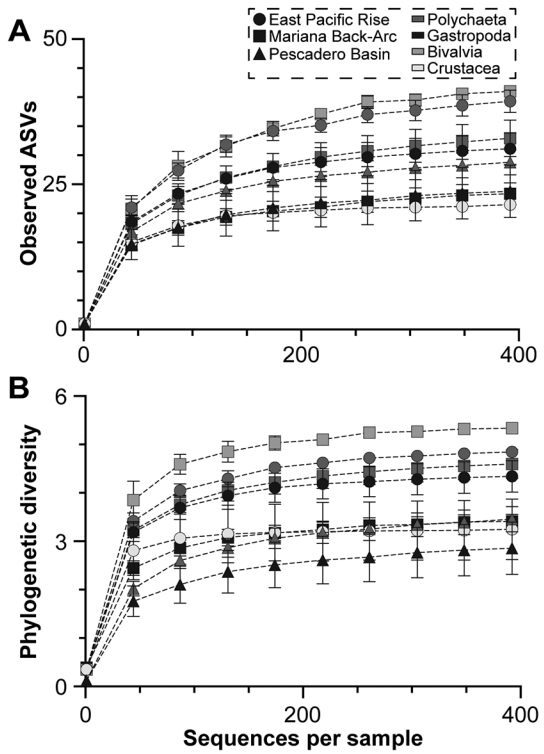

Figure 2. Alpha rarefaction curve for deep-sea invertebrate larvae. Alpha rarefaction curves based on observed amplicon sequence variants (ASVs) and phylogenetic diversity (mean ± standard deviation) for the bacterial community associated with bivalve, crustacean, gastropod, and polychaete larvae from the East Pacific Rise, Mariana Back-Arc, and Pescadero Basin. These estimates were based on a rarefaction depth of 392 sequences, and this sequence depth was used for all analyses.

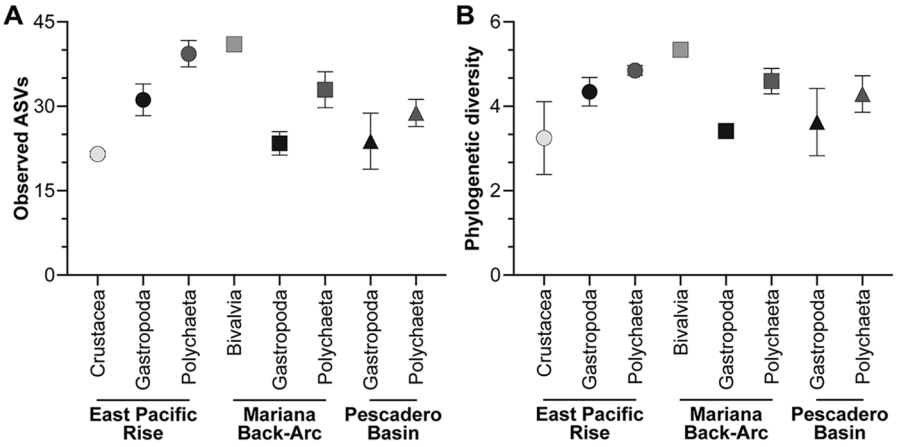

Figure 3. Alpha diversity for bacterial communities of deep-sea invertebrate larvae. Total amplicon sequence variants (ASVs; A; mean ± standard error) and phylogenetic diversity (B; mean ± standard error) of the bacterial communities associated with bivalve, crustacean, gastropod, and polychaete larvae from the East Pacific Rise, Mariana Back-Arc, and Pescadero Basin.

across the taxonomic groups and sampling location ($P > 0.05$ for each; Figs. 3, A2; Table S4, available online). Moreover, community diversity was also similar for gastropod and polychaete larvae across the three locations (Figs. 3, A2; Table S4).

