

1 Shift in bacterial taxa precedes morphological plasticity in a larval echinoid

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15

16 **Abstract**

17 Morphological plasticity is an adaptive response to heterogenous environments when
18 a fitness advantage is conferred. Larval sea urchins, for example, increase individual fitness
19 in dilute feeding environments by elongating their feeding structure. Morphological
20 plasticity for larval sea urchins is also coupled with significant shifts in the associated
21 bacterial community, but whether this response occurs before, during, or following the
22 expression of plasticity is unclear. Using the sea urchin *Lytechinus variegatus*, we define
23 the temporal pattern of the associated bacterial community throughout the expression of
24 morphological plasticity. From prefeeding through plasticity, we observed that *L.*
25 *variegatus* larvae exhibit a four-stage successional pattern and the relatedness of the larval-
26 associated bacterial community directly reflects morphological plasticity and does so prior
27 to expression of the environmental-specific morphology. Based on the structure of the
28 larval-associated bacterial communities, the expression of morphological plasticity
29 correlates short-arm larvae deviating from the microbial trajectory of pre-plastic siblings.
30 Taken together, these data suggest that a holobiont may exhibit shifts in the associated
31 bacterial community corresponding with the environmental variation in absence or
32 anticipation of morphological plasticity.

33

34

35 **1. Introduction**

36 As the environment ebbs and flows, animals from diverse phylogenetic lineages may
37 modify phenotypic traits to better match environmental conditions [1-3]. When phenotypic
38 plasticity—the potential to produce a range of phenotypes from a single genotype—is
39 induced and confers a fitness advantage, the response is considered adaptive and an
40 evolvable trait influenced by natural selection [1-3]. For morphological characters to
41 exhibit plasticity favored by selection, abiotic or biotic stimuli must be reliable and
42 detected at an appropriate time scale to elicit a developmental change in a structure that
43 results in a phenotype-environment match [1, 4, 5]. Plasticity in morphology and other life
44 history characters are, thus, viewed as a product of host genotype and the environment [1-
45 3, 6, 7]. Recent evidence, however, suggests that phenotypic state also correlates with the
46 composition of the symbiotic bacterial community [8, 9].

47 Host-associated microbial communities are fundamental to the metabolism, immunity,
48 and development of the host [10-12]. Symbiotic relationship between host and microbiota
49 have deep evolutionary origins, often exhibit co-divergence over evolutionary time, and
50 can be important for coping with environmental stressors [13, 14]. When faced with
51 heterogeneous environments that are known to induce phenotypic plasticity, an animal may
52 recruit, expel, and/or shuffle the membership and relative proportion of associated
53 microbiota to assemble a community with specific functional properties (*e.g.*, genes and
54 metabolic pathways) for the environmental conditions [12, 15-18]. When faced with
55 prolonged diet restriction, for example, the composition and diversity of invertebrate- and
56 vertebrate-associated bacterial communities shifts considerably to buffer against reduced
57 exogenous nutrients [9, 19].

58 Planktotrophic (feeding) larvae of benthic marine inhabit coastal seas, where the
59 abundance and distribution of exogenous nutrients are spatially and temporally
60 heterogeneous [20]. Several groups of planktotrophic larvae, including sea urchins
61 (phylum Echinodermata), respond to heterogeneous feeding environments by exhibiting
62 morphological plasticity [21]. To increase individual fitness in the face of diet-restriction,
63 larval sea urchins suppress development of the larval body and absorb stomach tissues to
64 allocate energetic resources towards the feeding structures that, in turn, increases the
65 capacity to collect particulates [22-27]. Moreover, morphological plasticity by echinoid
66 larvae is coupled with physiological plasticity [28, 29], whereby diet-restriction correlates
67 with reduced expression of genes associated with growth and metabolism and higher
68 expression of genes involved with neurogenesis, environmental sensing, immunity, and
69 longevity [29].

70 Larval sea urchins associate with bacterial communities that are diverse and dynamic
71 yet specific to host species [9, 30], variable between populations [31], and distinct from
72 the environmental microbiota [9, 30, 31]. Larval-associated bacterial communities shift
73 over the course of embryonic development [30], with disease [32], and in response to food
74 availability [9, 31, 33]. Specifically, the bacterial community associated with echinoid
75 larvae exhibits bi-directional shifts that correlate with phenotype [9]. This correlation
76 between morphological plasticity and the composition of the associated bacterial
77 community, thus far, is unique to echinoid larvae, but recent data suggests similar
78 responses in taxa from disparate clades [8]. What remains unclear is whether associating
79 with a phenotype-specific bacterial community occurs before, during, or following the
80 expression of morphological plasticity.

81 The expression of morphological plasticity by temperate Strongylocentrotids requires
82 a multi-week stimulus [9, 22, 23, 31], which is restrictive for defining the temporal
83 succession by the holobiont during morphological plasticity. Tropical and subtropical sea
84 urchins, on the other hand, develop at a relatively rapid pace and may express plasticity in
85 a few days [34-36]. This shortened developmental window permits a narrow time frame
86 for changes in morphology and associations with bacteria that can be studied at a finer
87 grain than species with prolonged larval durations. Using *Lytechinus variegatus*, a
88 subtropical/tropical sea urchin known to exhibit morphological plasticity in four days [35-
89 37], we define this temporal succession and test the hypothesis that shifts in the bacterial
90 taxa precede a morphological response. To test this, we performed a differential feeding
91 experiment on *L. variegatus* larvae and used amplicon sequencing to profile the larval-
92 associated bacterial communities over the course of morphological plasticity.

