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 the sea urchin *Strongylocentrotus droebachiensis* from the Atlantic and Pacific Oceans and the ability of their larvae to acclimate to heterogeneous feeding environments, we test the hypothesis that inter-population differences in the composition of animal-associated bacterial communities are more pronounced than intra-population variation. 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 populations in the Atlantic and Pacific Oceans, 20-30% of bacterial OTUs were specific to a single location while less than 10% were shared, and more than half of this community is predicted to contribute to metabolism of the larval holobiont. 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.

Importance

A central question in animal-microbiome research that remains largely unanswered concerns what factors govern taxonomic composition of symbiotic communities and what drives these differences. Efforts to answer this question primarily focus on variation within a single population or differences between geographically distant populations. Using sea urchin larvae, we compare whether variation in bacterial communities is larger between locations than within when larvae are cultured at different food concentrations. While each population of sea urchin larvae associated with specific bacterial communities when cultured at different food concentration, these differences were relatively minor compared to variation based on geography. Our comparisons suggest that variation between populations d is more pronounced than variation within populations, and that larger scale ecological components may mask factors at smaller scales. Future experiments explicitly testing for the potential of host genotype using common garden experiments and functional manipulations will help determine mechanisms driving this variation.

60 **Introduction**

61 Acclimating to environmental variability through morphological, developmental,
62 and/or physiological plasticity is a common trait of animals (1-7). Over the past decade,
63 the appreciation for the role that animal-associated microbiota play in responding to
64 environmental stressors has grown profoundly (8-14). When experiencing heterogeneous
65 environments, an animal may recruit, expel, and/or shuffle the relative proportion of
66 associated microbiota (15, 16) to assemble a community with particular molecular
67 pathways for the environmental conditions (17-19).

68 Microbial communities associated with animals often vary in response to a multitude
69 of abiotic and biotic factors, including temperature, salinity, diet quality and quantity,
70 season, and habitat-type (see reviews by 8, 12). Of these, dietary responses are best studied
71 and shown to have a major impact on the composition and functions of this community
72 (20-23). When faced with prolonged diet restriction, the composition and diversity of
73 microbiota associated with both invertebrate and vertebrate hosts shift considerably (13,
74 24), a response hypothesized to buffer against reduced exogenous nutrients.

75 Microbial communities associated with animals are also species-specific (*e.g.*, 13, 25,
76 26) and taxonomically variable across the geographical distribution of the host (27-31).
77 Population-specific microbial communities are partially dependent on environmental
78 conditions (32) and dispersal limitations (33). Despite this taxonomic variation, microbial
79 communities can remain functionally similar due to shared genes across bacterial taxa (18,
80 34). The microbial communities of the bromeliad *Aechmea nudicaulis*, for example, are
81 taxonomically dynamic but functionally consistent between individuals (19). Similarly, the
82 bacterial community associated with the green alga *Ulva* spp. are too spatially and

83 temporally variable to define a 'core' community; however, nearly 70% of the microbial
84 genes are conserved across this broad geography (18).

85 Efforts to classify the principles underlying the assembly of host-associated microbial
86 communities have primarily been within (*e.g.*, 13, 20, 35-38) or between (*e.g.*, 27-31, 39)
87 populations (but see 40). This conceptual discontinuity highlights a gap in understanding
88 the patterns and principles underlying community assembly. To test for differences in
89 assembly structure, direct comparisons of animal-associated microbial communities within
90 and between populations are needed to determine the relative strength of these factors. The
91 fields of sensory, behavioral, and circadian biology have shown that organismal responses
92 to ecological variables are unequal and may be masked based on spatial significance (41-
93 45). Factors within and between populations, therefore, may have different proportional
94 impacts on the composition of host-associated microbiota.

95 Planktotrophic (feeding) larvae are one biological system to compare ecological
96 components and how they influence the composition of animal-associated microbiota.
97 Many planktotrophic larvae (*e.g.*, the pluteus of sea urchins) inhabit heterogeneous
98 nutritional environments and are morphologically and physiologically plastic to food
99 availability (2, 46-53). Larvae of the echinoid *Strongylocentrotus droebachiensis*, for
100 example, associate with phenotype-, diet-, and developmental stage-specific bacterial
101 communities (13, 54). *S. droebachiensis*, in particular, has an Arctic-boreal distribution
102 composed of a multiple ocean population with limited gene flow (55-57) and signs of
103 potential local adaption (58, 59).

