Bacterial community dynamics during embryonic and larval development of three confamilial echinoids

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ABSTRACT

Development of some animals is influenced by and, in some cases, dependent on the associated microbiota. When the associated bacterial community is established by embryonic benthic marine invertebrates and their subsequent dynamics are still points of contention. Here, we assayed the bacterial communities of three confamilial echinoids from oocyte to juvenile. These data suggest that bacteria communities are established by the oocytes and change gradually during embryonic and larval development. Moreover, despite the differences between pre-metamorphic stages, ~33.6% of OTUs identified in the oocyte were present in the juveniles. During embryonic development, host-associated communities diverged from the environmental microbiota and converged following the onset of larval feeding. Taken together, the data presented here support that bacterial communities are established prior to fertilization and community composition shifts gradually thereafter, all while remaining distinct from the environment. Future work will need to determine the relative influence of the host and bacteria-bacteria interactions in shaping the associated microbiome to determine the potential functional importance on the development of larval sea urchins and benthic marine invertebrates more broadly.

INTRODUCTION

The journey from fertilized egg to adulthood has fascinated developmental biologists for centuries. A growing consensus in the field of animal-microbe symbioses suggest that these developmental programs are, at least in part, affected by the presence and activity of associated microbiota (McFall-Ngai & Ruby 2000, McFall-Ngai 2002, Fraune & Bosch 2010, Nyholm & McFall-Ngai 2014). The degree of dependency on these communities is highly variable: the removal of bacterial associates inhibits the differentiation of gut tissues in zebrafish (Bates et al. 2006) while other taxa are seemingly unaffected (Manahan et al. 1993). Nevertheless, several patterns have emerged from comparative studies of animal-bacteria interactions. First, animals tend to associate with stage-specific bacterial communities (Apprill et al. 2012, Carrier & Reitzel 2018). Second, changes in morphology and life history stage correlate with shifts in the composition and structure of this community (Fieth et al. 2016). Lastly, animal-associated bacterial communities may exhibit a 'winnowing,' or divergence from the environmental microbiota, during ontogenetic development (Nyholm & Mcfall-Ngai 2004).

Early development for many marine invertebrates includes several morphologically diverse life-history stages that are intricately tied to the environment. Regardless of taxonomy, the reproduction in most species involves an external fertilization, embryogenesis, and larval development before settling and metamorphosing into a benthic juvenile. When microbial communities are established during early development for marine invertebrates is currently a point of contention. Larvae of deep-sea vestimentiferans, for example, are symbiont free until settlement while several species of coral and other taxa are known to vertically inherent symbionts and diversify this

community by horizontal (environmental) acquisitions (Apprill et al. 2012, Sharp et al. 2012). Once established, the location of the bacterial symbionts can also vary. The embryos and larvae of eight echinoderm species, for example, have symbiotic bacteria (Cameron & Holland 1983, Bosch 1992, Cerra et al. 1997, Galac et al. 2016, Carrier & Reitzel 2018, Carrier et al. In Review) that are located broadly in the out-pockets of the extracellular matrix surrounding the larval body as well as a more restricted localization near or in gut epidermal cells (Bosch 1992, Cerra et al. 1997, Carrier et al. In Review).

The composition and structure of the bacterial communities associated with echinoderm larvae is highly variable and has been shown to be influenced by food availability (Carrier & Reitzel 2018), phenotype (Carrier & Reitzel 2018), cloning (Galac et al. 2016), and, potentially, disease (Carrier et al. 2018). Three confamilial echinoderms (*Strongylocentrotus purpuratus*, *Mesocentrotus franciscanus*, and *S. droebachiensis*) associate with bacterial communities that are specific to host species and between subsequent larval stages (Carrier & Reitzel 2018). Stage-specific bacterial communities during larval development may result from associations established in earlier embryonic stages and continue after metamorphosis or change dynamically based on each stage. Thus, determining when these bacterial communities are established early in development, to what extent these microbial populations are maintained, and the relative degree of similarity between confamilial species is important to identify the relative influence of each factor on animal-microbe symbioses.

To test when the associated bacterial community of early stage echinoderms is established, whether this community is maintained during embryonic and larval development, and for shared and species-specific patterning, we assayed the bacterial

communities of three confamilial echinoids from oocyte to benthic juvenile. Doing so, we test the hypothesis that a bacterial population is established before fertilization on mature oocytes and then shifts in the community occur gradually through metamorphosis, with these shifts in composition and structure correlating with changes in morphological complexity. Moreover, as the larval immune system is established and to remain differentiated from the environmental microbial community, we hypothesize each species exhibits a 'winnowing' response over development.

MATERIALS AND METHODS

Specimen collection and larval rearing

Adult urchins were collected from populations throughout the Salish Sea in April 2016, as described in Carrier & Reitzel (2018). Specifically, individual *Strongylocentrotus purpuratus* were hand-collected at low tide at Slip Point, Clallam Bay, WA (48°15'39" N, 124°15'03" W) and transferred directly to the Friday Harbor Laboratories (FHL; University of Washington; Friday Harbor, WA, USA). Similarly, *S. droebachiensis* were hand-collected at low tide at Cattle Point, San Juan Island, WA (48°27'00" N, 122°57'43" W), and were transferred to FHL within the hour. *M. franciscanus*, on the other hand, were collected by SCUBA off Bell Island, WA (48°35'49" N, 122°58'55" W) and transferred to FHL within two hours. All urchins were suspended in separate sub-tidal cages off the dock at FHL and drift fed *Nereocystis* spp. (sugar kelp) *ad libitum* until spawning two weeks later.