The bacterial taxa associated with deep-sea invertebrate larvae varied based on community membership but not composition (unweighted UniFrac: $P = 0.016$; weighted UniFrac: $P = 0.184$; Fig. 4; Table S5, available online). These bacterial communities did, however, tend to group more by taxonomic groups (*e.g.*, gastropods or polychaetes) than by sampling location (Fig. 4A). Pairwise comparison of the taxonomic groups suggests four differences between larval-associated communities: (i–ii) gastropods from the East Pacific Rise and polychaetes from both the Mariana Back-Arc and the Pescadero Basin, (iii) polychaetes from the East Pacific Rise and the Pescadero Basin, and (iv) polychaetes from the Mariana Back-Arc and gastropods from the Pescadero Basin (Fig. 4; Table S5, available online).

Taxonomic representation

The microbiota associated with deep-sea invertebrate larvae were primarily composed of 12 bacterial classes (each with >1% of the community; Fig. 5; Table S6, available online). Of these, the Bacteroidia, Alphaproteobacteria, and Gammaproteobacteria, on average, represented ~12.9%, ~19.2%, and ~40.7% of bacterial communities associated with these deep-sea invertebrate larvae, respectively (Fig. 5A). The other nine bacterial classes included the Acidimicrobiia, Actinobacteria, Bacilli, Campylobacteria, Deltaproteobacteria, Oxyphotobacteria, Phycisphaerae, Planctomycetacia, and Verrucomicrobiae (Fig. 5).

Community diversity of invertebrate larvae

Larvae of coastal invertebrates associated with an average of ~448 unique bacterial taxa (*i.e.*, OTUs or ASVs), ranging from 12 unique bacterial taxa for the asteroid *Mithrodia clavigera* to 1963 unique bacterial taxa for the echinoid *Strongylocentrotus purpuratus* (Fig. 6; Table 1). The community diversity of coastal invertebrate larvae was significantly more (~7.9 times) than that of larvae from the deep sea ($P = 0.005$; Fig. 6; Table 1). We were able to compare three of the six taxonomic groups (arthropods, molluscs, and poriferans) across habitats, and we observed a similar community diversity for each (arthropods: $P = 0.133$; molluscs: $P = 0.056$; poriferans: $P = 0.429$; Fig. 6).

Discussion

Symbiotic interactions between the developmental stages of marine invertebrates and microbes are widespread across animal phyla and are presumed to be an integral component of development (*e.g.*, Carrier and Reitzel, 2020; Rodrigues de Oliveira *et al.*, 2020). The richness of microbial symbionts associated with the developmental stages of marine invertebrates spans four orders of magnitude, from single mutualists to thousands of unique taxa (Fig. 6; Table 1). This understanding, however, is based on coastal invertebrates and does not concern species in the deep sea. Our primary finding from profiling the bacterial communities of various deep-sea invertebrate larvae is that the taxonomic and phylogenetic diversity is low and, on average, ~7.9 times less than that of larvae from coastal invertebrate larvae.

Animal-microbe symbioses range from single-mutualist (*e.g.*, *Euprymna scolopes* and *Vibrio fischeri*) to animal-associated microbial communities with hundreds or even thousands of distinct taxa based on sequence variation of the *16S rRNA* gene (*e.g.*, corals and sponges) (Nyholm and Mcfall-Ngai, 2004; Rosenberg *et al.*, 2007; Thomas *et al.*, 2016; O'Brien *et al.*, 2019). With an average of 30 unique bacterial taxa (*i.e.*, ASVs) per individual, deep-sea invertebrate larvae fall