93

94 **2. Materials and methods**

95 (a) *Specimen collection and larval rearing*

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

99 Within two days of collections adult sea urchins were spawned by a one to two mL
100 intracoelomic injection of 0.50 M potassium chloride. Gametes from five males and five
101 females were pooled separately and the fertilization of eggs (diameter: $97.9 \mu\text{m} \pm 1.07 \mu\text{m}$)
102 and larval rearing followed Strathmann [38], with the exception that embryos and larvae
103 were reared using 5.0 μm filtered seawater (FSW) to include the environmental microbiota.

104 Briefly, eggs were fertilized (verified using stereomicroscope) using dilute sperm in 100
105 mL of FSW at ambient temperature and salinity (Figure S1). After fertilization, embryos
106 were transferred to jars with 3 L of FSW (n=4; Table S1) and diluted to a density of eight
107 individuals•mL⁻¹.

108 For larval feeding, monocultures of the cryptophyte *Rhodomonas lens* were grown in
109 f/2 media at room temperature with a combination of ambient and artificial lighting for 24
110 hours per day [39].

111

112 *(b) Experimental feeding and tissue collection*

113 At 24 hours post-fertilization, each jar of prism stage larvae was sub-divided into four
114 replicates (n=16), diluted to two individuals•mL⁻¹ in 3 L of FSW, and, at random, were
115 provided growth medium-free *R. lens* at either 10,000, 1,000, 100, or 0 cells•mL⁻¹ (n=4 per
116 diet; Table S1). For the rest of the experiment, larval cultures had daily water changes of
117 90-95% volume and *R. lens* was replenished to the experimental density.

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

123

124 *(c) Larval morphology*

125 In addition to sampling the larval-associated bacterial community, twenty larvae
126 (n=20) from a single replicate of each dietary treatment were sampled for morphometric

127 analysis. Larvae were imaged using a compound microscope (Leitz Labovert; camera:
128 Olympus DP71) and morphometrics (length of larval body, MBL; post-oral arms, POA;
129 and stomach area; Figure 1) were performed using ImageJ (v. 1.9.2; [40]). Statistical
130 differences in larval morphology and stomach volume were compared using a two-way
131 analysis of variance (ANOVA, cut off $p=0.05$) in JMP Pro (v. 13) to test whether larval
132 morphology differed across time and between diets. When statistical differences were
133 detected, we then performed a Tukey's post-hoc test for pairwise comparisons.

134

135 *(d) Assaying bacterial communities*

136 Total DNA was extracted from larval samples using the GeneJet Genomic DNA
137 Purification Kit (Thermo Scientific), quantified using the NanoDrop 2000 UV-Vis
138 Spectrophotometer (Thermo Scientific), and diluted to $5 \text{ ng} \cdot \mu\text{L}^{-1}$ using RNase/DNase-free
139 water.

140 Bacterial DNA was amplified using primers for the V3/V4 regions of the 16S rRNA
141 gene (Table S2; [41]). Products were purified using the Axygen AxyPrep Mag PCR Clean-
142 up Kit (Axygen Scientific), indexed via PCR using the Nextera XT Index Kit V2 (Illumina
143 Inc.), and then purified again. At each of these three clean up states, fluorometric
144 quantitation was performed using a Qubit (Life Technologies) and libraries were validated
145 using a Bioanalyzer High Sensitivity DNA chip (Agilent Technologies). Illumina MiSeq
146 sequencing (v3, 2x300 bp paired-end reads) was performed at the University of North
147 Carolina at Charlotte. PCR recipe and thermal profiles are available in Table S2.

148

149 *(e) Computational analysis*

150 Raw reads, along with quality information, were imported into QIIME 2 (v. 2019.1;
151 [42]), where forward and reverse reads were paired using VSEARCH [43], filtered by
152 quality score, and denoised using Deblur [44]. QIIME 2-generated ‘features’ were grouped
153 into operational taxonomic units (OTUs) based on a minimum 99% similarity and were
154 assigned taxonomy using SILVA (v. 132; [45]). Sequences matching to Archaea as well as
155 samples with less than 1,000 reads were discarded and the filtered biom table was rarified
156 to 1,287 sequences (*i.e.*, the read count for the sample with the least remaining reads; Figure
157 S2).

158 To test whether community membership and composition shift over time, in response
159 to food availability, and relative to morphology, we calculated unweighted and weighted
160 UniFrac [46] values and compared them using principal coordinate analyses (PCoA).
161 Results from these analyses were then recreated and stylized using QIIME 1 (v. 1.9.1; [47])
162 and Adobe Illustrator CC. To test for differences in membership and composition over the
163 course of the differential feeding experiment, we used a two-way PERMANOVA and,
164 subsequently, performed pairwise comparisons. To complement UniFrac values, we also
165 calculated several measures of alpha diversity (*i.e.*, total OTUs, phylogenetic distance,
166 McIntosh evenness, and McIntosh dominance) over time and across diets, and compared
167 these values with a two-way analysis of variance (ANOVA) and, subsequently, performed
168 by a Tukey’s post-hoc test for pairwise comparisons between diets and times. Lastly, we
169 summarized the bacterial groups associated with *L. variegatus* larvae and determine which
170 differ with diet and time using a two-way ANOVA.

