Title

Geography, not food availability, reflects compositional differences in the bacterial communities associated with larval sea urchins

Running title

Population-specific microbiome of urchin larvae

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Abstract

Determining the principles underlying the assembly, structure, and diversity of symbiont communities remains a focal point of animal-microbiome research. Much of these efforts focus on taxonomic variation within or between animal populations, but rarely test the proportional impacts of ecological components that may affect animal-associated microbiota. Using larvae from the sea urchin *Strongylocentrotus droebachiensis* from the Atlantic and Pacific Oceans, we test the hypothesis that inter-population differences in the composition of animal-associated bacterial communities are more pronounced than intra-population variation due to a heterogeneous feeding environment. Despite significant differences in bacterial community structure within each *S. droebachiensis* larval population (based on food availability, time, development, and phenotype), variation in OTU membership and community composition correlated more strongly with geography. Moreover, across these three locations, 20-30% of OTUs were specific to a single population while less than 10% were shared. Taken together, these results suggest that inter-populational variation in symbiont communities is more pronounced than intra-populational variation. This difference may suggest that ecological variables over broad geographic scales may mask smaller scale ecological variables; however, explicit testing of this potential principle requires common garden experiments as well as molecular and functional manipulations.

41 **Introduction**

42 Acclimating to environmental variability through morphological, developmental,
43 and/or physiological plasticity is a common trait of animals (Bradshaw, 1965, Boidron-
44 Metairon, 1988, Sterns, 1989, DeWitt *et al.*, 1998, Schlichting & Smith, 2002, West-
45 Eberhard, 2003, Miner *et al.*, 2005). Over the past decade, the appreciation for the role that
46 animal-associated microbiota play in responding to environmental stressors has grown
47 profoundly (Kohl & Carey, 2016, Macke *et al.*, 2016, Shapira, 2016, Theis *et al.*, 2016,
48 Apprill, 2017, Carrier & Reitzel, 2017, Carrier & Reitzel, 2018). When experiencing
49 heterogeneous environments, an animal may recruit, expel, and/or shuffle the relative
50 proportion of associated microbiota (Zilber-Rosenberg & Rosenberg, 2008, Bordenstein &
51 Theis, 2015) to assemble a community with particular molecular pathways for the
52 environmental conditions (Burke *et al.*, 2011, Louca *et al.*, 2016, Roth-Schulze *et al.*,
53 2018).

54 Microbial communities associated with animals often vary in response to a multitude
55 of abiotic and biotic factors, including temperature, salinity, diet quality and quantity,
56 season, and habitat-type (see reviews by Kohl & Carey, 2016, Carrier & Reitzel, 2017). Of
57 these, dietary responses are best studied and shown to have a major impact on the
58 composition and function of this community (Kohl & Dearing, 2012, David *et al.*, 2014,
59 Rosenberg & Zilber-Rosenberg, 2016, Sonnenburg *et al.*, 2016). When faced with
60 prolonged diet restriction, the composition and diversity of microbiota associated with both
61 invertebrate and vertebrate hosts shift considerably (Kohl *et al.*, 2014, Carrier & Reitzel,
62 2018), a response hypothesized to buffer against reduced exogenous nutrients.

63 Microbial communities associated with animals are also species-specific (*e.g.*, Fraune
64 & Bosch, 2007, Schmitt *et al.*, 2012, Carrier & Reitzel, 2018) and taxonomically variable

65 across the geographical distribution of the host (Dishaw *et al.*, 2014, Marzinelli *et al.*, 2015,
66 Mortzfeld *et al.*, 2015, Marino *et al.*, 2017, Huang *et al.*, 2018). Population-specific
67 microbial communities are partially dependent on environmental conditions (Pantos *et al.*,
68 2015) and dispersal limitations (Moeller *et al.*, 2017). Despite this taxonomic variation,
69 microbial communities can remain functionally similar due to shared genes across bacterial
70 taxa (Louca *et al.*, 2018, Roth-Schulze *et al.*, 2018). The microbial communities of the
71 bromeliad *Aechmea nudicaulis*, for example, are taxonomically dynamic but functionally
72 consistent between individuals (Louca *et al.*, 2016). Similarly, the bacterial community
73 associated with the green alga *Ulva* spp. are too spatially and temporally variable to define
74 a 'core' community; however, nearly 70% of the microbial genes are conserved across the
75 broad geography of *Ulva* spp. (Roth-Schulze *et al.*, 2018).

76 Efforts to classify the principles underlying the assembly of host-associated microbial
77 communities have primarily been within (*e.g.*, Webster *et al.*, 2011, Webster *et al.*, 2011,
78 David *et al.*, 2014, Lokmer & Wegner, 2015, Carrier & Reitzel, 2018, Carrier *et al.*, 2018)
79 or between (*e.g.*, (Yatsunenko *et al.*, 2012, Dishaw *et al.*, 2014, Marzinelli *et al.*, 2015,
80 Mortzfeld *et al.*, 2015, Marino *et al.*, 2017, Huang *et al.*, 2018) populations (but see
81 Webster *et al.*, 2018). This conceptual discontinuity highlights a gap in understanding the
82 patterns and principles underlying community assembly. To test for differences in
83 assembly and structure, direct comparisons of animal-associated microbial communities
84 within and between populations are needed to determine the relevance of each factor. The
85 fields of sensory, behavioral, and circadian biology have shown that organismal responses
86 to ecological variables are unequal and may be masked based on spatial significance
87 (Beauchard *et al.*, 2017, Hodin *et al.*, 2018, Li *et al.*, 2018, Yang *et al.*, 2018, Kerr & Kemp,

88 In Press). Factors within and between populations, therefore, may have differential impacts
89 on the composition of host-associated microbiota.

90 Planktotrophic (feeding) larvae are one biological system to compare ecological
91 components and how they influence the composition of animal-associated microbiota.
92 Many planktotrophic larvae (*e.g.*, the pluteus of sea urchins) inhabit heterogeneous
93 nutritional environments and are morphologically and physiologically plastic to food
94 availability (Boidron-Metairon, 1988, Hart & Strathmann, 1994, Miner, 2004, Byrne *et al.*,
95 2008, Soars *et al.*, 2009, Adams *et al.*, 2011, Miner, 2011, Carrier *et al.*, 2015, McAlister
96 & Miner, 2018). Larvae of the echinoid *Strongylocentrotus droebachiensis*, for example,
97 associate with phenotype-, diet-, and developmental stage-specific bacterial communities
98 (Carrier & Reitzel, 2018, Carrier & Reitzel, 2019). *S. droebachiensis*, in particular, has an
99 Arctic-boreal distribution composed of a multiple ocean population with limited gene flow
100 (Addison & Hart, 2004, Addison & Hart, 2005, Scheibling & Hatcher, 2013) and evidence
101 for potential local adaption (Manier & Palumbi, 2008, Marks *et al.*, 2008).

102 Using the circumpolar distribution of *S. droebachiensis* and the ability of their larvae
103 to acclimate to heterogeneous feeding environments, we test the hypothesis that inter-
104 population differences in the composition of animal-associated bacterial communities are
105 more pronounced than intra-population variation. To test this, we performed differential
106 feeding experiments on *S. droebachiensis* larvae from populations in the Pacific and
107 Atlantic Oceans (Fig. 1) and used amplicon sequencing to profile the larval-associated
108 bacterial communities.

109

110 **Materials and Methods**

111 *Adult urchin collection and larval rearing*

112 Adult *S. droebachiensis* were collected from populations in the North Sea in March
113 2015, the Salish Sea in April 2016, and the Gulf of Maine in February 2017 (Fig. 1).
114 Individuals from the North Sea were collected by divers in Drøbak, Norway (59°39' N,
115 10°37' E) and transported in cold, aerated seawater to the Sven Lovén Centre for Marine
116 Infrastructure (Kristineberg, Sweden). Urchins were maintained in natural seawater and
117 fed *ad libitum* on a live mix of *Ulva lactuca* and *Laminaria* spp. collected from the
118 Kristineberg shoreline. Urchins from the Salish Sea were hand-collected at low tide at
119 Cattle Point, San Juan Island, USA (48°27' N, 122°57' W), transferred to the Friday Harbor
120 Laboratories (FHL; Friday Harbor, WA, USA) within one hour, suspended in sub-tidal
121 cages off the dock at FHL, and fed *Nereocystis* spp. *ad libitum*. Lastly, individuals from
122 the Gulf of Maine were collected by divers in Frenchman Bay, Maine (44°25' N 68°12'
123 W), shipped overnight to the Darling Marine Center (Walpole, ME, USA), and were
124 maintained in flow-through aquaria and fed *Saccharina latissima ad libitum*.
125 Within two weeks of collections, adult urchins were spawned by a one to two mL
126 intracoelomic injection of 0.50 M potassium chloride. For each population, gametes from
127 three males and females were pooled separately. Fertilization of eggs and larval rearing
128 followed Strathmann (1987), with the exception that embryos and larvae were reared using
129 5.0-µm filtered seawater (FSW) to include the environmental microbiota. Briefly, embryos
130 were incubated in 1 L of FSW at ambient temperature and salinity. Two hours post-
131 fertilization, seawater was decanted to remove excess sperm. Embryos were then
132 transferred to triplicate 3 L jars and diluted to a density of two individuals•mL^{-1}. Beginning
133 at the prism stage, larvae were provided *Rhodomonas lens* at 10,000 cells•mL^{-1}, for which
134 was made growth medium-free by centrifugation and resuspension in FSW prior to the
135 introduction to larval cultures. Larval cultures were subsequently diluted to one larva per

136 two mL at advanced developmental stages. Larval cultures were given 90-95% water
137 changes every other day and *R. lens* was replenished at 10,000 cells•mL^{-1}.
138 Monocultures of *R. lens* were grown in f/2 media at room temperature with a
139 combination of ambient and artificial lighting for 24 hours per day (Guillard, 1975).
140
141 *Experimental feeding and larval morphometrics*
142 At 48 hours post-fertilization, prism-stage larvae were divided into twelve replicate jars
143 that were sub-divided into four experimental feeding treatments: 10,000, 1,000, 100, or 0
144 cells•mL^{-1} of *R. lens*. For each experiment, larvae fed 10,000 cells•mL^{-1} were reared
145 through metamorphosis while diet-restricted (1,000 and 100 cells•mL^{-1}) and unfed larvae
146 were cultured until developmental stasis (Table S1). Larvae (n=100) from each replicate
147 for each treatment were sampled weekly. Immediately after sampling, larval samples were
148 concentrated into a pellet using a microcentrifuge, the FSW was removed with a sterile
149 glass pipette, and pelleted larvae were then preserved in RNAlater (Thermo Scientific,
150 Massachusetts, USA) and stored at -20°C before DNA extractions.
151 Complementary to sampling *S. droebachiensis* larvae, the environmental microbiota
152 from the seawater was also sampled. When larval cultures were sampled, triplicate ~1-L
153 of 5.0-µm FSW was filtered onto a 0.22-µm Millipore filter to retain the environmental
154 microbiota. Full filter disks were then preserved in RNAlater and stored at -20 °C before
155 DNA extractions.
156 In addition to sampling larvae to assay the associated bacterial communities, twenty
157 larvae (n=20) from a single replicate of each dietary treatment were sampled for
158 morphometric analysis. Larvae were imaged using a compound microscope (Salish Sea:
159 Nikon Eclipse E600; camera: QImaging MicroPublisher 5.0 RTV; Gulf of Maine: Zeiss