Urchins were spawned by an intracoelomic injection of 0.50 M KCl. For each species, gametes from up to three males and three females were separately pooled.

102 Fertilization of eggs and larval rearing followed Strathmann (Strathmann 1987), except, to
103 include the environmental microbiota, embryos and larvae were reared using 5.0-µm
104 filtered seawater (FSW). Briefly, embryos were incubated in one-liter of FSW at ambient
105 conditions. Two hours post-fertilization, embryos were transferred to triplicate one-liter
106 jars at a density of two individuals•mL^{-1}, and subsequently diluted as larvae reached
107 advanced developmental stages. Larval cultures were given 90 to 95% water changes every
108 other day, and food was replenished at this time to 10,000 cells•mL^{-1}.
109 Monocultures of *Rhodomonas lens* were grown at room temperature with f/2 media
110 and a combination of ambient and artificial lighting (Guillard 1975).
111
112 **Sampling across larval development**
113 Each urchin species was cultured from egg to juvenile. During this developmental
114 course, we sampled ten stages: (i) unfertilized eggs, (ii) cleavage, (iii) pre-hatched blastula,
115 (iv) hatched blastula, (v) gastrula, (vi) pre-feeding prism, (vii) 4-, (viii) 6-, and (ix) 8-armed
116 larvae, and (x) post-metamorphic juvenile. Developmental stages were identified using a
117 compound microscope (Nikon Eclipse E600; camera: QImaging MicroPublisher 5.0 RTV).
118 At each of these developmental landmarks, 100 individuals per triplicate jar and per species
119 were sampled at random. Immediately after sampling, larval samples were concentrated
120 into a pellet using a microcentrifuge, and all seawater was removed. Pelleted larvae were
121 then preserved in RNAlater and stored at -20 °C before DNA extractions.
122 Complementary to sampling of the developmental stages, the environmental
123 microbiota from the seawater was also sampled. When cultures were sampled, triplicates
124 of ~1-L of seawater was filtering onto a 0.22-µm Millipore filter to retain the environmental

125 microbiota. Full filter disks were then preserved in RNAlater and stored at -20 °C before
126 DNA extractions.
127
128 *(a) Assaying microbial communities*
129 Total DNA was extracted from larval samples using the GeneJet Genomic DNA
130 Purification Kit (Thermo Scientific). For filtered seawater samples, we extracted eDNA
131 using the FastDNA Spin Kit for Soil (MP Biomedical). DNA was then quantified using the
132 NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific) and diluted to 5 ng•μL^{-1}
133 using RNase/DNase-free water.
134 Bacterial sequences were amplified using universal primers for the V3/V4 regions
135 of the 16S rRNA gene (Forward: 5′ CTACGGGNGGCWGCAG, Reverse: 5′
136 GACTACHVGGGTATCTAATCC) (Klindworth et al. 2013). Products were purified
137 using the Axygen AxyPrep Mag PCR Clean-up Kit (Axygen Scientific), indexed via PCR
138 using the Nextera XT Index Kit V2 (Illumina Inc.), and then purified again. At each of
139 these three clean up states, fluorometric quantitation was performed using a Qubit (Life
140 Technologies) and libraries were validated using a Bioanalyzer High Sensitivity DNA chip
141 (Agilent Technologies). Illumina MiSeq sequencing (v3, 2x300 bp paired-end reads) was
142 performed at the University of North Carolina at Charlotte.
143 Forward and reverse sequences were paired and trimmed using PEAR(Zhang et al.
144 2014) and Trimmomatic (Bolger et al. 2014), respectively, converted from fastq to fasta
145 using custom script, and, prior to analysis of bacterial 16S rRNA sequences, chimeric
146 sequences were detected using USEARCH (Edgar et al. 2011) and removed using
147 filter_fasta.py. Using QIIME 1.9.1 (Caporaso et al. 2010), bacterial 16S rRNA sequences

148 were analyzed and grouped into operational taxonomic units (OTUs) based on a minimum
149 97% similarity. The biom table generated by the pick_open_reference_otus.py script was
150 filtered of OTUs with less than ten reads as well as sequences matching chloroplast for
151 cryptophytes (*i.e.*, *R. lens*; (Carrier & Reitzel 2018).

152 Using the filtered biom table and "biom summarize-table" function to count total
153 sequences per sample, the rarefaction depth of 25,396 was determined and applied to all
154 subsequent analyses (Supplemental Figure 1). Beta diversity was calculated using the
155 weighted UniFrac (Lozupone & Knight 2005), and principal coordinate analyses (PCoA)
156 were visualized in EMPeror (Vazquez-Baeza et al. 2013) and stylized in Adobe Illustrator
157 CS6. Community composition was generated using summarize_taxa_through_plots.py
158 script and visualized using Prism 7 (GraphPad Software). Community similarity across
159 phenotypes, dietary states, developmental stages, and their decoupling were compared
160 statistically using an ANOSIM as part of the compare_categories.py script.