171 The QIIME2 pipeline used to convert raw reads to OTUs for visualization of this
172 data is presented in detail in Note S1.

173

174 **3. Results**

175 As a prefeeding *L. variegatus* larva, the associated bacterial community is comparably
176 ‘simple’ and significantly different in membership and composition from all later larval
177 stages (Figure 2; Tables S3-4). At the prefeeding stage, the associated bacterial community
178 has less OTUs ($\sim 135 \pm 5$), is less phylogenetically diverse, and is less taxonomically
179 dominant (*i.e.*, more even) than the bacterial community at each ensuing day (Figure 3A-
180 B, Figure S3).

181 Following one day of development and differential feeding, prefeeding larvae from
182 each diet became two-arm larvae (Table S5), with the POA:MBL of larvae fed 10,000 and
183 0 cells•mL⁻¹ was significantly lower than those fed 1,000 and 100 cells•mL⁻¹ (Figure 1B;
184 Tables S6). The membership, composition, phylogenetic diversity, and community
185 evenness of the larval-associated bacterial community was not significantly different
186 across feeding regimes while total OTUs and community dominance varied slightly
187 between dietary treatments (Figures 2, 3, S3; Table S7).

188 A second day of differential feeding resulted in more rapid development for larvae in
189 the highest food concentration. Larvae fed 10,000 cells•mL⁻¹ were four-arm larvae while
190 those fed 1,000, 100, and 0 cells•mL⁻¹ were maintained as two-arm larvae (Tables S5).
191 Despite this difference in developmental stage, larvae fed 10,000 and 1,000 cells•mL⁻¹ had
192 a similar POA:MBL, which was lower than those fed 100 and 0 cells•mL⁻¹ (Figures 1;
193 Tables S6). Dietary treatments also resulted in differences in bacterial communities, where
194 algal concentrations induced diet-specific bacterial communities in both membership and
195 composition (Figure 2; Tables S3-4). This led to larval-associated communities with more

196 OTUs and a higher phylogenetic diversity but similar community evenness and dominance
197 (Figure 3A-B, S3; Table S7), with larvae fed 100 cells•mL⁻¹ being the most diverse and
198 those fed 10,000 and 1,000 cells•mL⁻¹ being the least diverse (Figure 3A-B, S3; Table S7).

199 Independent of diet quantity, *L. variegatus* larvae fed at each algal concentration for
200 three days were all four-armed with statistically similar POA:MBL (Figure 1; Table S5,
201 S6). The membership and composition of the bacterial communities associated with larvae
202 fed 1,000, 100, and 0 cells•mL⁻¹ were similar while those fed 10,000 cells•mL⁻¹ differed
203 significantly (Figure 2; Tables S3-4). A reduction in total OTUs and phylogenetic distance
204 as well as a more even and less taxonomically dominant community resulted in clear
205 differential structuring in the bacterial community for larvae fed 10,000 cells•mL⁻¹
206 (Figures 3, S3; Table S7). This response was not observed for larvae fed 1,000, 100, and 0
207 cells•mL⁻¹, as these treatments maintained their community structure from the day prior
208 (Figures 3, S3; Table S8).

209 As expected based on previous literature for *L. variegatus* [35, 36], morphological
210 plasticity was observed on the fourth day of differential feeding (Figure 1; Tables S5, S6),
211 where larvae fed 1,000, 100, and 0 cells•mL⁻¹ had a higher POA:MBL than those fed
212 10,000 cells•mL⁻¹. Like the day prior, larvae fed 1,000, 100, and 0 cells•mL⁻¹ associated
213 with bacterial communities that were similar in membership and composition while larvae
214 fed 10,000 cells•mL⁻¹ differed significantly (Figure 2; Tables S3-4). As morphological
215 plasticity was expressed, the number of OTUs for larvae in these three lower food
216 concentrations had similar phylogenetic distance (Figure 3; Table S7); however, larvae fed
217 10,000 cells•mL⁻¹ continued to associate with a comparatively more even and less
218 dominant community.

219 Consistent with the previous day, *L. variegatus* maintained expression of
220 morphological plasticity (Figure 1; Table S6), a result that was not confounded by a
221 continuation in larval developmental stage (Table S5). On day five of differential feeding,
222 patterns of community membership and composition (Figure 2; Tables S3-4) as well as
223 total OTUs, phylogenetic distance, evenness, and dominance all reflected the differences
224 observed in larval morphology (Figure 3; Table S7). Specifically, larvae fed 10,000
225 cells•mL⁻¹ associated with less OTUs that span less phylogenetic distance while
226 maintaining a more even and less dominant community than larvae fed 1,000, 100, and 0
227 cells•mL⁻¹ (Figure 3; Table S7).

228 Spanning the induction and expression of morphological plasticity, prefeeding and
229 feeding larval stages of *L. variegatus* consisted primarily of three Bacteroidetes (23.5%)
230 families and eleven Proteobacteria (64.0%) families (Figure S4). Over the three days where
231 morphological plasticity was expressed, we observed the abundance of all 14 bacterial
232 families from Bacteroidetes and Proteobacteria differ significantly with time while 11 (of
233 14) of these bacterial families differed across diets (Table S9). Moreover, we observed that
234 the abundance of α - and γ -proteobacteria were inversely proportional (Figure 3C, S4; Table
235 S10), such that α -proteobacteria increased following plasticity while γ -proteobacteria
236 decreased.