160 Primo Star HD digital microscope; North Sea: Leica stereomicroscope) and morphometrics
161 (length of larval body, post-oral arms, and stomach area; Figs. 1, S1-2) were performed
162 using ImageJ (v. 1.9.2; Schneider *et al.*, 2012). Statistical differences in larval size and
163 stomach volume were compared using a two-way analysis of variance (ANOVA; JMP Pro
164 v. 13) to test for differences over time and across feeding conditions. Moreover, differences
165 between sites were compared with a one-way ANOVA. Where statistical differences were
166 observed ($p<0.05$), a Tukey's post-hoc test were used to determine the affect at each time
167 point and for each diet.
168
169 *Assaying microbial communities*
170 Total DNA was extracted from larval samples using the GeneJet Genomic DNA
171 Purification Kit (Thermo Scientific, Massachusetts, USA). For FSW samples, eDNA was
172 extracted using the FastDNA Spin Kit for Soil (MP Biomedical, Illkirch, France). DNA
173 was then quantified using the NanoDrop 2000 UV-Vis Spectrophotometer (Thermo
174 Scientific, Massachusetts, USA) and diluted to 5 $ng \bullet \mu L^{-1}$ using RNase/DNase-free water.
175 Bacterial sequences were amplified using universal primers for the V3/V4 regions of
176 the 16S rRNA gene (Forward: 5′ CTACGGGNGGCWGCAG, Reverse: 5′
177 GACTACHVGGGTATCTAATCC; Klindworth *et al.*, 2013). Products were purified
178 using the Axygen AxyPrep Mag PCR Clean-up Kit (Axygen Scientific, New York, USA),
179 indexed via PCR using the Nextera XT Index Kit V2 (Illumina, California, USA), and then
180 purified again. At each of these three steps, fluorometric quantitation was performed using
181 a Qubit (Life Technologies, California, USA) and libraries were validated using a
182 Bioanalyzer High Sensitivity DNA chip (Agilent Technologies, California, USA). Illumina

183 MiSeq sequencing (v3, 2x300 bp paired-end reads) was performed at the University of
184 North Carolina at Charlotte. See, Table S2 for PCR recipe and thermal profiles.

185 Forward and reverse sequences (that are freely available on the Dryad Digital
186 Repository) were paired and trimmed using PEAR (Zhang *et al.*, 2014) and Trimmomatic
187 (Bolger *et al.*, 2014), respectively, and converted from fastq to fasta using custom script
188 (see, Note S1). Chimeric sequences were then detected using USEARCH (Edgar *et al.*,
189 2011) and removed using filter_fasta.py prior to analysis of bacterial 16S rRNA sequences.
190 Using QIIME 1.9.1 (Caporaso *et al.*, 2010) and Greengenes (v. 13.5), bacterial 16S rRNA
191 sequences were analyzed and grouped into operational taxonomic units (OTUs) based on
192 a minimum 97% similarity. The biom table generated by pick_open_reference_otus.py was
193 filtered of OTUs with ten or less sequences as well as sequences matching the cryptophytes
194 (*i.e.*, *R. lens*; as per Carrier & Reitzel, 2018).

195 Using the filtered biom table and "biom summarize-table" to count total sequences per
196 sample, the rarefaction depth of 3,193 reads was determined and applied to all subsequent
197 analyses (Fig. S3). Alpha diversity (*i.e.*, Fisher's alpha, Shannon equitability, Faith's
198 phylogenetic distance, and observed OTUs) was calculated using alpha_diversity.py and
199 compared statistically using one-way ANOVAs in JMP. Beta diversity was calculated
200 using the unweighted and weighted UniFrac (Lozupone & Knight, 2005) as part of
201 jackknifed_beta_diversity.py. These values were compared using principal coordinate
202 analyses (PCoA), recreated using make_2d_plots.py, and stylized for presentation in
203 Adobe Illustrator CS6. Community similarity across phenotypes, dietary states,
204 developmental stages, and biogeography were compared statistically using an analysis of
205 similarity (ANOSIM), permutational multivariate analysis of variance using distance
206 matrices (ADONIS), and permutational multivariate analysis of variance (PERMANOVA)

207 using compare_categories.py. Shared OTUs between urchin populations were determined
208 using compute_core_microbiome.py and shared_phylotypes.py. Average bacterial
209 community for each urchin population as well as across populations and the shared
210 community was generated using summarize_taxa_through_plots.py, visualized with Prism
211 7 (GraphPad Software), and stylized for presentation in Adobe Illustrator CS6.
212 A step-by-step listing of QIIME scripts used to convert raw reads to OTUs for
213 visualization of these data is located in Note S1.
214
215 **Results**
216 *Morphological plasticity of larval sea urchin*
217 Diet-induced morphological plasticity was observed for *S. droebachiensis* larvae from
218 each population (Figs. S1-2; Tables S3-4), with the extent and direction of expression being
219 location-specific (ANOVA, $p<0.0001$; Fig. 2). Diet-restricted larvae from the Salish Sea
220 and Gulf of Maine, in general, exhibited a higher post-oral arm to mid-body line ratio than
221 *ad libitum* counterparts (ANOVA, $p<0.0001$; Figs. S1-2; Tables S3-4), even though
222 analyses of Gulf of Maine larvae were partially confounded by developmental stage (Table
223 S1). Larvae from the North Sea, however, exhibited the opposite response: *ad libitum*
224 feeding induced a higher post-oral arm to mid-body line ratio than diet-restricted
225 individuals (ANOVA, $p<0.0001$; Fig. S1-2; Tables S3-4).
226
227 *Structural comparisons of bacterial communities within populations*
228 *S. droebachiensis* larvae from each population associated with a diet-specific bacterial
229 community (Figs. 3A-C, S4-7; Table S6). Larvae from the Salish Sea and Gulf of Maine
230 exhibited similar diet-specific community-level patterns in both alpha- and beta-diversity

231 (Figs. 3A-B, S4-6; Table S6), where the bacterial community associated with food
232 restricted larvae were more similar to each other than to well-fed counterparts. Larvae from
233 the North Sea, on the other hand, exhibited the opposite response (Figs. 3C, S4, S7; Table
234 S6), where diet-specific bacterial communities were observed (Table S6) except that all
235 food concentrations were more similar to each other than to starved larvae (Fig. S7; Table
236 S6).

237 The bacterial communities associated with larvae from each sea urchin population also
238 varied temporally in both alpha- and beta-diversity (Figs. 3D-F, S4, S8-10; Table S6).
239 Temporal changes in community diversity (*i.e.*, alpha-diversity) were reflective of
240 culturing environment: larval populations from the Atlantic Ocean increased in weeks one
241 through three and declined in week four while the larval population from the Pacific Ocean
242 decreased in the first three weeks and increased in week four (Table S5). Compositional
243 changes (*i.e.*, beta-diversity) in the associated bacterial communities for each larval
244 population, on the other hand, experienced similar time-dependent shifts from week one
245 though four (Figs. 3D-F, S4).

246 Complementary to dietary and temporal shifts in community structure and
247 composition, larval-associated bacterial communities for *S. droebachiensis* varied
248 significantly with larval morphology (Fig. S11; Table S7) and developmental stage (Figs.
249 S8-10; Table S7). Variation in the larval-associated bacterial communities across food
250 availability, time, morphology, and development, however, appeared to not co-depend on
251 the bacterial taxa observed in the seawater, as the composition of larval-associated and
252 environmental microbiota were significantly different (Fig. S12; Table S7).

253

254 *Differences in the bacterial communities of urchin populations*

255 Despite differences in bacterial community structure within each *S. droebachiensis*
256 larval population, variation in OTU membership and composition correlated best with
257 geography (ANOSIM, unweighted UniFrac, $p<0.001$; ANOSIM, weighted UniFrac,
258 $p<0.001$; Figs. 4A-B, S13-15; Table S8). Community relatedness of larval-associated
259 microbiota in the Western and Eastern Atlantic Ocean was more similar to each other than
260 to the Pacific Ocean population (Fig. 4 A-B). Moreover, bacterial community diversity and
261 structure were significantly different between these three populations (Fisher's alpha: F-
262 ratio = 142.2, $p<0.001$; Shannon equitability: F-ratio = 110.1, $p<0.001$; Faith's
263 phylogenetic distance: F-ratio = 159.2, $p<0.001$; observed OTUs: F-ratio = 124.2, $p<0.001$;
264 Fig. 4C; Table S8). For each of the tested indices, bacterial communities of Gulf of Maine
265 larvae were taxonomically richest and most diverse while Salish Sea larvae were the least
266 rich and diverse, leaving North Sea larvae as intermediate (Tukey's test, $p<0.05$ for each;
267 Fig. 4C; Table S8).
268 *S. droebachiensis* larvae from these populations primarily associated with seven
269 bacterial classes from three phyla: Saprospirae and Flavobacteriia (Bacteroidetes), Alpha-
270 , Beta-, Delta-, and Gammaproteobacteria (Proteobacteria), and Verrucomicrobiae
271 (Verrucomicrobia) (Fig. 5A). Of these classes, Flavobacteriia, Alpha- and
272 Gammaproteobacteria represented at least 10% of the relative community for each
273 population, where Gammaproteobacteria was the most abundant clade at 20-50% (Fig.
274 5A). Of the hundreds of OTUs associated with *S. droebachiensis* larvae (Fig. S1), less than
275 10% were shared across populations, with 20-30% being specific to a single population
276 and 3-11% being shared between two populations (Fig. 5B). This shared community was
277 similar to the full communities for each population, in that it was composed primarily by
278 Flavobacteriia (24.7%), Alpha- (28.1%) and Gammaproteobacteria (34.9%) (Fig. 5A).

279

280 **Discussion**

281 Principles underlying the assembly and diversity of host-associated microbial
282 communities has remained a focal point of host-microbiome research over the last few
283 decades (Ley *et al.*, 2006, Ley *et al.*, 2008, Zilber-Rosenberg & Rosenberg, 2008,
284 Rosenberg *et al.*, 2009, Bright & Bulgheresi, 2010, Bordenstein & Theis, 2015, Coyte *et*
285 *al.*, 2015, Gilbert *et al.*, 2015, Marzinelli *et al.*, 2015, Brooks *et al.*, 2016, Macke *et al.*,
286 2016, Shapira, 2016, Carrier & Reitzel, 2017, Huang *et al.*, 2018, Miller *et al.*, 2018).
287 Efforts to partition and categorize variation in community composition have suggested that
288 evolutionary history (Ley *et al.*, 2006, Ley *et al.*, 2008, Zilber-Rosenberg & Rosenberg,
289 2008, Brooks *et al.*, 2016, Shapira, 2016), physiology (Kohl *et al.*, 2016, Kohl & Carey,
290 2016, Buckley & Rast, 2017), and environmental conditions (Alberdi *et al.*, 2016, Kohl &
291 Carey, 2016, Carrier & Reitzel, 2017, Miller *et al.*, 2018) primarily drive these differences.
292 The majority of this previous work, however, focuses on two spatiotemporal scales: within
293 (Webster *et al.*, 2011, Webster *et al.*, 2011, David *et al.*, 2014, Lokmer & Wegner, 2015,
294 Carrier & Reitzel, 2018, Carrier *et al.*, 2018) or between (Dishaw *et al.*, 2014, Marzinelli
295 *et al.*, 2015, Mortzfeld *et al.*, 2015, Marino *et al.*, 2017, Huang *et al.*, 2018) host
296 populations (but see Webster *et al.*, 2018). The potential to distinguish and discern intra-
297 and inter-population processes for a single species highlights a potential conceptual gap in
298 understanding the patterns and principles underlying microbiome assembly.