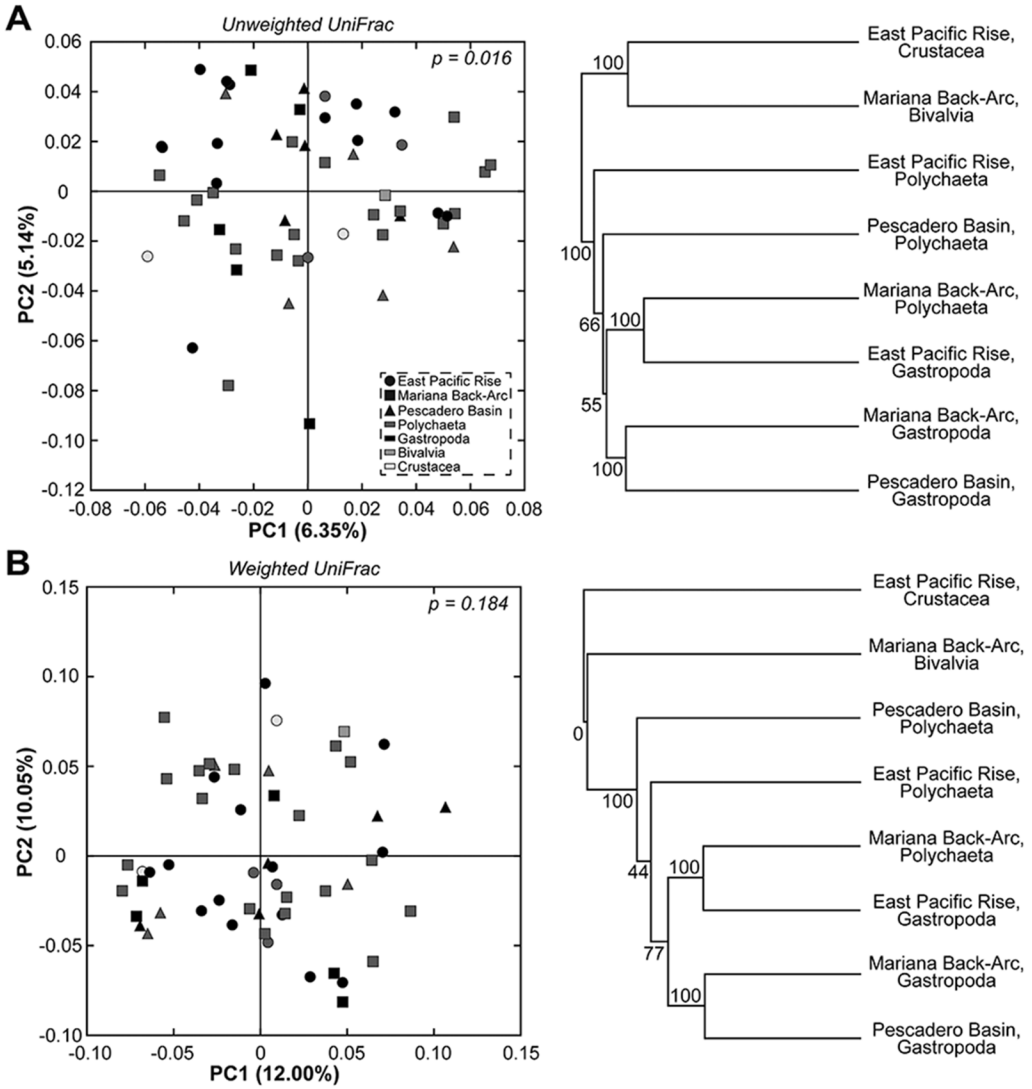

Figure 4. Relatedness of the bacterial communities of deep-sea invertebrate larvae. Similarity between the bacterial communities associated with bivalve, crustacean, gastropod, and polychaete larvae from the East Pacific Rise, Mariana Back-Arc, and Pescadero Basin based on membership (unweighted UniFrac; A) and composition (weighted UniFrac; B) with corresponding microbial dendrograms.

toward the low-complexity end of this symbiosis spectrum (Hammer *et al.*, 2019; O'Brien *et al.*, 2019). Even though the rarefaction depth used to estimate the diversity of these communities was low (Caporaso *et al.*, 2012), the rarefaction curves for both taxonomic and phylogenetic diversity had largely plateaued, suggesting that the majority of the present bacterial diversity was captured despite the limited sequencing depth.

These diversity estimates may be an overestimation. There are numerous sources of, and opportunities for, microbial contamination during field collections and processing (Hammer *et al.*, 2019). This contamination is currently unquantified for field-based sampling of marine invertebrate larvae. What can be accounted for were the microbial contaminants from the molecular processing. This source is widely recognized, suspected to significantly influence the low-tissue samples, and can be accounted through sequencing controls (Salter *et al.*, 2014; de Goffau *et al.*, 2018; Eisenhofer *et al.*, 2019). Due to the lack of controls during field collections and processing, we suspect that microbial contamination had some influence on the diversity estimates reported here and, thus, that our ~30 ASV average for deep-sea invertebrate larvae may be an overestimation. The extent of this overestimation remains in question. One possibility is that deep-sea invertebrate larvae without obligate chemoautotrophic bacteria harbor few, if any, other microbial residents, as is observed in a diverse array of animal taxa (Hammer *et al.*, 2017, 2019).