237 Across the same three days where morphological plasticity was observed, larvae fed
238 10,000 cells•mL⁻¹ stably associated with 114 OTUs (43.7%) while 16-40 OTUs (6.1-
239 17.6%) were specific to a single day and 19-34 OTUs (7.3-13.0%) were shared between
240 successive days (Figure 3). Larvae fed 1,000, 100, and 0 cells•mL⁻¹, on the other hand,
241 stably associated with an average of 190 OTUs (56.6%) while 15-19 OTUs (4.5-5.7%)

242 were specific to a single day and 36-39 OTUs (10.7-11.6%) were shared between
243 successive days (Figure 3). Despite the shifts in community membership, the larval stages
244 of *L. variegatus* maintained a 'core' of 31 OTUs from the Bacteroidetes (12.9%),
245 Epsilonbacteraeota (3.2%), and Proteobacteria (83.9%).

246

247 **4. Discussion**

248 Morphological plasticity is an adaptive response to heterogenous environments when
249 a fitness advantage results relative to an individual with no response [1-3]. Larval sea
250 urchins, in particular, increase individual fitness in dilute feeding environments by
251 elongating their feeding structure that, in turn, allows for a greater capacity to filter
252 particulates and reduce development time [21-23, 26, 34, 36]. Morphological plasticity for
253 larval sea urchins is also coupled with significant shifts in the associated bacterial
254 community [9, 31]. The timing of morphological plasticity and associating with a
255 phenotype-specific bacterial community, however, remains unclear.

256 Daily profiling of the bacterial communities associated with *L. variegatus* larvae over
257 the course of early development and through morphological plasticity supports three
258 primary findings. First, a four-stage successional pattern is followed as larvae transition
259 towards phenotype-specific bacterial communities. Second, the relatedness of the larval-
260 associated bacterial community directly reflects morphological plasticity and does so prior
261 to the expression of the phenotype. Third, relatedness of the bacterial communities prior to
262 and following morphological plasticity implies that the long-arm is the default phenotype.

263 From the initiation of differential feeding through the expression of morphological
264 plasticity and associating with phenotype-specific bacterial communities, we observed four

265 specific stages for *L. variegatus* larvae. First, ‘Stage 1’ followed one-day of differential
266 feeding where the bacterial communities across diets were similar in membership and
267 composition (Figure 4). Second, ‘Stage 2’ followed two-days of differential feeding where
268 the membership and composition of the larval-associated bacterial communities were diet-
269 specific (Figure 4). Third, ‘Stage 3’ was observed after three-days of differential feeding
270 where the membership and composition of the larval-associated bacterial communities
271 reflected the larval phenotypes while the host had yet to (Figure 4). Lastly, ‘Stage 4’ was
272 observed following four- and five-days of differential feeding and was where the
273 relatedness of the bacterial communities correlated with morphological plasticity (Figure
274 4).

275 The expression of morphological plasticity by temperate Strongylocentrotids [9] show
276 similar ‘Stages’ to the patterns observed with subtropical/tropical *L. variegatus* larvae.
277 Specifically, over four weeks of differential feeding for Strongylocentrotids, it was
278 observed that these larvae exhibit ‘Stages 1, 2, and 4’ [9]. The temporal pattern of these
279 ‘Stages’ was, however, unclear. Timing by *L. variegatus* larvae provides a fine grain
280 temporal organization to compare with Strongylocentrotid larvae as well as implies another
281 stage that was potentially missed. Similarly, differential feeding of a larval sea star
282 (*Acanthaster* sp.) showed both ‘Stages 1 and 2’ [33], which suggests that these stages may
283 be broadly conserved in these two distinct classes (Echinoidea and Asteroidea). Consistent
284 observation of these stages in the literature may imply that these four stages (Figure 4) are
285 common for echinoderm larvae. Whether these ‘Stages’ are observed for other taxonomic
286 Classes known to also exhibit morphological plasticity (e.g., Ophiuroidea [48] and
287 Holothuroidea [49]) is unknown but merits testing.

288 Time lags between experiencing and responding to an environmental stimulus are
289 common, advantageous, and hypothesized to be under strong selection for species
290 expressing morphological plasticity [4, 5]. For plasticity to be advantageous, time lags must
291 be short relative to the time scale of the environmental variant but long enough to minimize
292 false-positive expression [4, 5, 50]. When faced with fine grain variability in food
293 abundance, *L. variegatus* larvae are unable to match phenotype with feeding environment,
294 as the lag for morphological responses exceeds two days [51]. Despite a multi-day time
295 lag, *L. variegatus* larvae are capable of associating with bacterial communities reflective
296 of morphological plasticity prior to expressing the morphological trait. This, in principle,
297 implies that instances where a time lag exceeds the morphological response (e.g., [51]), a
298 holobiont may exhibit shifts in the associated bacterial community corresponding with the
299 environmental variation in absence or anticipation of morphological plasticity. The
300 expression of morphological plasticity also comes with the inherent energetic cost of
301 producing and maintaining an alternate phenotype [7]; thus, shifts in the bacterial
302 community may be energetically favored over morphological plasticity in heterogenous
303 environments.