299 Comparing the bacterial communities associated with *S. droebachiensis* larvae across
300 feeding environments and geographical location supports two primary findings. First,
301 larvae from each population associate with bacterial communities correlated with food
302 availability, time, development, and phenotype. Second, despite variation in the microbiota

303 within each population of urchin larvae, the composition of *S. droebachiensis* larval-
304 associated bacterial communities best correlated with geography.

305 Larval marine invertebrates associate with microbial communities that are diverse and
306 dynamic yet specific to host species and distinct from the environmental microbiota
307 (Webster *et al.*, 2011, Carrier *et al.*, 2018, Carrier & Reitzel, 2018, Carrier *et al.*, 2018,
308 Carrier & Reitzel, 2019). The bacterial community associated with larval echinoderms, in
309 particular, shifts with food availability (Carrier & Reitzel, 2018, Carrier *et al.*, 2018),
310 embryonic and larval development (Carrier & Reitzel, 2018, Carrier & Reitzel, 2019),
311 phenotype (Carrier & Reitzel, 2018), disease (Carrier *et al.*, 2018), and physiology
312 (Buckley & Rast, 2017). Common approaches for investigating larval-microbe interactions
313 involve single location studies of a widely distributed taxa (see Webster *et al.*, 2011, Carrier
314 & Reitzel, 2018, Carrier *et al.*, 2018, Carrier & Reitzel, 2019). The data presented here
315 suggest that community-level shifts in response to biotic (development and phenotype) and
316 abiotic (food availability and time) factors occur at multiple locations across a population
317 with limited gene flow. Three widely-distributed sea urchin species (including *S.*
318 *droebachiensis*) have previously been shown to associate with food-elicited phenotype-
319 specific bacterial communities (Carrier & Reitzel, 2018). It may, therefore, be
320 hypothesized that phenotype-specific microbiota for these and, potentially, other species
321 of larval echinoderms (*e.g.*, *Acanthaster* sp. in the Pacific Ocean, including on the Great
322 Barrier Reef; Wolfe *et al.*, 2015, Wolfe *et al.*, 2015, Wolfe *et al.*, 2017, Carrier *et al.*, 2018)
323 occur throughout the geographical distribution of the species.

324 Marine invertebrate larvae are not unique in their ability to respond to environmental
325 variability by exhibiting shifts in the associated bacterial community (Kohl & Carey, 2016,
326 Carrier & Reitzel, 2017). Several groups of marine and terrestrial vertebrates (*e.g.*,

327 mammals, reptiles, and fish) respond to diet variability and abiotic stress by restructuring
328 their gut microbiota that, in turn, are likely to differ in community function (Muegge *et al.*,
329 2011, Kohl & Dearing, 2012, David *et al.*, 2014, Schmidt *et al.*, 2015, Kohl *et al.*, 2016,
330 Kohl & Carey, 2016, Kohl *et al.*, 2018). Similar to larval marine invertebrates, marine and
331 terrestrial vertebrates can be widely-distributed and, thus, multiple genetically distinct
332 populations may exhibit similar community-levels shifts in microbiota at multiple locations
333 across these populations. To our knowledge, however, parallel responses between
334 populations for vertebrates and other taxa have not yet been documented.

335 Inter-populational variation in the composition of *S. droebachiensis* larval-associated
336 bacterial communities was more pronounced than intra-populational differences. While the
337 mechanisms driving this result is unknown, genetic and ecological differences between *S.*
338 *droebachiensis* populations are likely contributing factors (Pantos *et al.*, 2015, Moeller *et*
339 *al.*, 2017). *S. droebachiensis* from the Salish Sea, Gulf of Maine, and North Sea have some
340 degree to genetic isolation (Addison & Hart, 2004, Addison & Hart, 2005, Cowen &
341 Sponaugle, 2009, Norderhaug *et al.*, 2016). Moreover, each urchin population inhabit
342 environments that differ in physical (Otto *et al.*, 1990, Pettigrew *et al.*, 2005, Sutherland &
343 MacCready, 2011) and biological (Winter *et al.*, 1975, Thomas *et al.*, 2003, McQuatters-
344 Gollop *et al.*, 2007) factors. Inter-populational variation in the bacterial communities
345 associated with *S. droebachiensis* larvae may, therefore, have been due to differences in
346 host genetics and the oceanographic environment, a result that requires future explicit
347 testing using common garden experiments and molecular manipulations (Williams &
348 Carrier, 2018).

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

351 across host geography (Dishaw *et al.*, 2014, Marzinelli *et al.*, 2015, Mortzfeld *et al.*, 2015,
352 Marino *et al.*, 2017, Huang *et al.*, 2018). Studies using diverse animal taxa (*e.g.*, sponges,
353 corals, fish, and humans) support that the environment, not genotype, principally drives
354 community differences (Sullam *et al.*, 2012, Burgsdorf *et al.*, 2014, Pantos *et al.*, 2015,
355 Rothschild *et al.*, 2018). Genetic comparison of *S. droebachiensis* indicates that individuals
356 from the Salish Sea and Gulf of Maine sub-populations are more related to each other than
357 to the North Sea sub-population (Addison & Hart, 2004, Addison & Hart, 2005, Scheibling
358 & Hatcher, 2013). Microbial communities from these populations, however, suggest the
359 contrary: larvae from the Gulf of Maine and North Sea are more similar to each other than
360 to Salish Sea larvae. This result reinforces the hypothesis that environment (or, here,
361 geography) is a larger influence on community assembly than genetic similarity. The
362 mechanistic linkage between host genetics, the environment, and microbial community,
363 however, is unknown.

364 By comparing host-associated bacterial communities across feeding environments and
365 biogeography in larval sea urchins, we propose that inter-populational variation in
366 symbiont communities is more biologically significant than intra-populational variation.
367 This difference suggests that larger scale ecological variables that correspond to different
368 geographic locations may mask smaller scale ecological variables (*e.g.*, feeding, time,
369 development, and phenotype). Explicit testing with diverse animal taxa by experimental
370 manipulations and functional characterization are merited to understand the priority of
371 exogenous factors in the composition of host-associated microbial communities.

372

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386 **Figure Legends**

387

388 **Figure 1.** Location of *Strongylocentrotus droebachiensis* experiments. Cartoon
389 representation of adult populations and the geographical distribution for where differential
390 feeding experiments were conducted.

391

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

396

397 **Figure 3.** Dietary and temporal shifts in the bacterial communities associated with
398 *Strongylocentrotus droebachiensis* larvae. Community similarity based on *S.*
399 *droebachiensis* larval-associated bacterial communities based on food availability (A-C)
400 over a multi-week exposure (D-F) for populations from the Salish Sea (A, D), Gulf of
401 Maine (B, E), and North Sea (C, F). Comparisons between food availability (A-C) and over
402 time (D-F) are based on weighted UniFrac comparisons.

403

404 **Figure 4.** Structure of the bacterial communities associated with *Strongylocentrotus*
405 *droebachiensis* larvae from three populations. Community similarity of *S. droebachiensis*
406 larval-associated bacterial communities between three geographic locations based on (A)
407 unweighted and weighted UniFrac comparisons, with the relatedness of the weighted
408 UniFrac represented by a microbial dendrogram (B). Diversity within each population was

409 estimated using four (C) alpha metrics: observed OTUs, Faith's phylogenetic distance,
410 Fisher's alpha, and Shannon diversity.
411
412 **Figure 5.** Bacterial taxa associated with *Strongylocentrotus droebachiensis* larvae. (A)
413 Class-level profiles of the bacterial communities associated with *S. droebachiensis* larvae
414 from the Salish Sea, Gulf of Maine, and North Sea as well as the mean community across
415 populations and the shared taxa between each population. (B) Distribution of OTUs
416 associated with *S. droebachiensis* larvae uniquely found in each population, common
417 between two populations, and shared between all populations.
418