104 Using the circumpolar distribution of *S. droebachiensis* and the ability of their larvae
105 to acclimate to heterogeneous feeding environments, we test the hypothesis that inter-
106 population differences in the composition of animal-associated bacterial communities are

more pronounced than intra-population variation. To test this, we performed differential feeding experiments on *S. droebachiensis* larvae from populations in the Pacific and Atlantic Oceans (Fig. 1) and used amplicon sequencing to profile the larval-associated bacterial communities. We then used these sequences to predict the functional gene profiles of the larval-associated bacterial communities to preliminarily test for similar community function between urchin populations.

Results

Morphological plasticity of larval sea urchin

Diet-induced morphological plasticity was observed for *S. droebachiensis* larvae from each population, with the pattern of expression being location-specific (ANOVA, $p<0.0001$; Figs. S2-3). Diet-restricted larvae from the Salish Sea and Gulf of Maine, in general, exhibited a higher post-oral arm to mid-body line ratio than *ad libitum* counterparts (ANOVA, $p<0.0001$; Figs. S1-2; Tables S3-4), even though analyses of Gulf of Maine larvae were partially confounded by developmental stage (Table S1). Larvae from the North Sea, however, exhibited the opposite response: *ad libitum* feeding induced a higher post-oral arm to mid-body line ratio than diet-restricted individuals (ANOVA, $p<0.0001$; Fig. S1-2; Tables S3-4).

Structural comparisons of bacterial communities within populations

S. droebachiensis larvae from each population associated with a diet-specific bacterial community (Figs. 2A-C, S4-7; Table S6). Larvae from the Salish Sea and Gulf of Maine exhibited similar diet-specific community-level patterns in both alpha- and beta-diversity (Figs. 2A-B, S4-6; Table S6), where the bacterial consortium of food-restricted larvae were

131 more similar to each other than to well-fed counterparts. Larvae from the North Sea, on the
132 other hand, exhibited the opposite response (Figs. 2C, S4, S7; Table S6), where diet-
133 specific bacterial communities were observed (Table S6) except that all food
134 concentrations were more similar to each other than to starved larvae (Fig. S7; Table S6).

135 The bacterial communities associated with larvae from each sea urchin population also
136 varied temporally in both alpha- and beta-diversity (Figs. 2D-F, S4, S8-10; Table S6).
137 Temporal changes in community diversity was reflective of culturing environment: larval
138 populations from the Atlantic Ocean increased in weeks one through three and declined in
139 week four while the larval population from the Pacific Ocean decreased in the first three
140 weeks and increased in week four. Compositional changes in the associated bacterial
141 communities for each larval population, on the other hand, experienced similar time-
142 dependent shifts from week one though four (Figs. 2D-F, S4).

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

150

151 *Differences in the bacterial communities of urchin populations*

152 Despite differences in bacterial community structure within each *S. droebachiensis*
153 larval population, variation in OTU membership and composition correlated best with
154 geography (ANOSIM, unweighted UniFrac, $p<0.001$; ANOSIM, weighted UniFrac,

155 p<0.001; Figs. 3, S13-15; Table S8). Relatedness of these communities supports that the
156 larval-associated microbiota in the Western and Eastern Atlantic Ocean were more similar
157 to each other than to the Pacific Ocean population (Figs. 3, S13). Moreover, bacterial
158 community diversity and structure were significantly different between these three
159 populations (Fisher's alpha: F-ratio = 142.2, $p<0.001$; Shannon equitability: F-ratio =
160 110.1, $p<0.001$; Faith's phylogenetic distance: F-ratio = 159.2, $p<0.001$; observed OTUs:
161 F-ratio = 124.2, $p<0.001$; Table S8). For each of the tested indices, bacterial communities
162 of Gulf of Maine larvae were taxonomically richest and most diverse while Salish Sea
163 larvae were the least rich and diverse, leaving North Sea larvae as intermediate (Tukey's
164 test, $p<0.05$ for each; Table S8).