304 Provided the substantial energetic requirement for planktotrophic echinoids to undergo
305 larval development [52], laboratory culturing has traditionally simulated conditions
306 equivalent to phytoplankton blooms. This, in turn, has instilled the general idea that
307 morphological plasticity requires food deprivation and plasticity is the elongation of the
308 feeding apparatus [21, 23, 35]. Field culturing, however, suggests the opposite, that
309 echinoid larvae are naturally food-limited [20, 53, 54] and ‘plasticity’ is shortening the
310 larval arm and expanding stomach volume in response to uncommon food-rich

311 environments [23]. The molecular mechanisms underlying morphological plasticity have
312 suggested that algal chemosensations inhibit growth of the larval arms [28] and, thus, the
313 long-arm phenotype would likely be the default under natural conditions.

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

323 Taken together, the data presented here suggests: (i) that phenotype-specific bacterial
324 communities for larval *L. variegatus* follow a four-stage progression (Figure 4); (ii) that
325 shift in bacterial taxa precedes morphological plasticity and occur during the time lag; and
326 (iii) that the long-arm phenotype is most similar to pre-plasticity larval siblings and the
327 short-arm phenotype correlates with a restructuring of the bacterial community.
328 Determining if and how this community contributes to larval fitness during before, during,
329 and following the expression of morphological plasticity merits future investigation and
330 would require multi-omic comparisons [55] between axenic and germ-rich siblings (e.g.,
331 [56-58]) as well as add-back experiments of individual bacterial taxa (e.g., [59-61]).
332

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339

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342

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346

347 **Figure legend**

348

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

354

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

363

364 **Figure 3.** Structural changes to the bacterial community associated with *Lytechinus*
365 *variegatus* larvae. (A) Enumeration of operational taxonomic units (OTUs) and
366 phylogenetic distance of those OTUs for *L. variegatus* larvae from prefeeding through five
367 days of feeding on either (10,000, 1,000, 100, and 0 $\text{cells} \cdot \text{mL}^{-1}$ of a phytoplankton
368 represented by blue, red, green and purple, respectively). (B) Unweighted distribution of
369 bacterial taxa for *L. variegatus* larvae from either of the larval phenotypes, and the (C) total

370 sequences (from the rarefied table) of α - and γ -proteobacteria for these time points and
371 diets.

372

373 **Figure 4.** Dynamics of the bacterial community during morphological plasticity. Visual
374 model representation of morphological plasticity for echinoid larvae and the four
375 successive stages towards associations with a phenotype-specific bacterial community.

376

377

378 **Literature**

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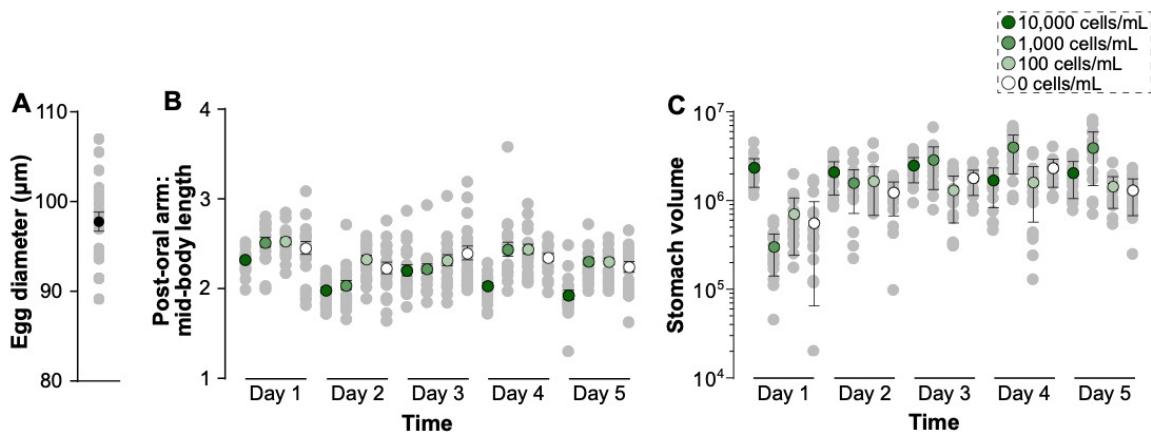
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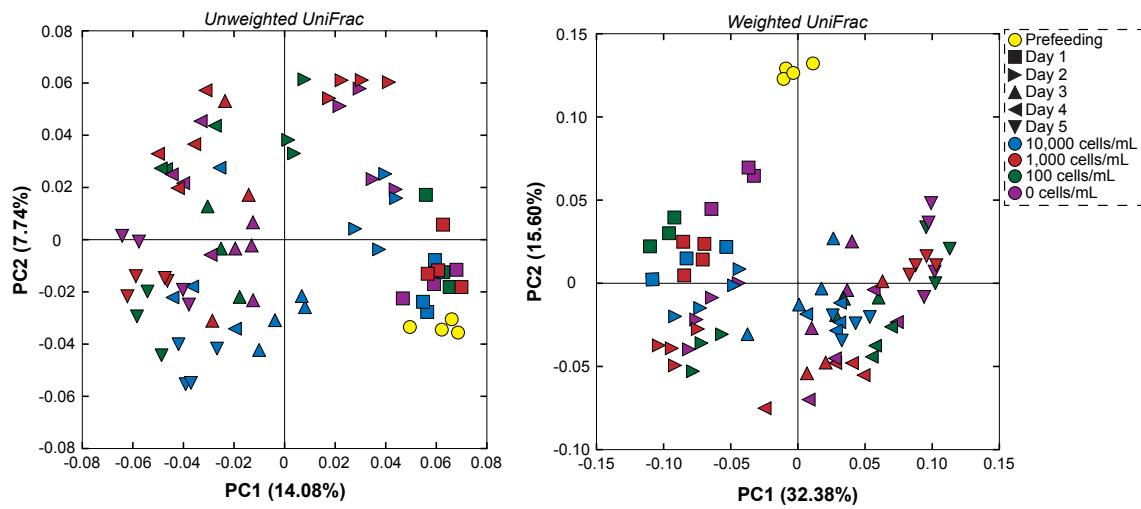
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6 **Figure 2**