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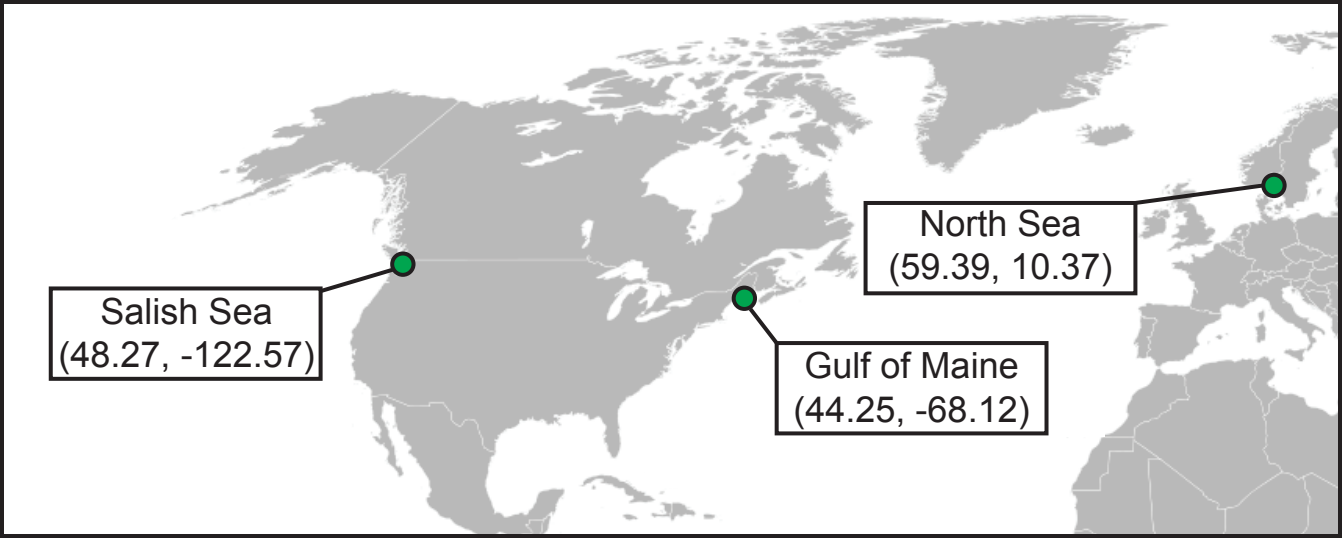
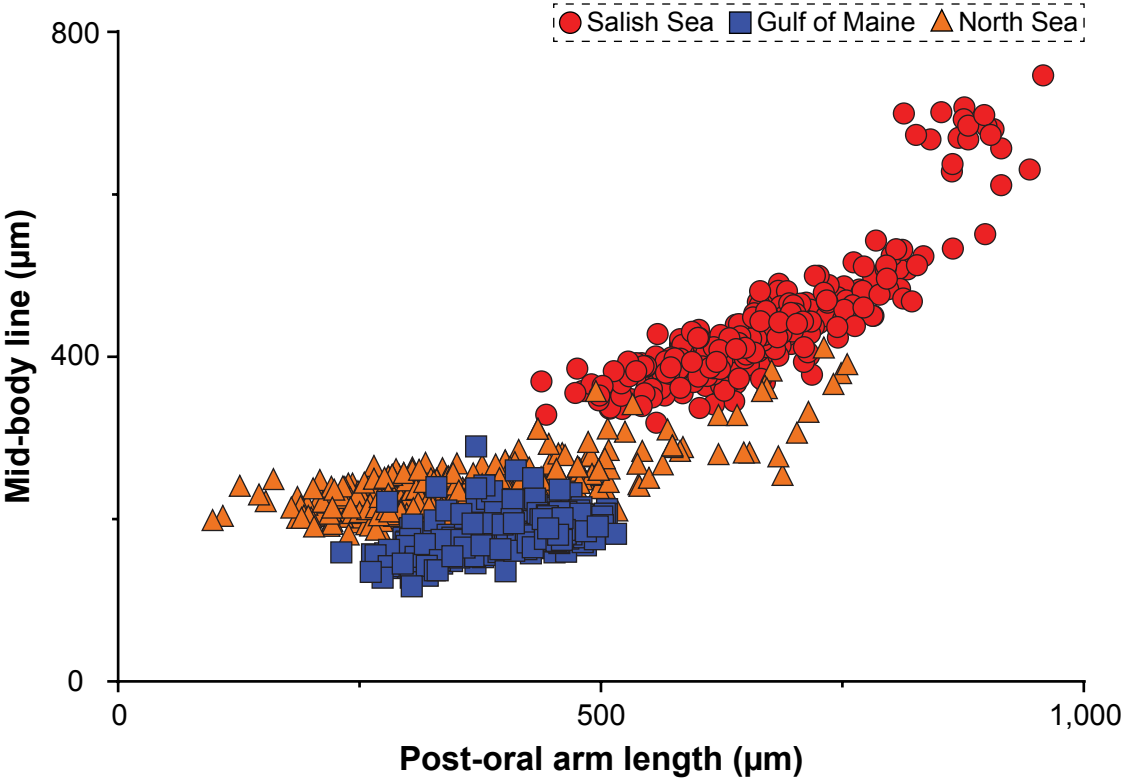
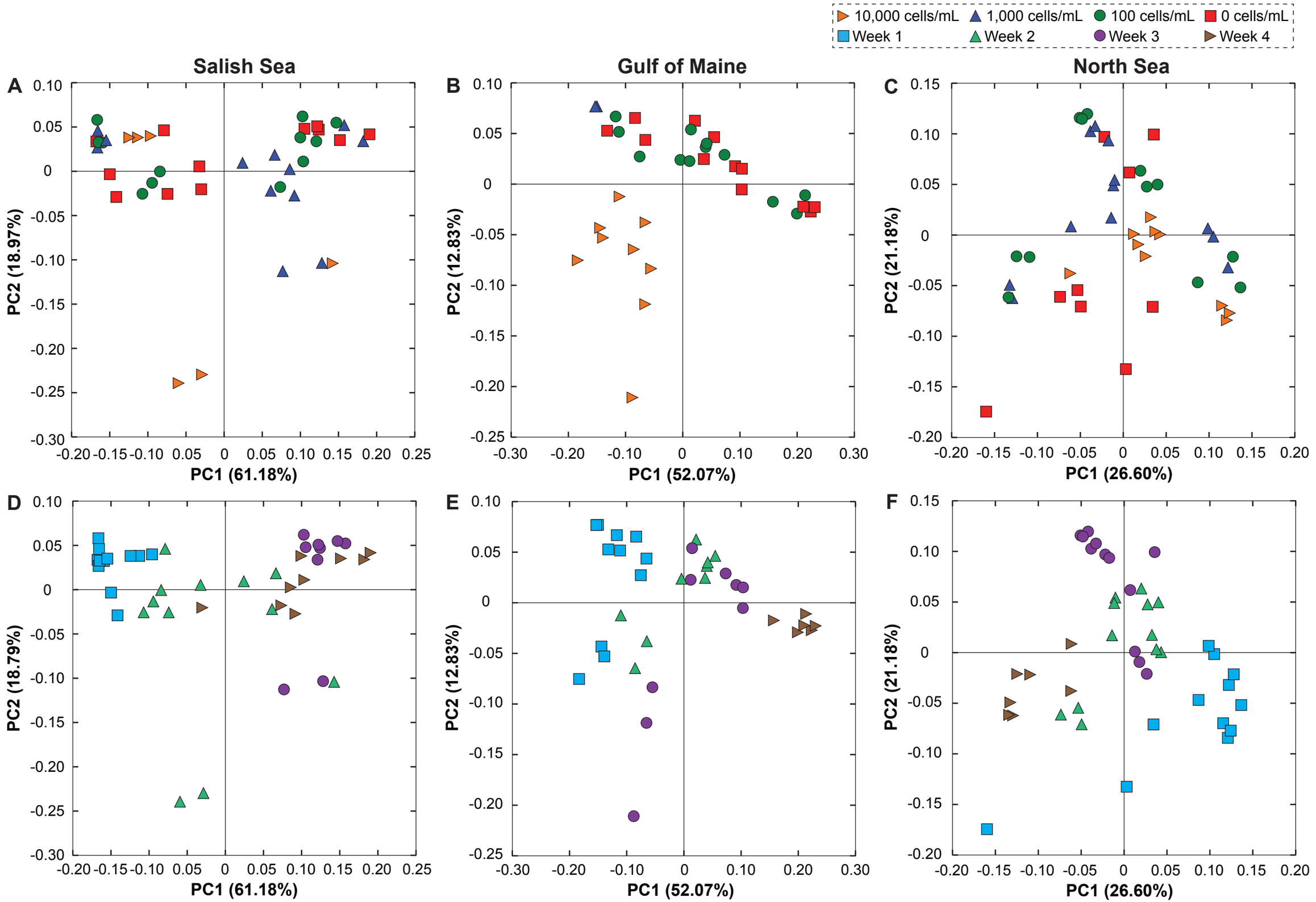
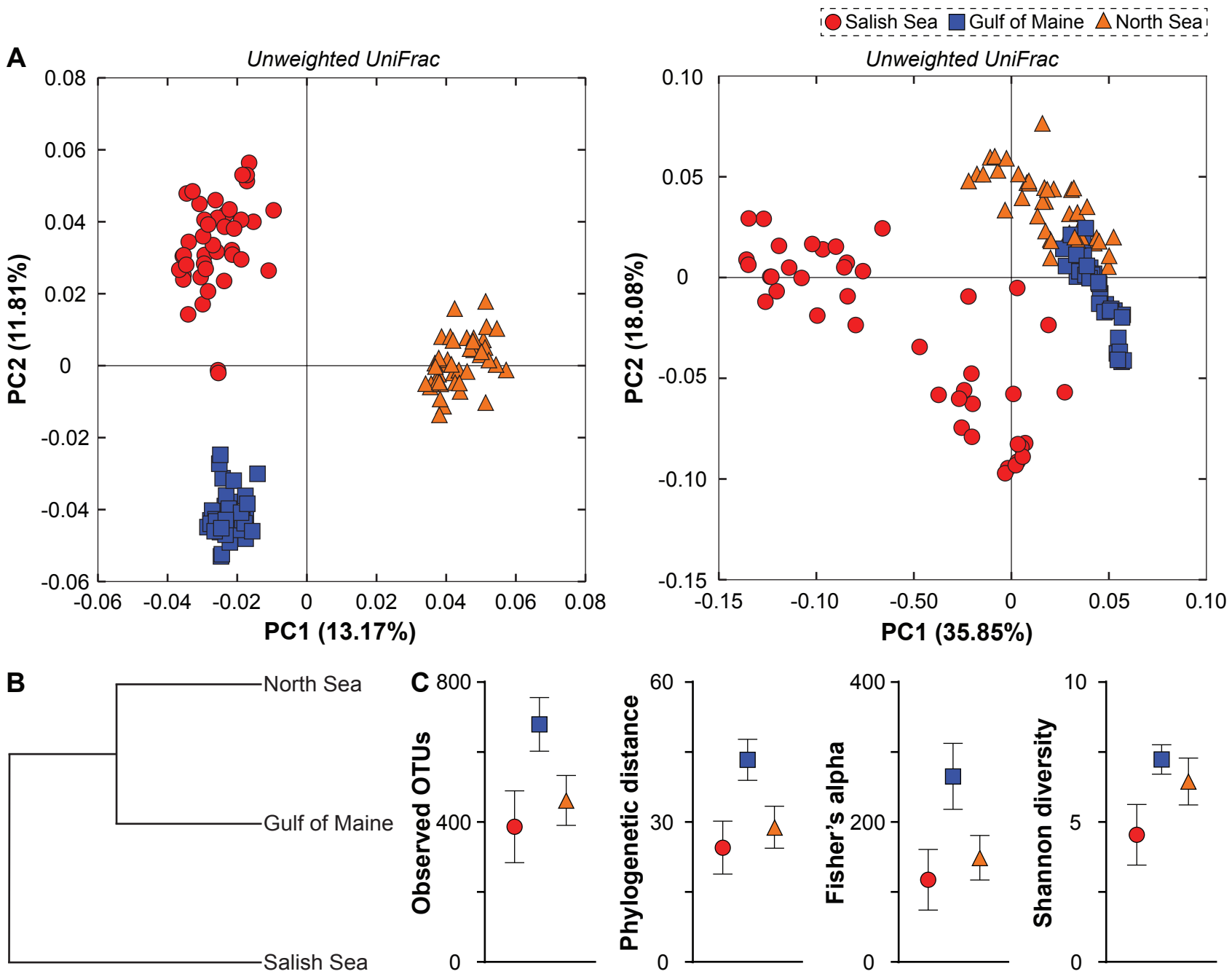
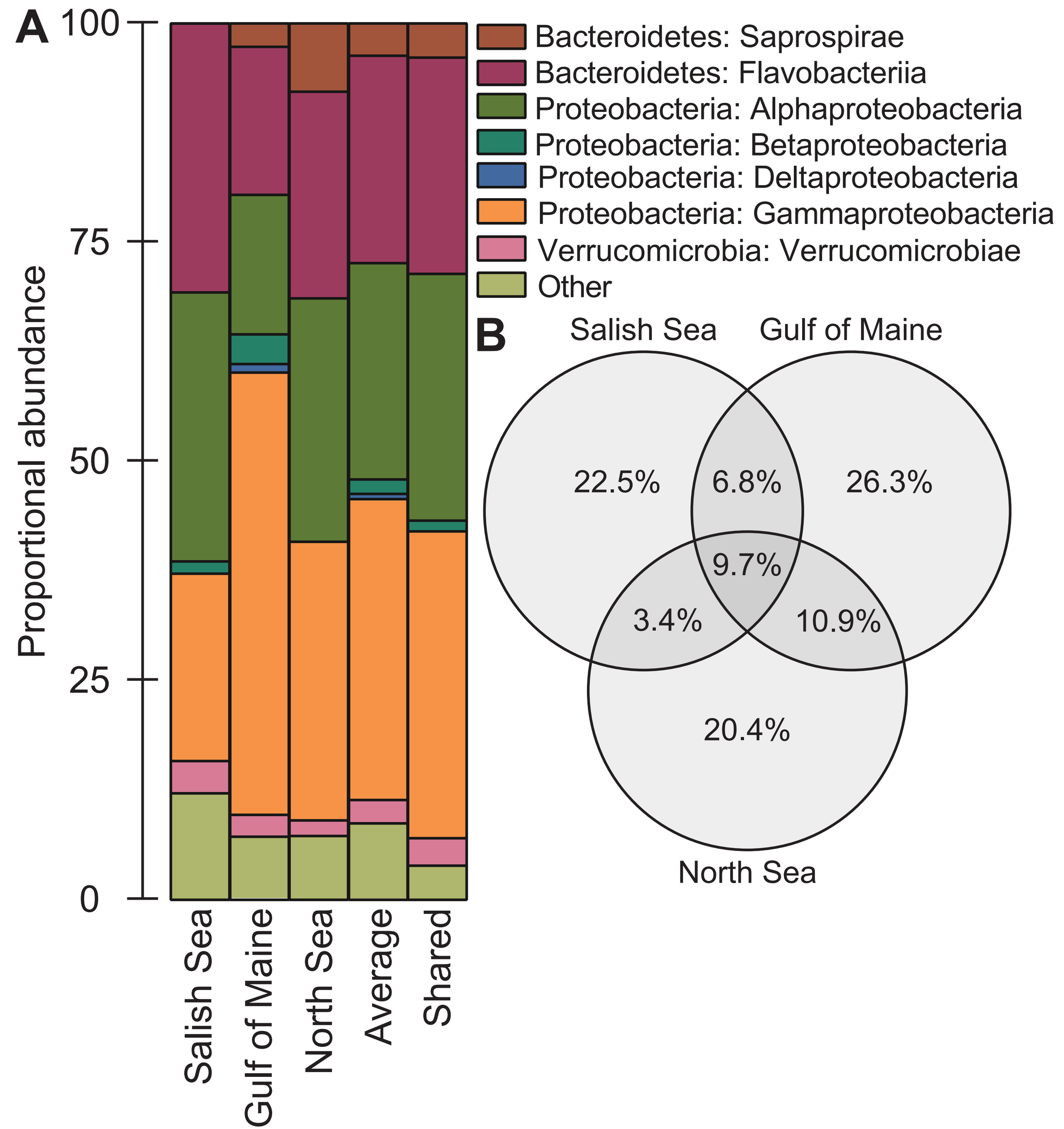