165 *S. droebachiensis* larvae from these populations primarily associated with seven
166 bacterial classes from three phyla: Saprospirae and Flavobacteriia (Bacteroidetes), Alpha-
167 , Beta-, Delta-, and Gammaproteobacteria (Proteobacteria), and Verrucomicrobiae
168 (Verrucomicrobia) (Fig. 4A). Of these classes, Flavobacteriia, Alpha- and
169 Gammaproteobacteria represented at least 10% of the relative community for each
170 population, where Gammaproteobacteria was the most abundant clade at 20-50% (Fig.
171 4A). Of the hundreds of OTUs associated with *S. droebachiensis* larvae (Fig. S1), less than
172 10% were shared across populations, with 20-30% being specific to a single population
173 and 3-11% being shared between two populations (Fig. 4B). This shared community was
174 similar to the full communities for each population, in that it was composed primarily by
175 Flavobacteriia (24.7%), Alpha- (28.1%) and Gammaproteobacteria (34.9%) (Fig. 4A).
176

177 *Predicted community function and representative taxa*

178 Similar to the 16S rRNA sequence diversity, PICRUSt-generated (predicted)
179 metagenomic gene profiles for *S. droebachiensis* larvae were specific to urchin population
180 (ANOVA, $p<0.0001$; Fig. 5A). Predicted gene contents of PICRUSt-generated
181 metagenomes were, on average, ~54.9% metabolism, ~17.5% genetic information
182 processing, ~13.0% environmental information processing, and ~7.3% cellular processes
183 and signaling (Figs. 5B, S16). Total gene content was significantly different between
184 locations for metabolism (ANOVA, $p<0.0001$), cellular processes and signaling (ANOVA,
185 $p<0.0001$), and organismal systems (ANOVA, $p<0.0001$) (Fig. S16).
186 More than half of the genes of the predicted *S. droebachiensis* larval metagenome
187 related to metabolism (Figs. 5B, S15). Gammaproteobacteria (42.6%),
188 Alphaproteobacteria (31.4%), and Flavobacteriia (13.3%) were the major taxa presumed
189 to have metabolic functionality (Fig. 5C). Moreover, Betaproteobacteria (2.9%),
190 Verrucomicrobiae (1.7%), Deltaproteobacteria (1.2%), and Planctomycetia (1.2%) were
191 also minor taxa in this metabolism-prediction subset of the community (Fig. 5C).
192
193 **Discussion**
194 Principles underlying the assembly and diversity of host-associated microbial
195 communities has remained a focal point of host-microbiome research over the last few
196 decades (9, 10, 12, 15, 16, 28, 30, 60-67). Efforts to partition and categorize variation in
197 community composition have suggested that evolutionary history (10, 16, 61, 64, 65),
198 physiology (8, 68, 69), and environmental conditions (8, 12, 66, 70) primarily drive these
199 differences. The majority of this work, however, focuses on two spatiotemporal scales:
200 within (13, 20, 35-38) or between (27-31) host populations (but see 40). The potential to

201 distinguish and discern intra- and inter-location processes highlights a conceptual gap in
202 understanding the patterns and principles underlying microbiome assembly.

203 Comparing the bacterial communities associated with *S. droebachiensis* larvae across
204 feeding environments and geographical location supports three primary findings. First,
205 larvae from each population associate with bacterial communities correlated with food
206 availability, time, development, and phenotype. Second, despite variation in the microbiota
207 within each population of urchin larvae, the composition of *S. droebachiensis* larval-
208 associated bacterial communities best correlated with geography. Third, predicted
209 metagenomic profiles for *S. droebachiensis* larval-associated bacterial communities were
210 population-specific, with more than half the predicted gene pool relating to metabolism.

211 Larval marine invertebrates associate with a microbial community that is diverse and
212 dynamic yet specific to host species and distinct from the environmental microbiota (13,
213 35, 38, 54, 71). The bacterial community associated with larval echinoderms, in particular,
214 shifts with food availability (13, 35), embryonic and larval development (13, 54),
215 phenotype (13), disease (71), and physiology (68). Common practice for investigating
216 larval-microbe interactions involve single location studies of a widely distributed taxa (see
217 13, 35, 38, 54). The data presented here suggest that community-level shifts in response to
218 biotic (development and phenotype) and abiotic (food availability and time) factors occur
219 at multiple locations across a population with limited gene flow. Three widely-distributed
220 sea urchin species (including *S. droebachiensis*) have previously been shown to associate
221 with food-elicited phenotype-specific bacterial communities (13). It may, therefore, be
222 hypothesized that phenotype-specific microbiota for these and, potentially, other species
223 of larval echinoderms (*e.g.*, *Acanthaster* sp. in the Pacific Ocean, including on the Great
224 Barrier Reef; 35, 72-74) occur throughout the geographical distribution of the species.