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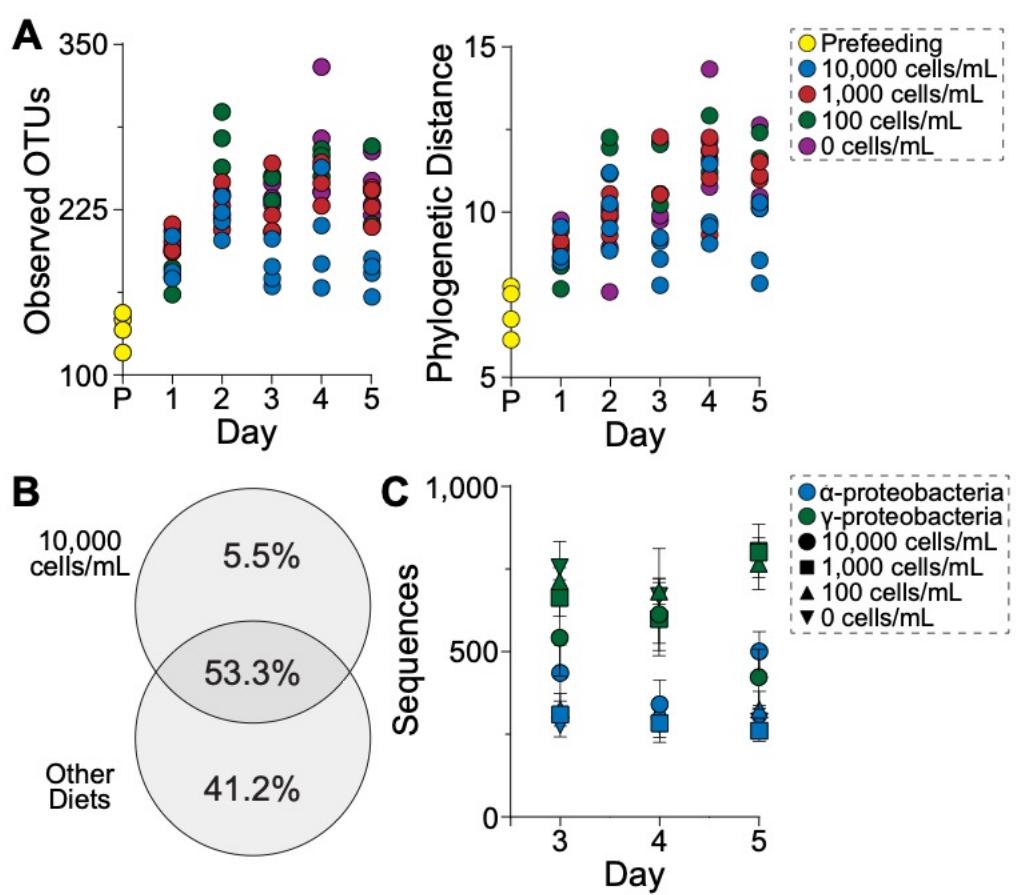
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11 **Figure 3**

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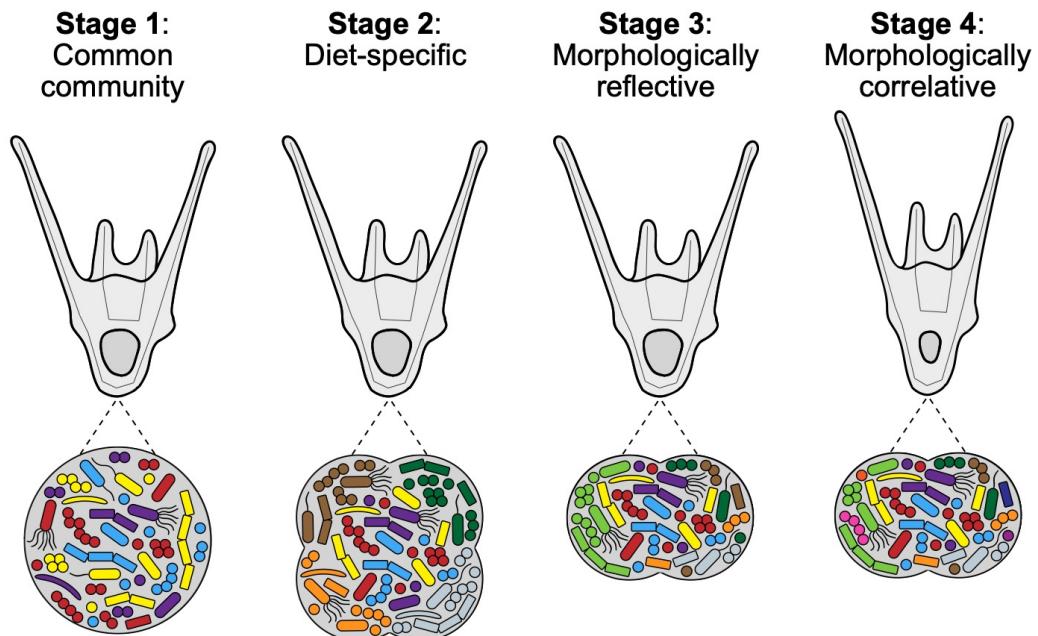
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16 **Figure 4**