North Sea
(59.39, 10.37)
Salish Sea
(48.27, -122.57)
Gulf of Maine
(44.25, -68.12)


Salish Sea
Gulf of Maine
North Sea
800
400
0
Mid-body line (µm)
0
500
1,000
Post-oral arm length (µm)


10,000 cells/mL
1,000 cells/mL
100 cells/mL
0 cells/mL
Week 1
Week 2
Week 3
Week 4
Salish Sea
Gulf of Maine
North Sea
A
B
C
D
E
F
PC2 (18.97%)
PC1 (61.18%)
PC2 (12.83%)
PC1 (52.07%)
PC2 (21.18%)
PC1 (26.60%)
PC2 (18.79%)
PC1 (61.18%)
PC2 (12.83%)
PC1 (52.07%)
PC2 (21.18%)
PC1 (26.60%)


Salish Sea
Gulf of Maine
North Sea
A
Unweighted UniFrac
PC2 (11.81%)
PC1 (13.17%)
0.08
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
Unweighted UniFrac
PC2 (18.08%)
PC1 (35.85%)
0.10
0.05
0
-0.05
-0.10
-0.15
-0.50
B
North Sea
Gulf of Maine
Salish Sea
C
Observed OTUs
800
400
0
Phylogenetic distance
60
30
0
Fisher's alpha
400
200
0
Shannon diversity
10
5
0


A
100
75
50
25
0
Proportional abundance
Salish Sea
Gulf of Maine
North Sea
Average
Shared
Bacteroidetes: Saprospirae
Bacteroidetes: Flavobacteriia
Proteobacteria: Alphaproteobacteria
Proteobacteria: Betaproteobacteria
Proteobacteria: Deltaproteobacteria
Proteobacteria: Gammaproteobacteria
Verrucomicrobia: Verrucomicrobiae
Other
B
Salish Sea
Gulf of Maine
North Sea
22.5%
6.8%
26.3%
9.7%
3.4%
10.9%
20.4%

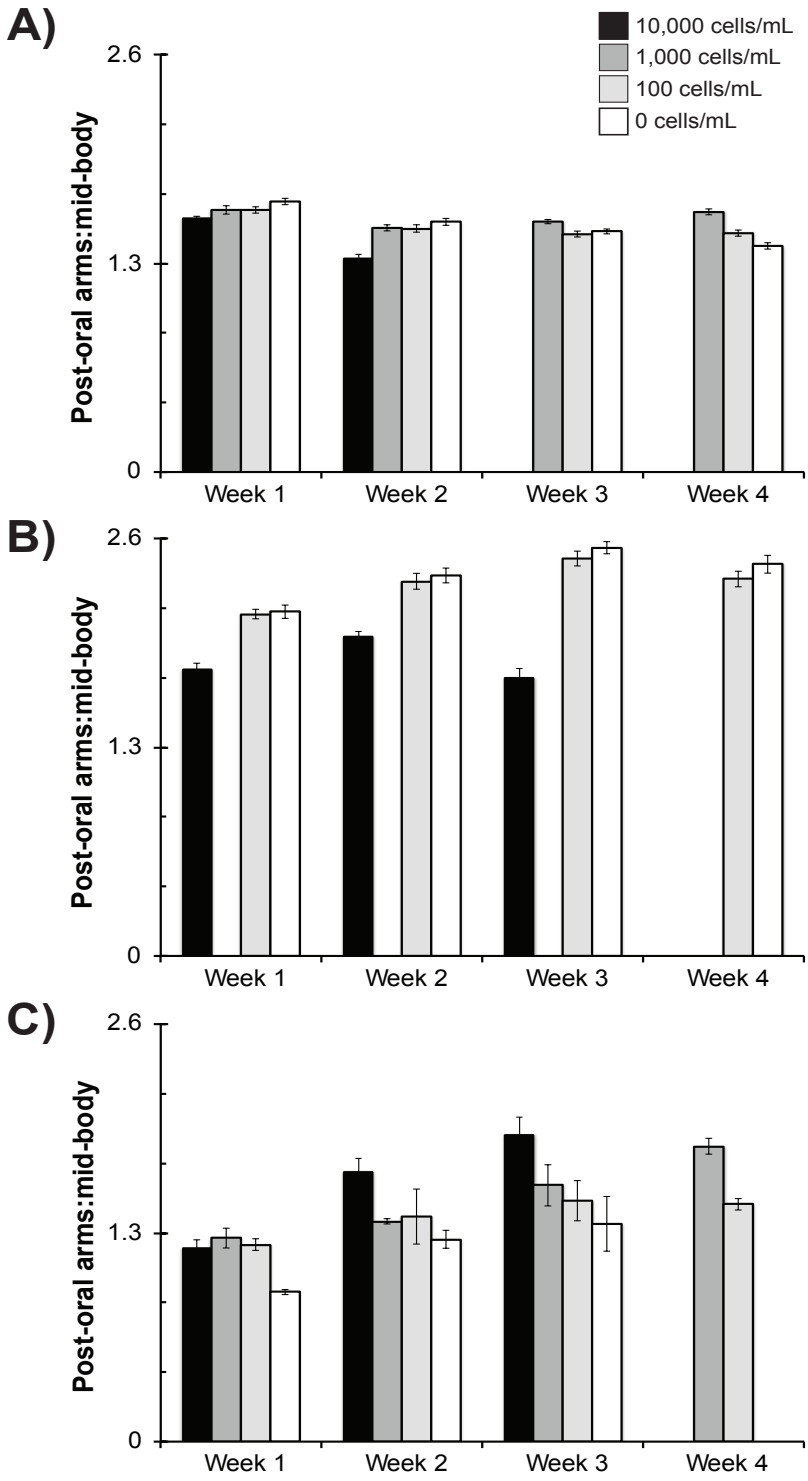


Figure S1. *Strongylocentrotus droebachiensis* larvae alter phenotype to feeding environment. Ratio between the post-oral arm and mid body line (mean ± standard error; n=20) for *S. droebachiensis* from the Salish Sea (A), Gulf of Maine (B), and North Sea (C) larvae having been fed either 10,000 (black), 1,000 (dark grey), 100 (grey), and 0 cells•mL^{-1} (white).

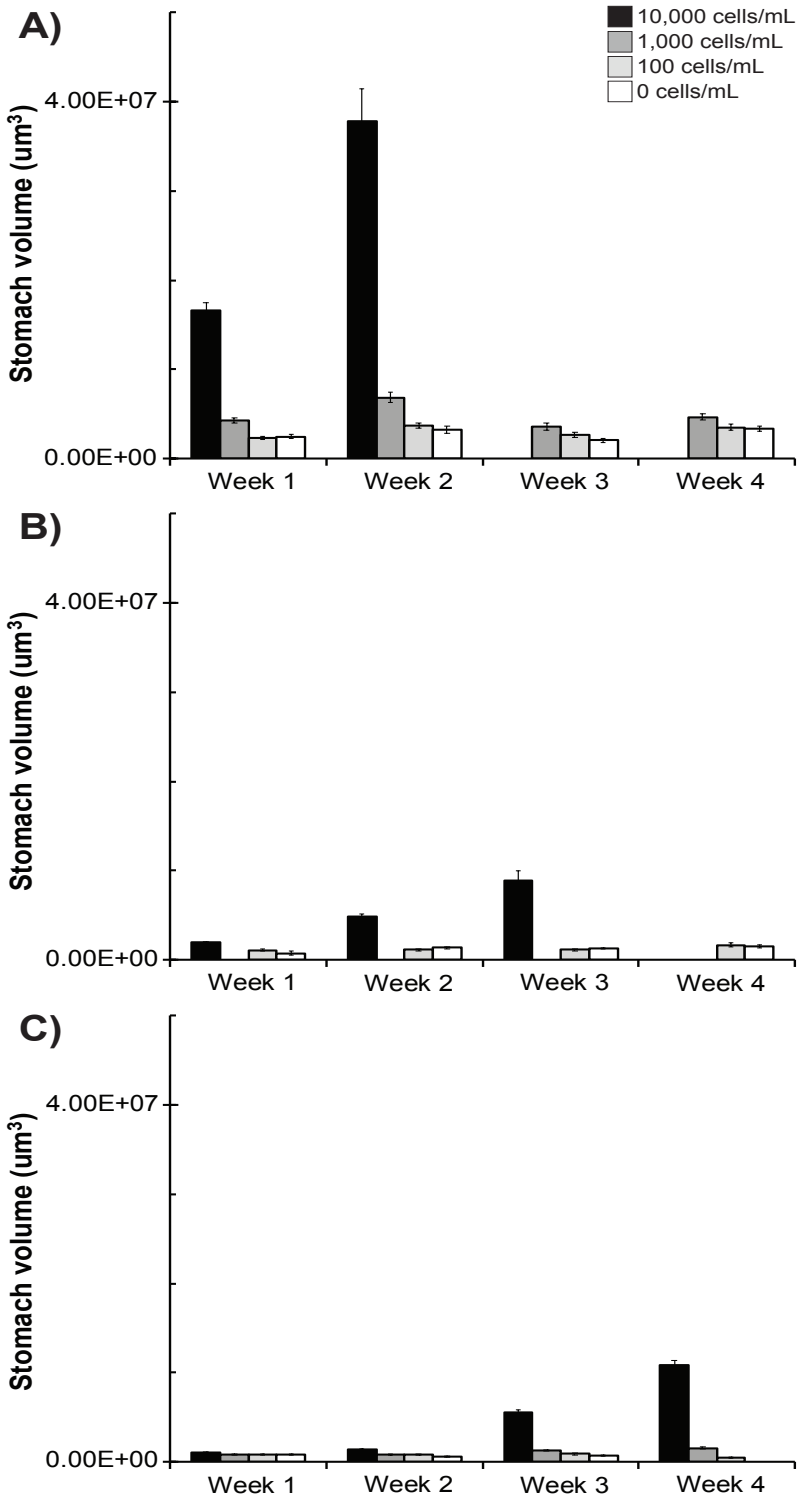


Figure S2. *Strongylocentrotus droebachiensis* larvae alter gut volume to feeding environment. Gut volume (mean ± standard error; n=20) for larval *S. droebachiensis* from the Salish Sea (A), Gulf of Maine (B), and North Sea (C) having been fed either 10,000 (black), 1,000 (dark grey), 100 (grey), and 0 cells•mL^{-1} (white).