225 Marine invertebrate larvae are not unique in their ability to respond to environmental
226 variability by exhibiting shifts in the associated bacterial community (8, 12). Several clades
227 of marine and terrestrial vertebrates (*e.g.*, mammals, reptiles, and fish) respond to diet
228 variability and abiotic stress by restructuring their gut microbiota that, in turn, are likely to
229 differ in community function (8, 20, 21, 69, 75-77). Similar to larval marine invertebrates,
230 marine and terrestrial vertebrates can be widely-distributed and, thus, multiple genetically
231 distinct populations may exhibit similar community-levels shifts in microbiota at multiple
232 locations across these populations. To our knowledge, however, parallel responses between
233 populations for vertebrates and other taxa have not yet been documented.

234 Inter-populational variation in the composition of *S. droebachiensis* larval-associated
235 bacterial communities was more pronounced than intra-populational differences. While the
236 mechanisms driving this result is unknown, genetic and ecological differences between *S.*
237 *droebachiensis* populations are likely contributing factors (32, 33). *S. droebachiensis* from
238 the Salish Sea, Gulf of Maine, and North Sea diverged following larval-mediated restricted
239 gene flow (55, 56, 78, 79). Moreover, each urchin population inhabit environments that
240 differ in physical (80-82) and biological (83-85) factors. Inter-populational variation in the
241 bacterial communities associated with *S. droebachiensis* larvae may, therefore, have been
242 due to differences in host genetics and the oceanographic environment, a result that
243 requires future explicit testing using common garden experiments and molecular
244 manipulations (86).

245 Population-specific bacterial communities for *S. droebachiensis* larvae is consistent
246 with the hypothesis that host-associated microbial communities are taxonomically variable
247 across host geography (27-31). Studies using diverse animal taxa (*e.g.*, sponges, corals,
248 fish, and humans) support that the environment, not genotype, principally drives

249 community differences (87-90). Genetics of *S. droebachiensis* indicates that individuals
250 from the Salish Sea and Gulf of Maine sub-populations are more related to each other than
251 to the North Sea sub-population (55-57). Microbial communities from these populations,
252 however, suggest the contrary: larvae from the Gulf of Maine and North Sea are more
253 similar to each other than to Salish Sea larvae. This reinforces that environment (or, here,
254 geography) is a larger influence on community assembly than genetic similarity. The
255 mechanistic linkage between host genetics, the environment, and microbial community is
256 unknown, but based on the predicted larval metagenomics, we suspect it is related to the
257 metabolic activity of and between the larval host and bacterial symbionts.

258 Metagenomes predicted from 16S rRNA profiles for *S. droebachiensis* larvae also
259 suggest population specificity. From these data, what was perhaps most notable is that the
260 predicted metagenomes primarily include genes relating to metabolism. Planktotrophic
261 (feeding) larvae, such as the echinopluteus of *S. droebachiensis*, are traditionally thought
262 to acquire the energy for larval development by capturing exogenous particulates (*e.g.*,
263 phytoplankton and bacteria; 91). A growing body of research, however, has suggested that
264 planktotrophic larvae also utilize diverse 'alternative' nutritional resources, such as neutral
265 amino acids (92, 93), kelp detritus (94), and, perhaps, phototrophic symbionts (35, 95, 96).
266 Our predicted metagenomic gene profiles suggest the modes of nutrient acquisition for
267 larvae may relate to the bacterial symbiont community. This observation may be of
268 particular importance because planktotrophic larvae often inhabit food-limited
269 environments (97-99). To decrease mortality due to starvation and prolonged exposure to
270 pelagic predators (100-102), a metabolic input from the symbiont community may aid in
271 accelerating development and serve as a physiological buffer that complements metabolic
272 depression induced by diet-restriction (48).