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

163

164 **RESULTS**

165 **Community-level shifts across development**

166 The associated bacterial communities of *S. purpuratus*, *M. franciscanus*, and *S.*
167 *droebachiensis* shifted considerably in OTU membership (ANOSIM, p=0.0013; Figure
168 1A; Supplemental Figures 2-3) and the relative abundance of those taxa (ANOSIM,
169 p=0.0011; Figure 1B; Supplemental Figures 2-3) during embryonic and larval
170 development, with community dynamics being specific to urchin species (ANOSIM,

171 unweighted: p=0.0011; weighted: p<0.001; Figure 1). Moreover, community evenness
172 (Shannon and Robbins), diversity (Simpson), richness (Chao1), phylogenetic distance
173 (Faith's), and Fishers alpha varied significantly across developmental stages (p<0.0001 for
174 all tests) and between urchin species (p<0.0001 for all tests) (Supplemental Figure 4;
175 Supplemental Table 2).

176 Weighted UniFrac comparisons of the associated bacterial communities for each
177 urchin species suggests that community similarity largely grouped by developmental stage
178 (Figure 2). Inferred bacterial communities may be grouped into clusters of sequential
179 developmental stages with some specific separation for: (i) earliest embryological stages,
180 (ii) late embryogenesis and early larval development, and (iii) later larval stages. The
181 benthic juveniles had more variable positioning: the bacterial community of *M.*
182 *franciscanus* was most similar to oocytes while *S. purpuratus* was intermediate to groups
183 early and late larval development (Figure 2).

184 The embryos and larvae of these confamilial echinoids associated with nine classes
185 of bacteria within the phyla Bacteroidetes, Proteobacteria, and Verrucomicrobiae. Of these,
186 the relative abundance of the γ-proteobacteria (Phylum: Proteobacteria), α-proteobacteria
187 (Phylum: Proteobacteria), and Flavobacteriia (Phylum: Bacteroidetes) were, on average,
188 more than ten-percent of the community (Figure 2). First, for the γ-proteobacteria, the
189 relative abundance ranged from 26.6% to 61.0% for *S. purpuratus*, 12.2% to 62.9% for *M.*
190 *franciscanus*, and 39.6% to 88.7% for *S. droebachiensis* (Figure 2). Second, for the α-
191 proteobacteria, the relative abundance ranged from 7.7% to 42.4% for *S. purpuratus*,
192 11.0% to 31.1% for *M. franciscanus*, and 5.1% to 22.7% for *S. droebachiensis* (Figure 2).
193 Lastly, for the Flavobacteriia, the relative abundance ranged from 4.9% to 30.5% for *S.*

194 *purpuratus*, 5.1% to 36.1% for *M. franciscanus*, and 1.5% to 34.2% for *S. droebachiensis*
195 (Figure 2).
196
197 **Dynamics of the associated bacterial species**
198 Total OTUs in association with these confamilial echinoids differed significantly
199 during development ($p<0.0001$) for each species (*S. purpuratus*: $p<0.0001$; *M.*
200 *franciscanus*: $p<0.0001$; *S. droebachiensis*: $p<0.0001$; Figure 3A; Supplemental Table 1).
201 Unfertilized eggs, on average, associated with a similar number of OTUs (average: ~1,734
202 OTUs; $p=0.4410$), which increased during embryonic development (~2,043-2,086; Figure
203 3A). Following the onset of feeding and larval development, the number of OTUs
204 subsequently decreased until metamorphosis (~1,321 for 8-armed larvae; Figure 3A). The
205 maximum number of associated OTUs during development varied between species (*S.*
206 *purpuratus*: 2,387; *M. franciscanus*: 2,051; *S. droebachiensis*: 2,347) as well as at which
207 stages this occurred (*S. purpuratus*: hatched blastula; *M. franciscanus*: pre-hatched
208 blastula; *S. droebachiensis*: pre-hatched blastula) (Figure 3A).
209 A significant proportion of the OTUs associated with echinoid eggs did not remain
210 in association throughout embryonic and larval development for each urchin species (*S.*
211 *purpuratus*: $p<0.0001$; *M. franciscanus*: $p<0.0001$; *S. droebachiensis*: $p<0.0001$; Figure
212 3B; Supplemental Table 1). Following fertilization and early embryogenesis, ~57% of
213 OTUs remained in association while ~43% were lost (Figure 3B). In the subsequent
214 developmental stages, egg-associated OTUs were lost gradually, resulting in juvenile
215 urchins with ~33.6% of the originally acquired OTUs (Figure 3B). Moreover, when

216 transitioning between any two subsequent developmental stages, ~22.5% of OTUs are
217 retained while ~77.5% of OTUs were specific to either stage (Figure 3C).

218 Community dissimilarity (Bray-Curtis) between the larval host and environmental
219 microbiota showed significant differences during embryonic and larval developmental for
220 each species (*S. purpuratus*: $p<0.0001$; *M. franciscanus*: $p<0.0001$; *S. droebachiensis*:
221 $p<0.0001$; Figure 3D; Supplemental Figure 5; Supplemental Table 1). During development
222 from egg to hatched blastula, the associated bacterial community became more dissimilar
223 from the environmental microbiota (Figure 3D), with the magnitude of dissimilarity
224 varying between species (Figure 3D; egg through hatched blastula, each $p<0.0001$).
225 Furthermore, following maturation of the mouth and the onset of feeding, late embryos and
226 early stage larvae became more similar to the environmental bacterial community (Figure
227 3D), with the relative dissimilarity converging in the early larval stages, which was the last
228 sampled time point (Figure 3D; 4-armed larvae: $p=0.6845$). During divergence and
229 convergence events between the larval host and the environmental microbiota, the
230 environmental bacterial community was largely constant and did not co-vary with shifts in
231 the host-associated bacteria (Supplemental Figure 5).