One underlying principle, and potentially fundamental property, of animals that associate with microbiota is that the composition of these communities tends to be host specific (Gilbert *et al.*, 2012; McFall-Ngai *et al.*, 2013; Bordenstein and Theis,

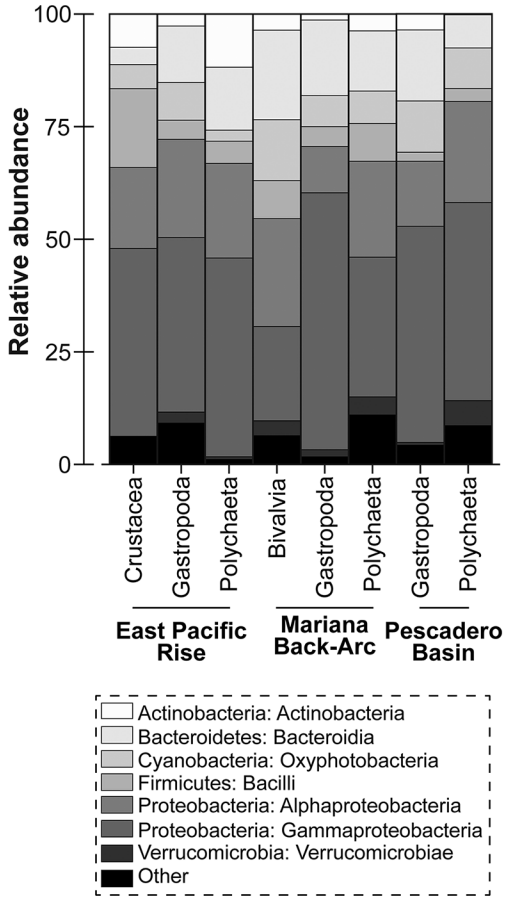

Figure 5. Bacterial taxa of deep-sea invertebrate larvae. Mean class-level taxonomic profiles of the total community associated with bivalve, crustacean, gastropod, and polychaete larvae from the East Pacific Rise, Mariana Back-Arc, and Pescadero Basin.

2015). While we are not fully certain of the species for these deep-sea larvae, the compositional similarity across three geographically distant sampling locations for multiple phyla suggests that these low-diversity communities are similar in composition and structure. The lack of a specific signature was also recently observed for eggs of the Caribbean echinoids *Echinometra lucunter* and *Echinometra viridis* (Carrier *et al.*, 2020). One possible reason why some deep-sea larvae associate with bacterial communities that are both low diversity and compositionally consistent is that they form bacterial partnerships by neutral or stochastic processes (Bordenstein and Theis, 2015; Sieber *et al.*, 2019).

If the bacterial taxa detected in our community profiles were the result of neutral or stochastic processes, then we would suspect that the taxa associated with these deep-sea invertebrate larvae resemble the seawater microbiota. A proper comparison of host and environment cannot be made here, because seawater samples were not collected alongside these larvae. A coarse comparison of the bacterial communities associated with these larvae and with that of seawater from these locations (Gulmann *et al.*, 2015; Espinosa-Asuar *et al.*, 2019; Trembath-Reichert *et al.*, 2019) suggests that there is some taxonomic overlap. However, identical and parallel molecular and bioinformatic sampling pipelines would be required to conclude whether deep-sea larvae associate with a microbial community that assembles by neutral processes. Alternatively, these larvae may be enriched with seawater taxa. Some of the most abundant bacterial genera in this dataset are environmental generalists (*e.g.*, *Pseudomonas*), and we suspect that they passively come in contact with these larvae.