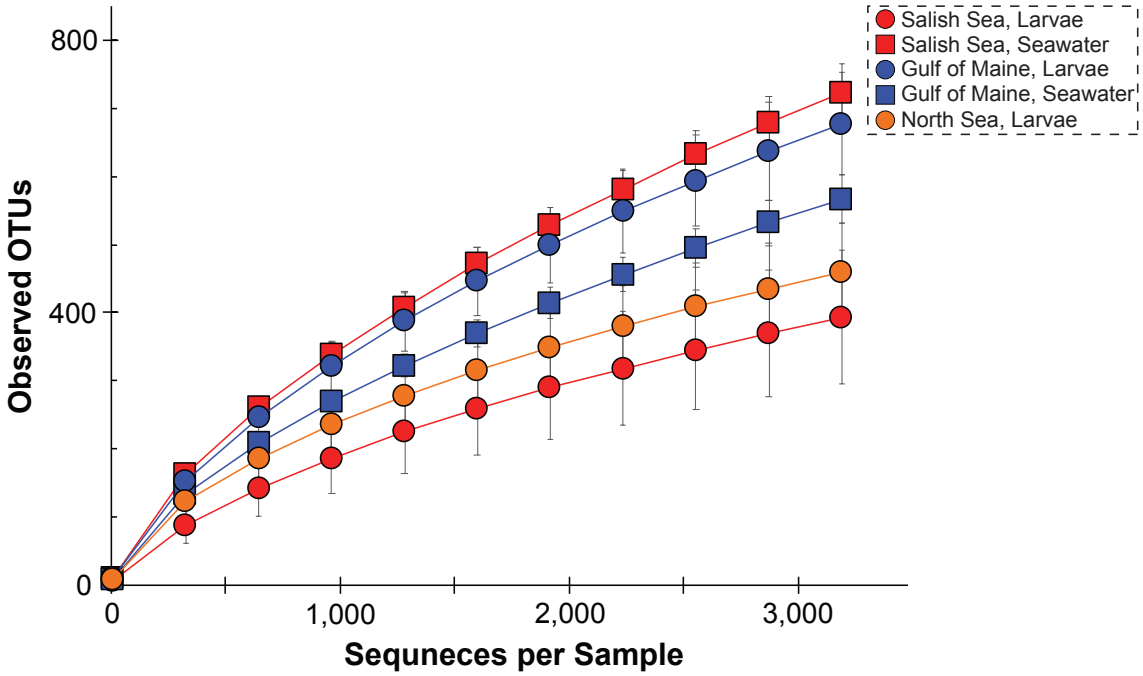


Figure S3. Alpha rarefaction curves for *Strongylocentrotus droebachiensis* larvae from three geographic locations and of the seawater. Alpha rarefaction curves for the associated microbiota for *S. droebachiensis* larvae (circles) from the Salish Sea (blue), Gulf of Maine (red), and North Sea (yellow) and seawater (squares) based on the rarefaction depth (3,193 sequences) used for all analyses.

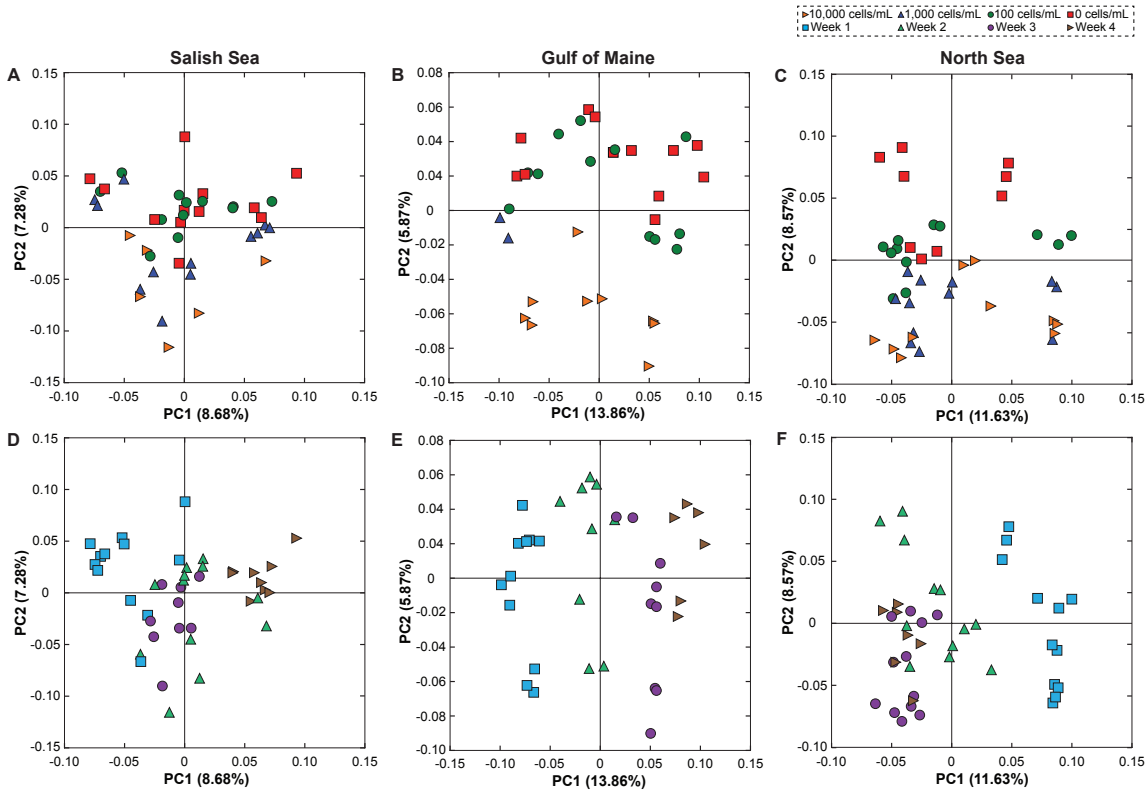


Figure S4. Dietary and temporal shifts in the bacterial communities associated with *Strongylocentrotus droebachiensis* larvae. Community similarity based on *S. droebachiensis* larval-associated bacterial communities based on food availability (A-C) over a multi-week exposure (D-F) for populations from the Salish Sea (A, D), Gulf of Maine (B, E), and North Sea (C, F). Comparisons between food availability (A-C) and over time (D-F) are based on unweighted UniFrac comparisons. (Note: this is the unweighted complement to Fig. 2.)

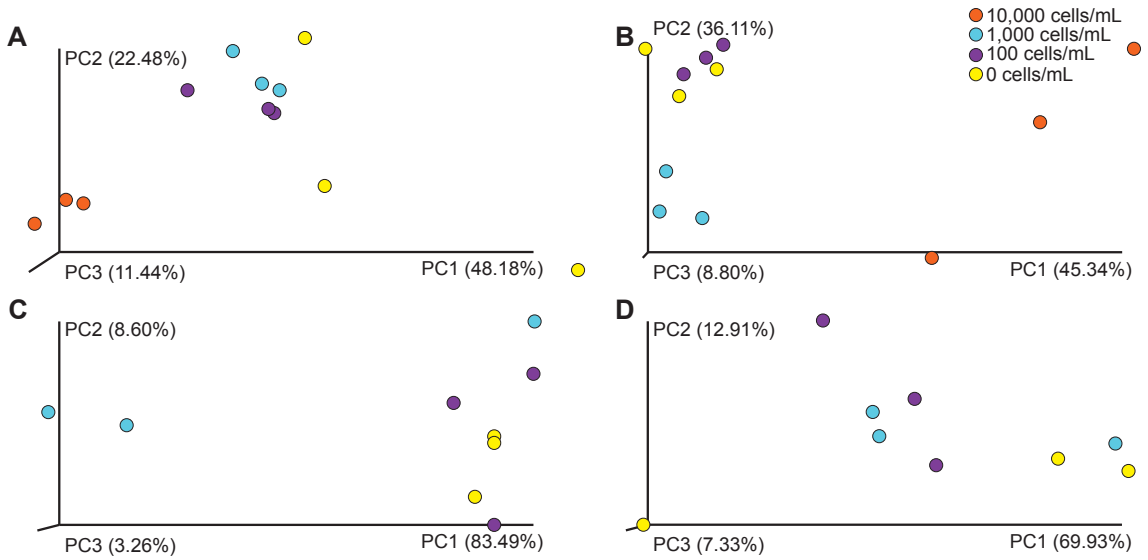


Figure S5. Community similarity of *Strongylocentrotus droebachiensis* larvae from the Salish Sea across time. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea for one (A; $p < 0.002$), two (B; $p < 0.001$), three (C; $p < 0.001$), or four (D; $p < 0.001$) weeks and having been fed 10,000 (orange), 1,000 (blue), 100 (purple), or 0 (yellow) cells•mL^{-1}.

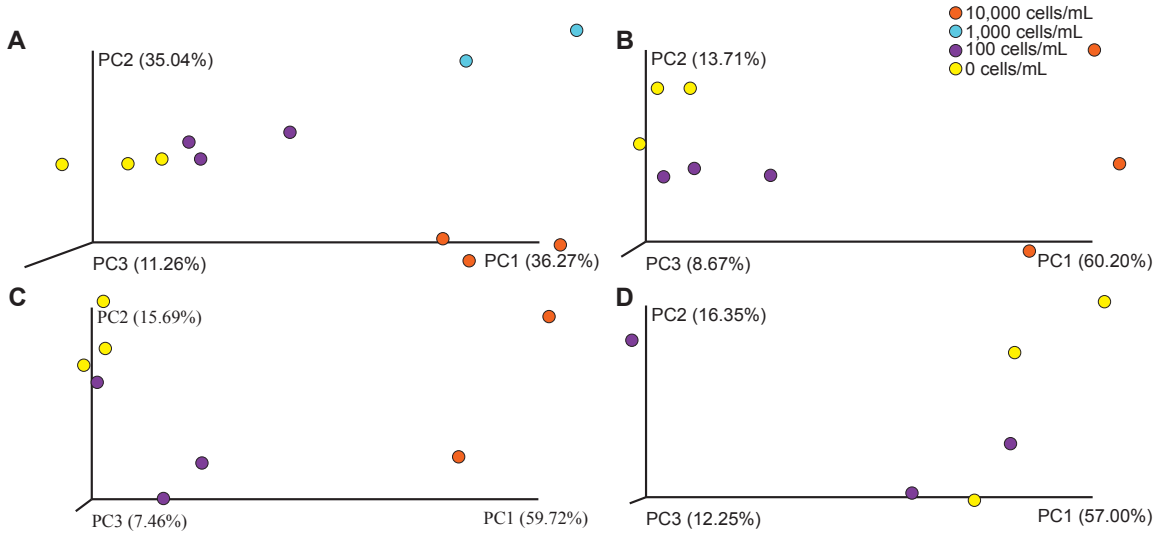


Figure S6. Community similarity of *Strongylocentrotus droebachiensis* larvae from the Gulf of Maine across time. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Gulf of Maine for one (A; $p < 0.001$), two (B; $p < 0.005$), three (C; $p < 0.007$), or four (D; $p = 0.430$) weeks and having been fed 10,000 (orange), 1,000 (blue), 100 (purple), or 0 (yellow) cells•mL^{-1}.