232

233 ***Psychromonas* and echinoid eggs**

234 Bacteria in the genus *Psychromonas* showed the highest abundance for any
235 bacterial genus for all three urchin species in at any one of the sampled stages. For each
236 urchin species, the abundance of *Psychromonas* was highest in the unfertilized eggs and
237 declined significantly following fertilization ($p<0.0001$), until it was undetectable in 4-
238 armed larvae and the subsequent developmental stages (Figure 4). The magnitude of

239 relative abundance varied between urchin species ($p<0.0001$) and was directly proportion
240 to egg size (r=0.951). The OTUs clustered within *Psychromonas* totaled 93 for *S.*
241 *purpuratus*, 61 for *M. franciscanus*, and 85 for *S. droebachiensis* (Figure 4), and of those,
242 nine of the ten most abundant *Psychromonas* OTUs for each urchin species were shared
243 between urchin species.
244
245 **DISCUSSION**
246 Like many marine invertebrates, echinoid life cycles involve a distinct embryonic,
247 larval, and juvenile stage. Our assay of the bacterial communities of three confamilial
248 echinoids suggests that the bacterial consortium is highly dynamic across this
249 developmental landscape, with taxonomic membership and the relative abundance of those
250 taxa shifting gradually from oocyte to juvenile. Furthermore, over the course of
251 development, the bacterial community of urchin embryos diverges from the environmental
252 microbiota until larvae begin feeding. Lastly, eggs of these echinoids are dominated by a
253 diverse OTUs of *Psychromonas*.
254 The bacterial communities associated with echinoplutei are highly dynamic when
255 the larval holobiont faces variation in the abiotic and biotic environment (Carrier & Reitzel
256 2018, Carrier et al. In Review). For example, following diet-restriction the bacterial
257 communities of *S. purpuratus*, *M. franciscanus*, and *S. droebachiensis* each shift
258 considerably from their well-fed counterparts, to potentially cope with reduced exogenous
259 resources (Carrier & Reitzel 2018). When diet-restriction is prolonged and the larval host
260 expresses phenotypic plasticity, the composition and structure of the bacterial partners are

261 specific to phenotype, a response correlated with the magnitude in which phenotypic
262 plasticity is expressed (Carrier & Reitzel 2018).

263 Phenotypic plasticity for echinoid larvae, more broadly, is the interaction between
264 phenotype, nutritional state, and development. Our assays across the developmental course
265 for these three confamilial echinoids extends our understanding of the network and
266 magnitude to which larval-oriented programs correlate with bacterial community
267 dynamics. Particularly after unfertilized eggs establish a bacterial community, the
268 memberships of these associates shifts gradually shifts during embryogenesis and during
269 transitions towards a feeding larva. In these gradual community transitions, it was
270 surprising that more coarse shifts were not observed between major developmental
271 milestones, such as when blastula hatch and shed the fertilization envelope as well as when
272 larvae metamorphose into benthic juveniles. The latter may be because echinoid larvae
273 undergoing metamorphosis reabsorb some of the larval digestive tract (Chia & Burke
274 1978). Thus, the juveniles may have retained, instead of shed (Fieth et al. 2016), a portion
275 of their microbial community present in late larval stages.

276 The influence of bacteria and bacterial communities on the development of
277 particular aquatic and terrestrial invertebrates is well-studied (McFall-Ngai & Ruby 2000,
278 Fraune & Bosch 2010, McFall-Ngai 2014, Nyholm & McFall-Ngai 2014). Established
279 invertebrate systems where bacteria play a significant functional role include *Vibrio fisheri*-
280 mediated maturation of the light organ in the bobtail squid *Euprymna scolopes* (McFall-
281 Ngai & Ruby 2000, McFall-Ngai 2014), immune regulation relating to the epithelial
282 bacteria associated with the freshwater cnidarian *Hydra* spp. (Fraune & Bosch 2010, Bosch
283 2013, Franzenburg et al. 2013), and the nutritional endosymbionts (*e.g.*, *Buchnera*) of

284 aphids (Douglas 1998). Provided the vast genomic and molecular foundation established
285 over the past several decades for the echinoid clade used in this study (Kudtarkar &
286 Cameron 2017), these confamilial urchins have the potential to be established as a
287 comparative system for beneficial animal-microbe interactions during development and in
288 bi-phasic life-histories. One route to testing this, and how marine invertebrates establish
289 symbiont communities, is through the egg-associated *Psychromonas*.