The low-diversity estimate of deep-sea invertebrate larvae was, on average, ~7.9 times less than larvae of coastal invertebrate species. This difference in diversity is notable, and we suspect that it varies between taxonomic groups. The latter stems from our observation that arthropod, mollusc, and poriferan larvae from each habitat associate with bacterial communities that are similar in diversity; this remains to be tested in annelids, cnidarians, and echinoderms. Our comparisons between these taxonomic groups are, however, confounded by sampling technique, molecular methods, sequencing platform, and bioinformatic pipeline (Knight *et al.*, 2018; Pollock *et al.*, 2018). Notably, some of the more recent studies that used identical molecular methods and computational techniques (*e.g.*, Carrier and Reitzel, 2019b; Carrier *et al.*, 2020) would suggest that the diversity of the bacterial communities associated with these deep-sea invertebrate larvae is lower than larvae from coastal invertebrates. Thus, the relationship of bacterial community diversity and habitat is suspected to

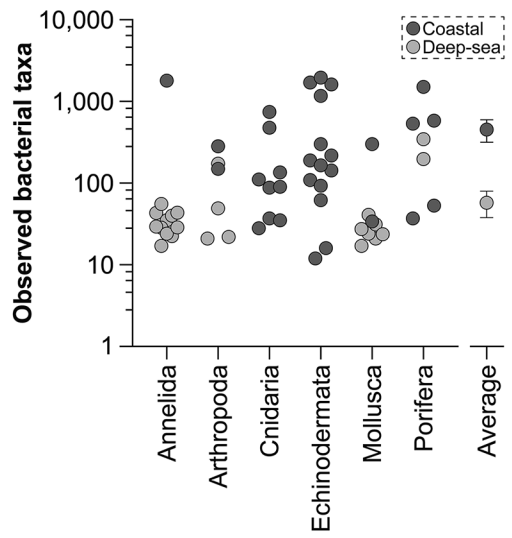

Figure 6. Bacterial diversity for marine invertebrate larvae. Estimated number of bacterial taxa (based on observed operational taxonomic units or amplicon sequence variants) for the bacterial communities associated with the developmental stages of coastal and deep-sea marine invertebrates, as partitioned by taxonomic group. Diversity estimates are presented as raw values (left) and average (± standard error; right). Values for all coastal species were taken directly from the literature as well as the deep-sea crab *Kiwa puravida*, shrimp *Rimicaris exoculata*, and sponges *Craniella zetlandica* and *C. infrequens*.

differ between taxonomic groups, and it should be investigated further.

Animal-associated bacterial communities are hypothesized to help buffer the host from environmental variation (Kohl and Carey, 2016; Carrier and Reitzel, 2017). Coastal invertebrate larvae can experience a wide array of abiotic and biotic stressors that require a physiological response (Thorson, 1950; Young and Chia, 1987; Byrne, 2011). Part of the system-wide response to food availability and temperature includes taxonomic and composition shifts in the diverse bacterial community associated with marine invertebrate larvae (Webster *et al.*, 2011; Kohl and Carey, 2016; Carrier and Reitzel, 2017, 2020). The magnitude of environmental heterogeneity in the deep sea is much less than that of shallow coastal ecosystems (Tyler, 1988). One potential explanation for why deep-sea invertebrate larvae harbor low-diversity bacterial communities could be that the decreased occurrence of environmental variation in the deep sea has relaxed the selective pressures for harboring taxonomically, and likely functionally, diverse microbial communities.

Taken together, data presented here suggest that deep-sea invertebrate larvae from multiple taxonomic groups associate with low-diversity bacterial communities and that these have little specificity. Moreover, the diversity of these communities is considerably lower than coastal invertebrate larvae, but this appears to depend on the taxonomic group. The extent to which larvae of some deep-sea invertebrates are functionally integrated with a symbiotic bacterial community is an open question (Hammer *et al.*, 2019). One approach to assess the potential of this is through quantitatively assessing the bacterial taxa and the identification of potential resident microbiota and characterizing the cross-talk with the larval host (Marsh *et al.*, 2001; Pradillon *et al.*, 2001; Zilber-Rosenberg and Rosenberg, 2008; Bordenstein and Theis, 2015).