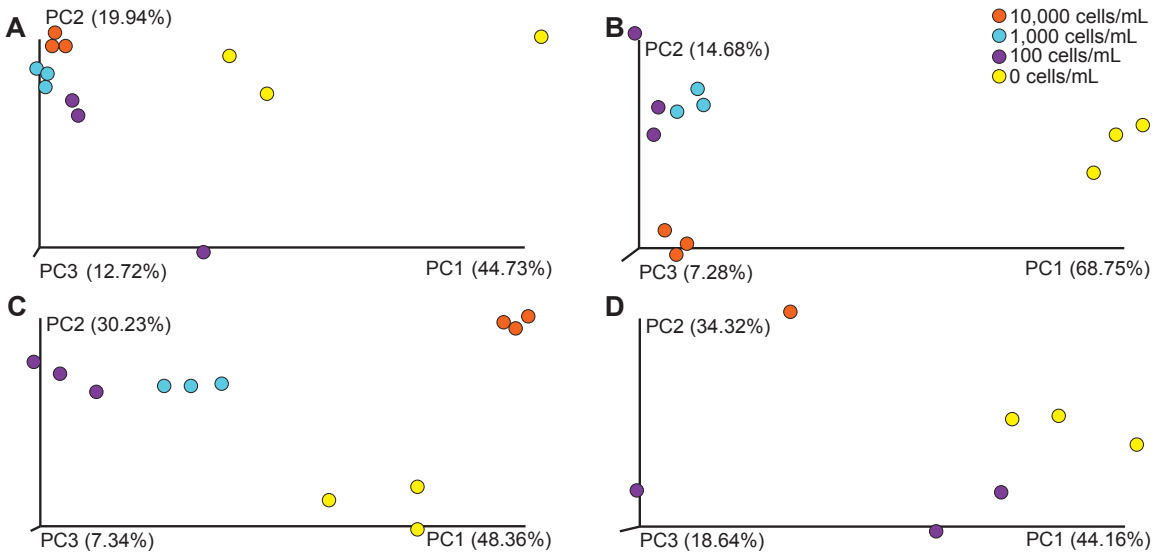


Figure S7. Community similarity of *Strongylocentrotus droebachiensis* larvae from the North Sea across time. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the North Sea for one (A; $p < 0.002$), two (B; $p < 0.001$), three (C; $p < 0.001$), or four (D; $p < 0.020$) weeks and having been fed 10,000 (orange), 1,000 (blue), 100 (purple), or 0 (yellow) cells•mL^{-1}.

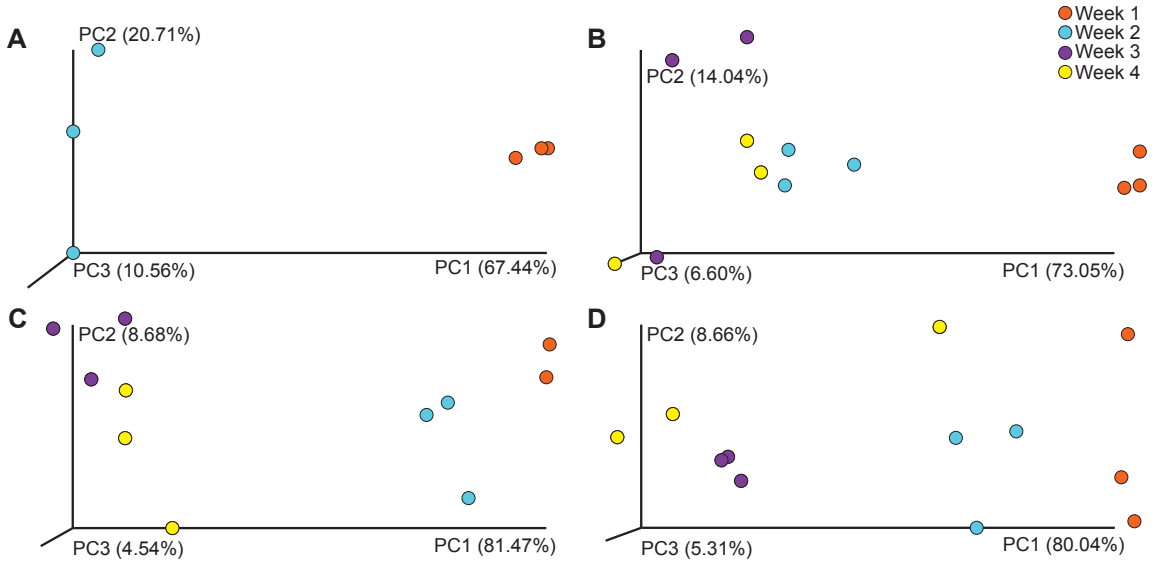


Figure S8. Community similarity of *Strongylocentrotus droebachiensis* larvae from the Salish Sea across diets. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea having been fed 10,000 (A; $p < 0.001$), 1,000 (B; $p < 0.001$), 100 (C; $p < 0.001$), or 0 (D; $p < 0.001$) cells•mL^{-1} for one (orange), two (blue), three (purple), or four (yellow) weeks.

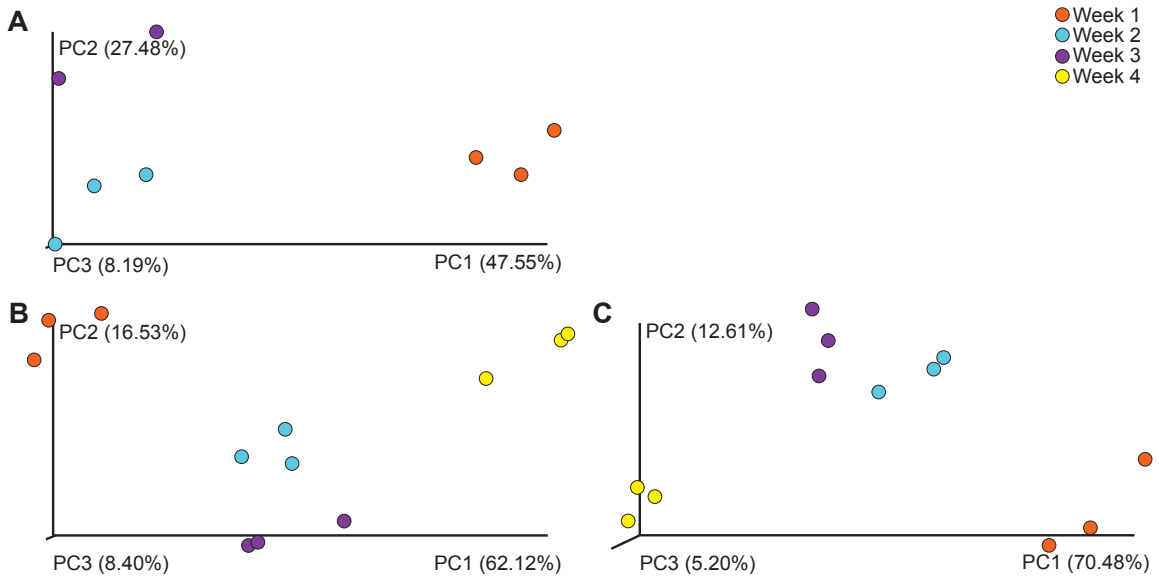


Figure S9. Community similarity of *Strongylocentrotus droebachiensis* larvae from the Gulf of Maine across diets. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Gulf of Maine having been fed 10,000 (A; $p < 0.005$), 100 (B; $p < 0.001$), or 0 (C; $p < 0.002$) cells•mL^{-1} for one (orange), two (blue), three (purple), or four (yellow) weeks.

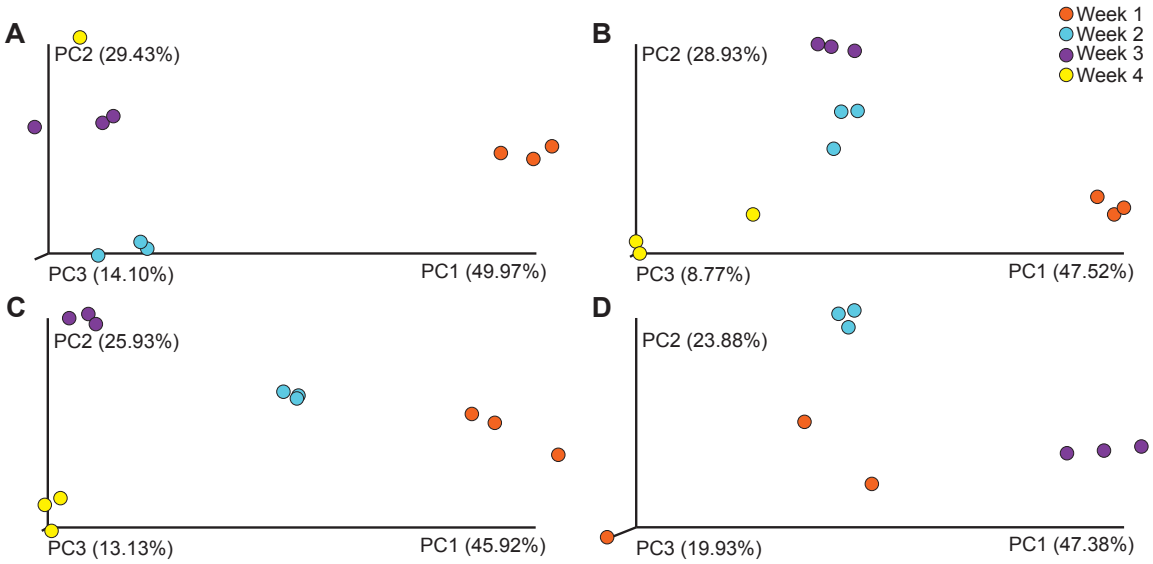


Figure S10. Community similarity of *Strongylocentrotus droebachiensis* larvae from the North Sea across diets. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the North Sea having been fed 10,000 (A; $p < 0.002$), 1,000 (B; $p < 0.002$), 100 (C; $p < 0.002$), or 0 (D; $p < 0.006$) cells•mL^{-1} for one (orange), two (blue), three (purple), or four (yellow) weeks.