290 Eggs of echinoids have long been used as a metric in larval biology and ecology
291 (Vance 1973, Jaeckle 1995, Moran & McAlister 2009, Marshall et al. 2012) to make
292 inferences about life history mode and transitions (Vance 1973, Collins & Moran 2018),
293 fertilization ecology (Pennington 1985, Levitan 1995), and maternal inheritance (Byrne et
294 al. 1999, Villinski et al. 2002). The high abundance of egg-associated *Psychromonas* and
295 the exponential loss and replacement over development may imply that this network of
296 bacterial associates primarily contribute to lipid metabolism (Riley et al. 2008).
297 Interestingly, egg size of echinoids is strongly correlated with total lipid content (Jaeckle
298 1995, Moran & McAlister 2009) and data here support that egg size is also directly
299 correlated with the abundance of *Psychromonas*. *Psychromonas*, therefore, may aid in lipid
300 metabolism during embryonic development or serve as a commensal symbiont and rely on
301 the lipids leaching off the maturing embryos, as egg-associated bacteria have been
302 suggested previously (Ikuta et al. 2016).

303 Over the course of embryonic development, we also observed that the urchin-
304 associated bacterial community diverged from the environmental microbiota. At the
305 surface, this appears to be a 'winnowing'-like response, whereby urchin embryos
306 seemingly take a step-wise elimination of potential non-symbionts to select for prokaryotic

307 partners (Nyholm & Mcfall-Ngai 2004). Winnowing was first established in the *E.*
308 *scolopes*-*V. fisheri* mutualism (Nyholm & Mcfall-Ngai 2004), and has since been well-
309 characterized by McFall-Ngai and colleagues (McFall-Ngai 2014). To test whether urchins
310 larvae exhibit a winnowing response during embryonic and larval development, a similar
311 multifaceted molecular and -omics approach would be required to characterize this
312 potential developmental feature (Williams & Carrier 2018).

313 Taken together, data presented here support that the bacterial community associated
314 with the developmental stages of three species of sea urchin are established by the oocytes
315 and shift in parallel. The functional importance and evolutionary significance of this
316 bacterial community and the mechanisms for these corresponding shifts remains unknown.
317 Future experiments involving germ-free culturing as well as manipulations of the larval
318 host, members of the microbial community, and culturing environment will be needed to
319 determine roles of associated microbiota. Results from these experiments would serve as a
320 key piece towards establishing echinoids as a comparative system for understanding the
321 interdependence of animal development and the associated bacterial communities.

322

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331
332

333 **FIGURE LEGEND**

334

335 **Figure 1**. Similarity between the associated bacterial community across development for
336 three species of echinoids. Community similarity for *Strongylocentrotus purpuratus*
337 (triangle), *Mesocentrotus franciscanus* (square), and *S. droebachiensis* (circle) for ten
338 developmental stages when considering the presence/absence of taxa (A) and their relative
339 abundance (B).

340

341 **Figure 2**. Relatedness of bacterial communities during development. Weighted
342 dendrograms and taxonomic plots (Class) for the bacterial communities of
343 *Strongylocentrotus purpuratus* (left), *Mesocentrotus franciscanus* (center), and *S.*
344 *droebachiensis* (right) across ten developmental stages.

345

346 **Figure 3**. Measures of OTU shifts during development. OTU dynamics across embryonic
347 and larval development for *Strongylocentrotus purpuratus* (purple), *Mesocentrotus*
348 *franciscanus* (red), and *S. droebachiensis* (green) when considering (A) total observed
349 OTUs, (B) percent of OTUs shared with unfertilized eggs, (C) proportion of OTUs unique
350 to and shared between developmental stages, and (D) weighted similarity with the
351 environmental microbiota.

352

353 **Figure 4**. Decay in abundance of *Psychromonas* during development. Relative abundance
354 of the bacterial genus *Psychromonas* across embryonic and larval development for

355 *Strongylocentrotus purpuratus* (purple), *Mesocentrotus franciscanus* (red), and *S.*
356 *droebachiensis* (green) and the number of operational taxonomic units.
357
358 **Supplemental Figure 1**. Alpha rarefaction curves for three species of echinoids and of the
359 seawater. Alpha rarefaction curves for *Strongylocentrotus purpuratus* (purple),
360 *Mesocentrotus franciscanus* (red), and *S. droebachiensis* (green) and seawater (blue) based
361 on rarefaction depth of 25,396.
362
363 **Supplemental Figure 2**. Similarity between the associated bacterial community across
364 development for three species of echinoids. Community similarity for *Strongylocentrotus*
365 *purpuratus* (top), *Mesocentrotus franciscanus* (middle), and *S. droebachiensis* (bottom) for
366 ten developmental stages when considering the presence/absence of taxa (*i*) and their
367 relative abundance (*ii*).
368
369 **Supplemental Figure 3**. Bacterial community dendrograms across development for three
370 echinoid species. Unweighted (*i*) and weighted (*ii*) bacterial community dendrograms for
371 *Strongylocentrotus purpuratus* (top), *Mesocentrotus franciscanus* (middle), and *S.*
372 *droebachiensis* (bottom).
373
374 **Supplemental Figure 4**. Various alpha diversity indices for three echinoid species across
375 development. Shannon (A), Simpson (B), Robbins (C), Chao1 (D), Fisher (E), and Faiths
376 (F) indices for *Strongylocentrotus purpuratus* (purple), *Mesocentrotus franciscanus* (red),
377 and *S. droebachiensis* (green) at ten developmental stages.