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Data Accessibility

The *16S rRNA* and *28S rRNA* gene sequences are accessible on the Dryad Digital Repository at https://doi.org/10.5061/dryad.sqv9s4n18 (Carrier *et al.*, 2021a). Sampling locations and identifications for larvae used in this study are accessible at the Biological and Chemical Oceanography Data Management Office (BCO-DMO) repository (Beaulieu *et al.*, 2021).

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Appendix

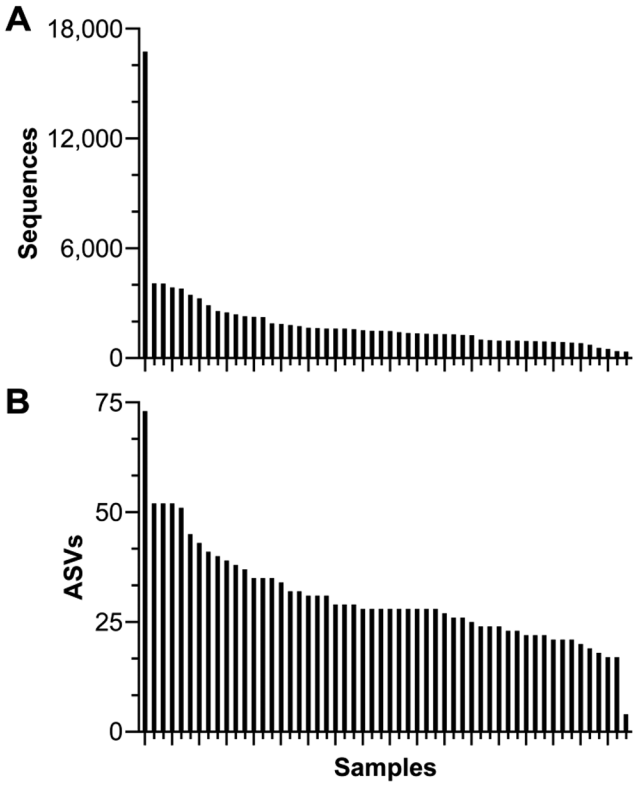

Figure A1. Sequence and amplicon sequence variant (ASV) distribution. The distribution of high-quality sequences (*i.e.*, those that were not filtered during pre-processing) and ASVs across the profiles for the bacterial communities associated with bivalve, crustacean, gastropod, and polychaete larvae from the East Pacific Rise, Mariana Back-Arc, and Pescadero Basin.

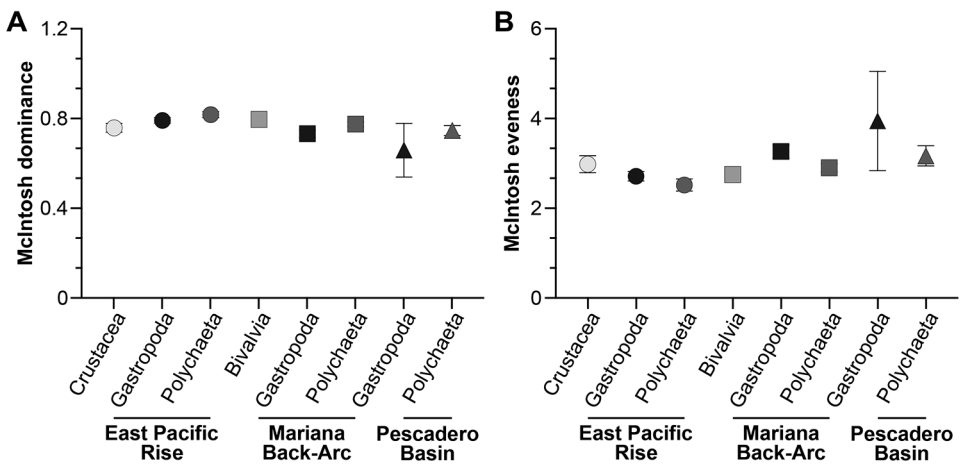

Figure A2. Alpha diversity for deep-sea larvae. McIntosh dominance (A; mean ± standard error) and McIntosh dominance (B; mean ± standard error) of the bacterial communities associated with bivalve, crustacean, gastropod, and polychaete larvae from the East Pacific Rise, Mariana Back-Arc, and Pescadero Basin.