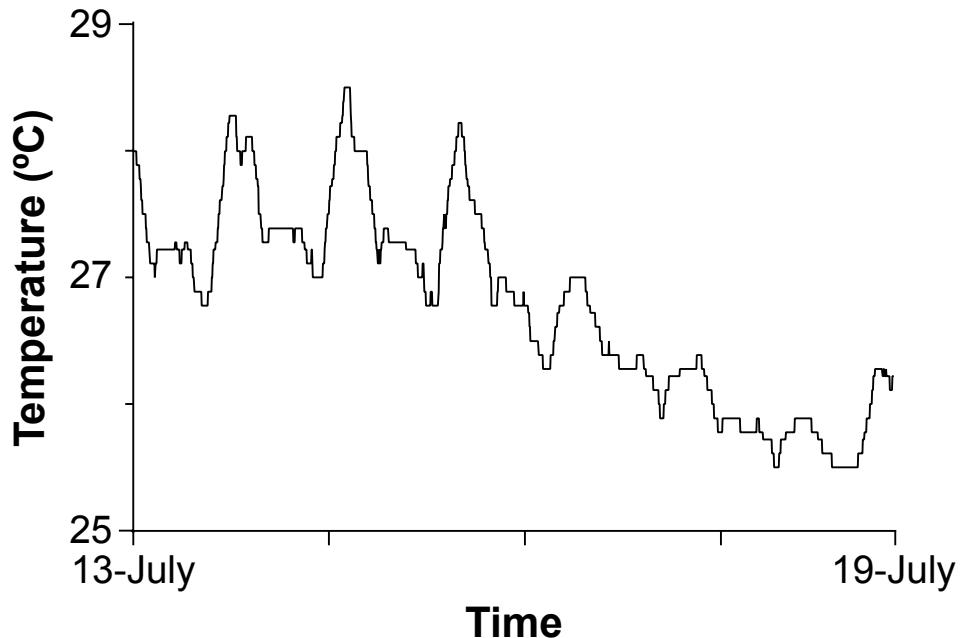
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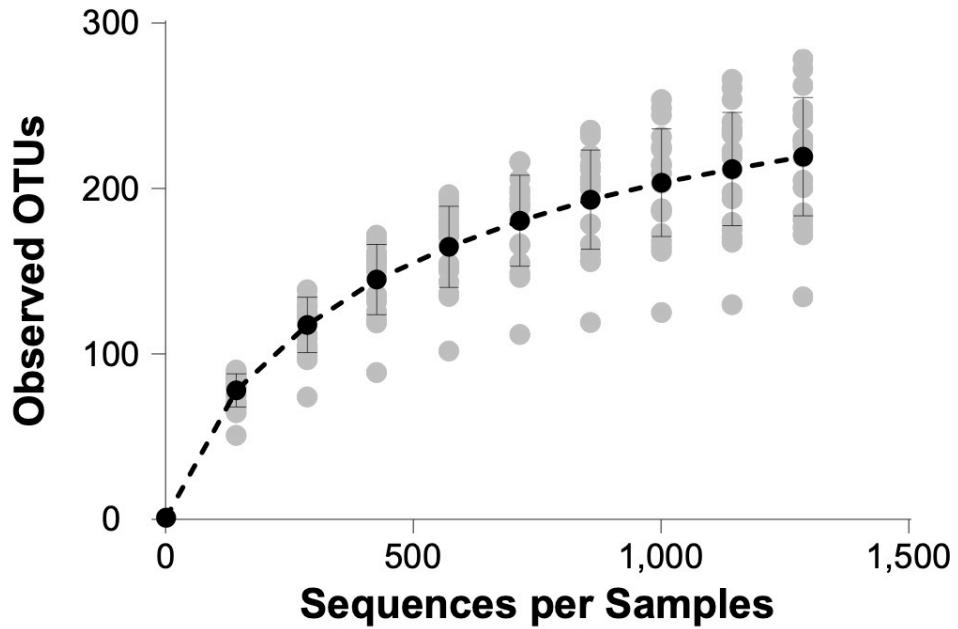
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3 **Figure S1.** *in situ* measurements of sea surface temperature at the Duke University Marine
4 Laboratory. Sea surface temperature at Beaufort, NC (NOAA station, BFTN7) for the
5 entirety of larval experimentation (13-19 July 2017).
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10 **Figure S2.** Alpha rarefaction curve for *Lytechinus variegatus* larvae. Alpha rarefaction
11 curve for the associated bacteria with for *L. variegatus* from prefeeding through five days
12 of feeding on either 10,000, 1,000, 100, and 0 cells•mL⁻¹ of a phytoplankton based on the
13 rarefaction depth (1,287 sequences) used for all analyses.