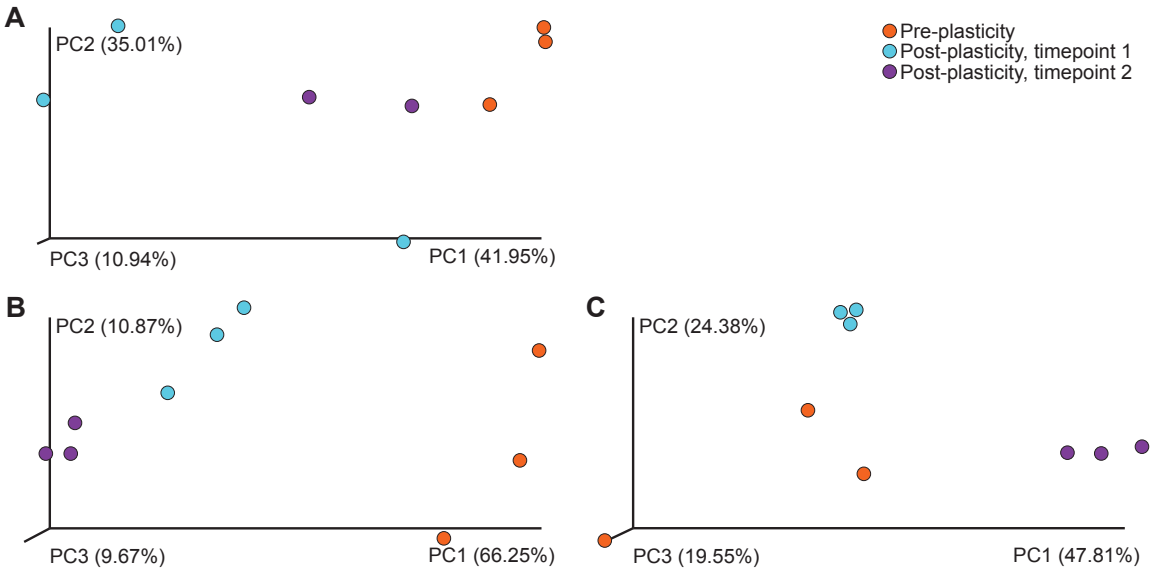


Figure S11. Community similarity of the associated microbial community along a phenotypic continuum for *Strongylocentrotus droebachiensis* from three geographic locations. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea (A; $p = 0.046$), Gulf of Maine (B; $p < 0.005$), and North Sea (C; $p < 0.004$) pre (orange) and post (blue and purple) expression of plasticity.

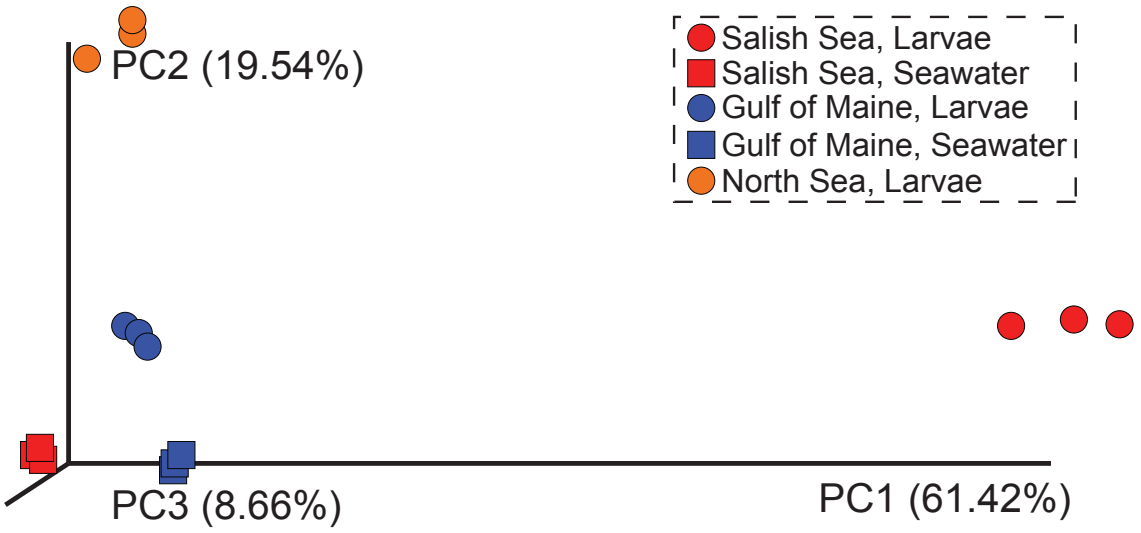


Figure S12. Similarity of the associated microbial community for *Strongylocentrotus droebachiensis* larvae from three geographic locations. Community similarity ($p < 0.001$) of the associated microbiota between *S. droebachiensis* larvae (circles) from the Salish Sea (blue), Gulf of Maine (red), and North Sea (yellow) and seawater (squares).

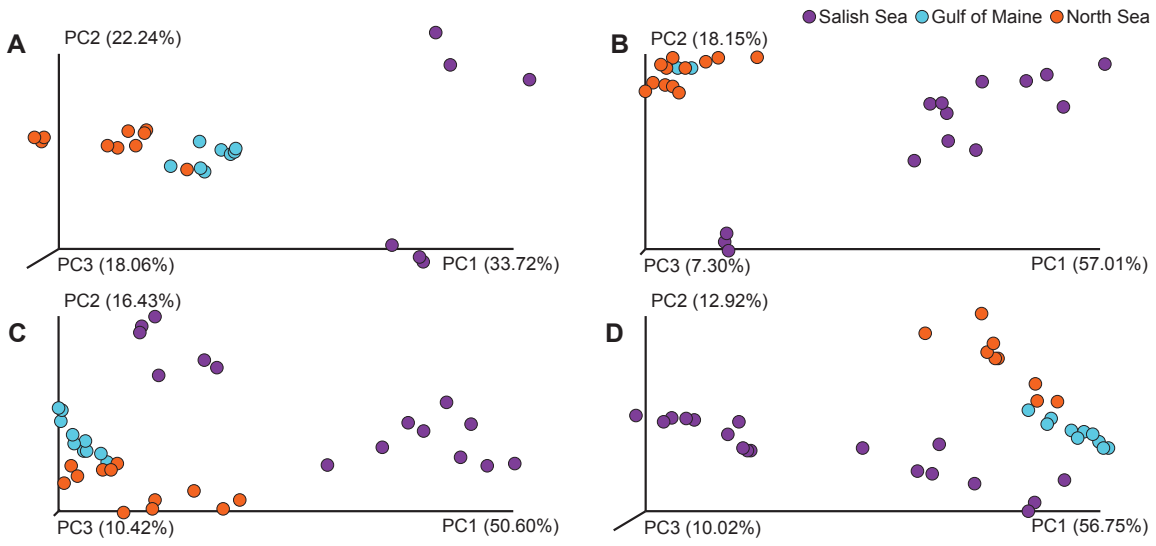


Figure S13. Community similarity of *Strongylocentrotus droebachiensis* larvae across diets. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea (purple), Gulf of Maine (blue), or North Sea (orange) and having been fed 10,000 (A; $p < 0.001$), 1,000 (B; $p < 0.001$), 100 (C; $p < 0.001$), and 0 (D; $p < 0.001$) cells•mL^{-1}.

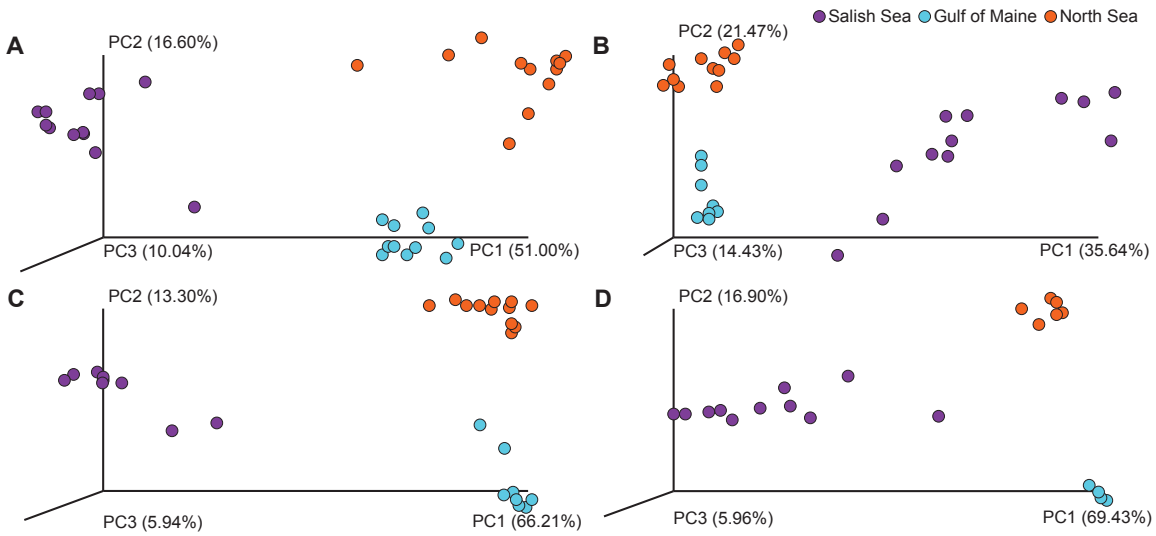


Figure S14. Community similarity of *Strongylocentrotus droebachiensis* larvae across time. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea (purple), Gulf of Maine (blue), or North Sea (orange) and having been differentially fed for one (A; $p < 0.001$), two (B; $p < 0.001$), three (C; $p < 0.001$), or four (D; $p < 0.001$) weeks.

Note S1

QIIME analysis

Starting with raw read files from MiSeq:

1) Pair forward and reverse files using PEAR (pear-0.9.10-bin-64)
2) Trim paired files using Trimmomatic (trimmomatic-0.36.jar)
3) Convert paired and trimmed .fastq to .fasta using the custom code: cat [input .fastq] | paste – - - - | cut -f 1,2 | sed 's/^/>/'| tr "\t" "\n" >[output .fasta]
4) Validate tab-delimited mapping file using "validate_mapping_file.py"
5) Generate meta-.fasta files using "add_qiime_labels.py"
6) Detect chimeras from meta-.fasta (called, "combined_seqs.fna")
7) Filter chimeras using "filter_fasta.py"
8) Pick OTUs using "pick_open_reference_otus.py"
9) Filter OTUs with >10 reads using "filter_otus_from_otu_table.py"
10) Filter 'o__Cryptophyta' using "filter_taxa_from_otu_table.py"
11) Determine rarefaction depth using "biom summarize-table"
12) Filtered .biom table was rarified using "single_rarefaction.py"
13) Filtered filtered .biom table was split using "split_otu_table.py" to test specific hypotheses
14) Alpha diversity was compared using "alpha_diversity.py"
15) Beta diversity was compared using "jackknifed_beta_diversity.py" and 2D PCoA plots of unweighted and weighted UniFrac values were generated using "make_2d_plots.py"
16) Statistical comparisons of unweighted and weighted UniFrac were performed with "compare_categories.py" using the '--method' "anosim," "adonis," and "permanova."
17) Taxonomic plots were generated using "summarize_taxa_through_plots.py"
18) Unique and shared OTUs were determined both using "compute_core_microbiome.py"and "shared_phylotypes.py"