378

379 **Supplemental Figure 5**. Microbial dendrogram of developmental stages and seawater.
380 Dendrogram of the bacterial communities based on weighted UniFrac for the
381 developmental stages of *Strongylocentrotus purpuratus*, *Mesocentrotus franciscanus*, and
382 *S. droebachiensis* as well as the respective environmental bacterial communities.

383

384

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Figure 1

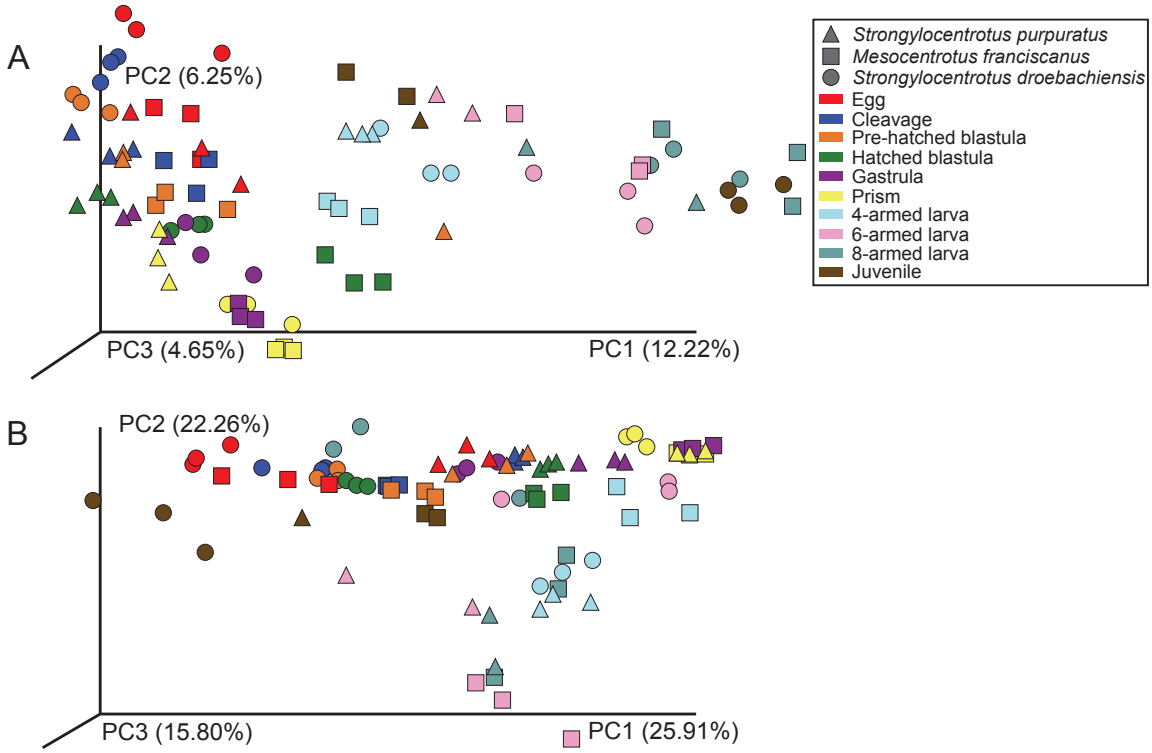

A
PC2 (6.25%)
PC3 (4.65%)
PC1 (12.22%)
B
PC2 (22.26%)
PC3 (15.80%)
PC1 (25.91%)
Strongylocentrotus purpuratus
Mesocentrotus franciscanus
Strongylocentrotus droebachiensis
Egg
Cleavage
Pre-hatched blastula
Hatched blastula
Gastrula
Prism
4-armed larva
6-armed larva
8-armed larva
Juvenile