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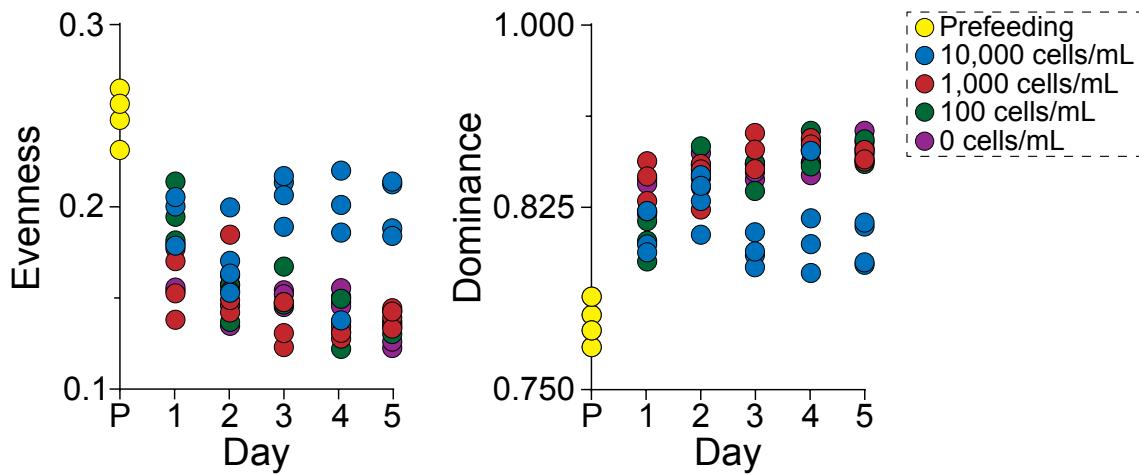
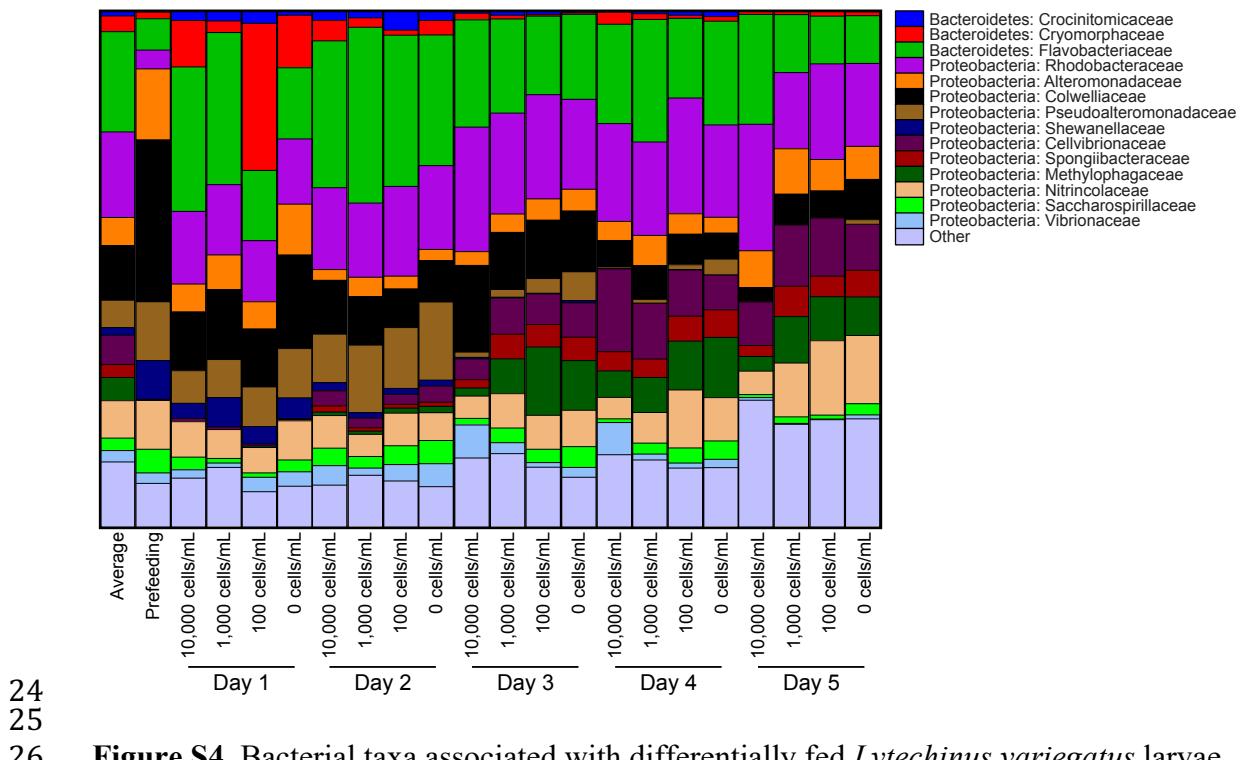


Figure S3. Changes in alpha diversity metrics to the bacterial community associated with *Lytechinus variegatus* larvae. Evenness and dominance of bacterial taxa for *L. variegatus* larvae from prefeeding through five days of feeding on either 10,000, 1,000, 100, and 0 cells•mL⁻¹ of a phytoplankton (represented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

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Figure S4. Bacterial taxa associated with differentially fed *Lytechinus variegatus* larvae.
Bacterial family associated with *L. variegatus* larvae fed either fed either 10,000, 1,000,
100, or 0 cells mL⁻¹ over the course of five days of differential feeding.