Figure 2

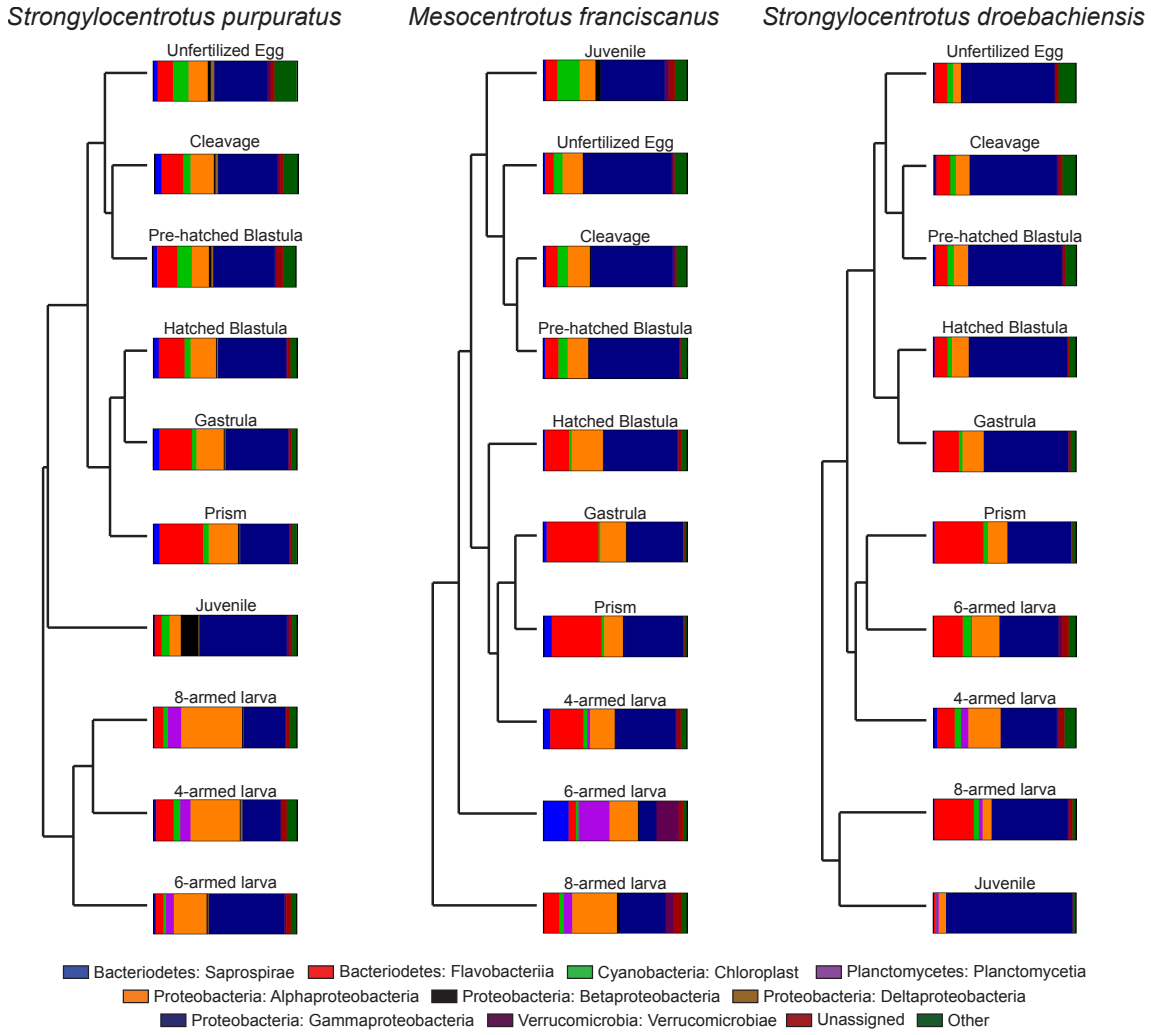
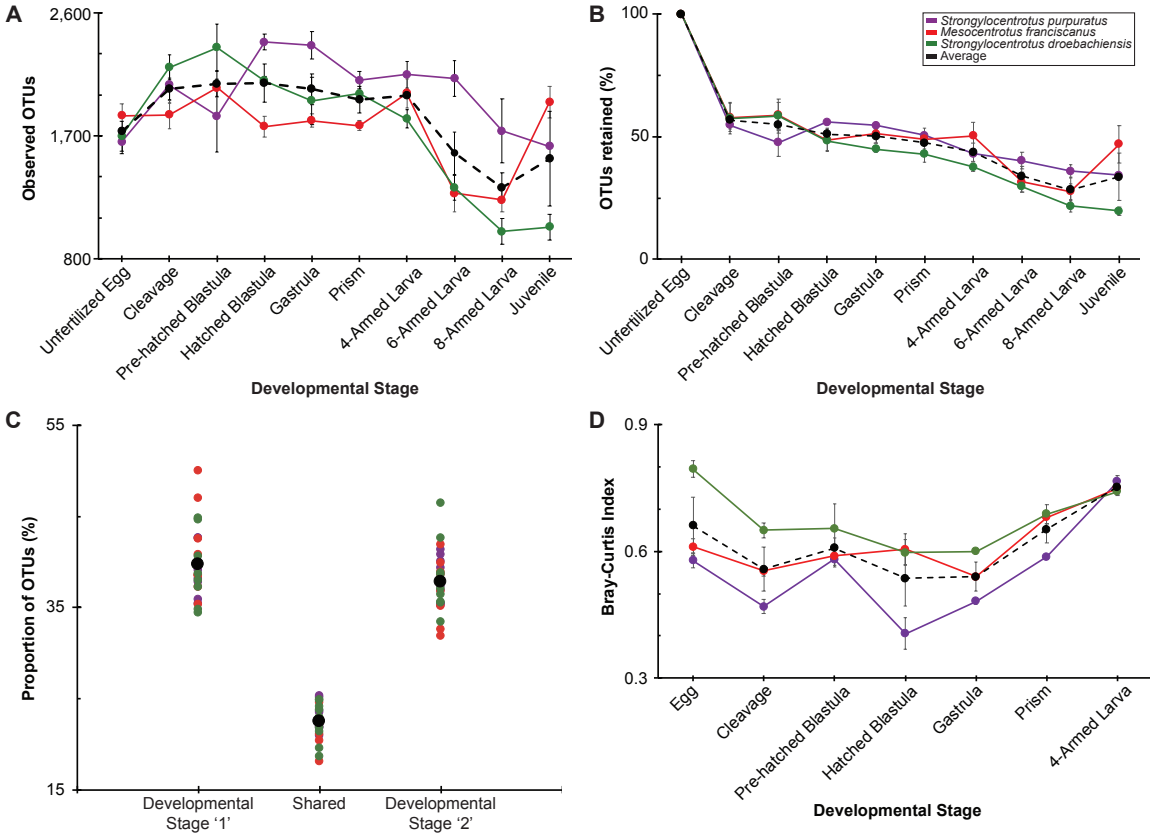
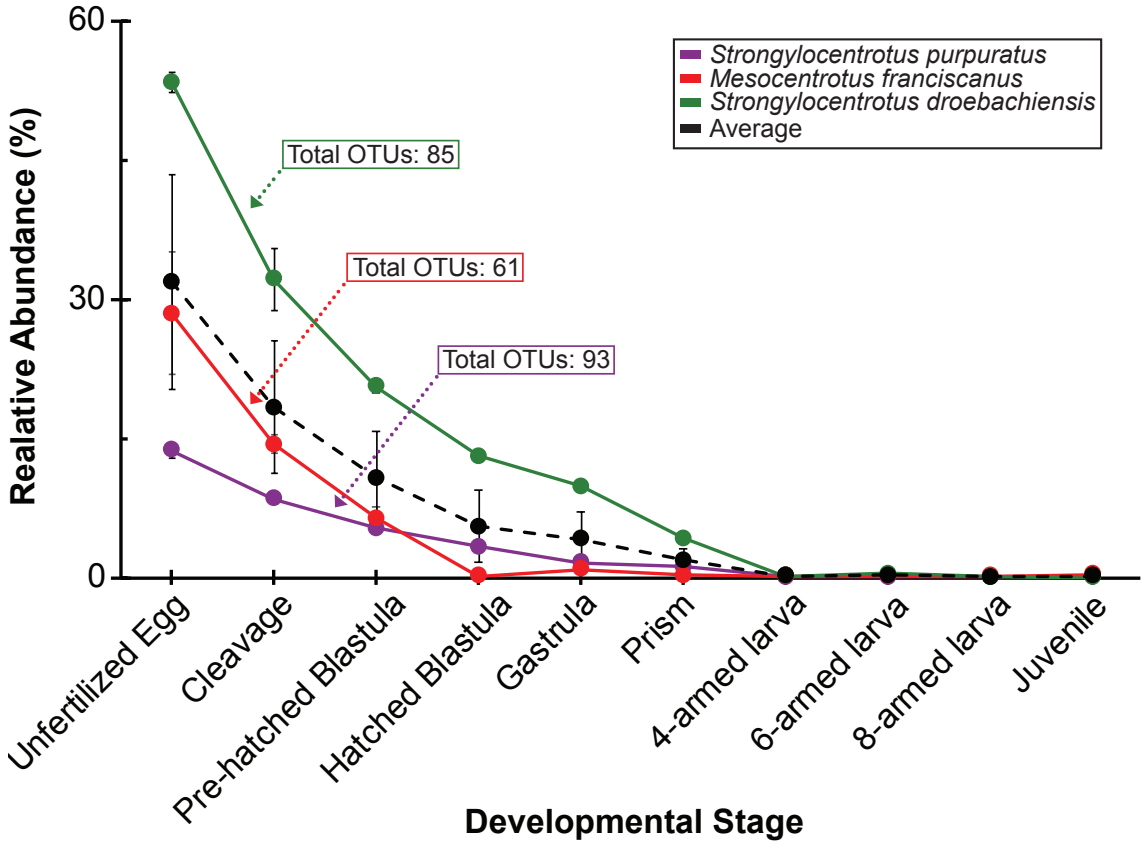

Strongylocentrotus purpuratus
Unfertilized Egg
Cleavage
Pre-hatched Blastula
Hatched Blastula
Gastrula
Prism
Juvenile
8-armed larva
4-armed larva
6-armed larva
Mesocentrotus franciscanus
Juvenile
Unfertilized Egg
Cleavage
Pre-hatched Blastula
Hatched Blastula
Gastrula
Prism
4-armed larva
6-armed larva
8-armed larva
Strongylocentrotus droebachiensis
Unfertilized Egg
Cleavage
Pre-hatched Blastula
Hatched Blastula
Gastrula
Prism
6-armed larva
4-armed larva
8-armed larva
Juvenile
Bacteriodetes: Saprospirae
Bacteriodetes: Flavobacteriia
Cyanobacteria: Chloroplast
Planctomycetes: Planctomycetia
Proteobacteria: Alphaproteobacteria
Proteobacteria: Betaproteobacteria
Proteobacteria: Deltaproteobacteria
Proteobacteria: Gammaproteobacteria
Verrucomicrobia: Verrucomicrobiae
Unassigned
Other

Figure 3


A
2,600
1,700
800
Observed OTUs
Unfertilized Egg
Cleavage
Pre-hatched Blastula
Hatched Blastula
Gastrula
Prism
4-Armed Larva
6-Armed Larva
8-Armed Larva
Juvenile
Developmental Stage
B
100
50
0
OTUs retained (%)
Strongylocentrotus purpuratus
Mesocentrotus franciscanus
Strongylocentrotus droebachiensis
Average
Developmental Stage
C
55
35
15
Proportion of OTUs (%)
Developmental Stage '1'
Shared
Developmental Stage '2'
D
0.9
0.6
0.3
Bray-Curtis Index
Egg
Cleavage
Pre-hatched Blastula
Hatched Blastula
Gastrula
Prism
4-Armed Larva
Developmental Stage

16 **Figure 4**
17



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