273 Metabolic activity by animal-associated microbial communities benefiting the host is
274 widespread. The growth and reproduction of aphids, for example, is reliant on the amino
275 acids provisioned by the obligate endosymbiont *Buchnera* (103) while autotrophic and
276 heterotrophic bacteria drive carbon metabolism in sponges from oligotrophic seas (26).
277 Several bacterial phyla associated with the woodrat *Neotoma* gut (21, 104) and *Daphnia*
278 (105), on the other hand, are responsible for toxin degradation, allowing the host to access
279 and subsequently absorb nutrients from otherwise inaccessible diet. While a microbe-based
280 metabolism aiding in the digestion of diverse diets is not supported functionally for marine
281 invertebrate larvae, the wealth of data from a diversity of animal system and our 16S rRNA-
282 derived metagenomes merit testing.

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

291

292 **Experimental Procedures**

293 *Adult urchin collection and larval rearing*

294 Adult *S. droebachiensis* were collected from populations in the North Sea in March
295 2015, the Salish Sea in April 2016, and the Gulf of Maine in February 2017 (Fig. 1).
296 Individuals from the North Sea were collected by divers in Drøbak, Norway (59°39' N,

297 10°37’ E) and transported in cold, aerated seawater to the Sven Lovén Centre for Marine
298 Infrastructure (Kristineberg, Sweden). Urchins were maintained in natural seawater and
299 fed *ad libitum* on a live mix of *Ulva lactuca* and *Laminaria* spp. collected from the
300 Kristineberg shoreline. Urchins from the Salish Sea were hand-collected at low tide at
301 Cattle Point, San Juan Island, USA (48°27' N, 122°57' W), transferred to the Friday Harbor
302 Laboratories (FHL; Friday Harbor, WA, USA) within one hour, suspended in sub-tidal
303 cages off the dock at FHL, and fed *Nereocystis* spp. *ad libitum*. Lastly, individuals from
304 the Gulf of Maine were collected by divers in Frenchman Bay, Maine (44°25' N 68°12'
305 W), shipped overnight to the Darling Marine Center (Walpole, ME, USA), and were
306 maintained in flow-through aquaria and fed *Saccharina latissima ad libitum*.

307 Within two weeks of collections, adult urchins were spawned by a one to two mL
308 intracoelomic injection of 0.50 M potassium chloride. For each population, gametes from
309 three males and females were separately pooled. Fertilization of eggs and larval rearing
310 followed Strathmann (106), with the exception that embryos and larvae were reared using
311 5.0-µm filtered seawater (FSW) to include the environmental microbiota. Briefly, embryos
312 were incubated in 1 L of FSW at ambient temperature and salinity. Two hours post-
313 fertilization, seawater was decanted to remove excess sperm. Embryos were then
314 transferred to triplicate 3 L jars and diluted to a density of two individuals•mL^{-1}. Beginning
315 at the prism stage, larvae were provided *Rhodomonas lens* at 10,000 cells•mL^{-1}, for which
316 was made growth medium-free by centrifugation and resuspension in FSW prior to the
317 introduction to larval cultures. Larval cultures were subsequently diluted to one larva per
318 two mL at advanced developmental stages. Larval cultures were given 90-95% water
319 changes every other day and *R. lens* was replenished at 10,000 cells•mL^{-1}.

320 Monocultures of *R. lens* were grown in f/2 media at room temperature with a
321 combination of ambient and artificial lighting for 24 hours per day (107).
322
323 *Experimental feeding and larval morphometrics*
324 At 48 hours post-fertilization, prism-stage larvae were divided into twelve replicate jars
325 that were sub-divided into four experimental feeding treatments: 10,000, 1,000, 100, or 0
326 cells•mL^{-1} of *R. lens*. For each experiment, larvae fed 10,000 cells•mL^{-1} were reared
327 through metamorphosis while diet-restricted (1,000 and 100 cells•mL^{-1}) and starved larvae
328 were cultured until developmental stasis (Table S1). Larvae (n=100) from each replicate
329 for each treatment were sampled weekly. Immediately after sampling, larval samples were
330 concentrated into a pellet using a microcentrifuge, the FSW was removed with a sterile
331 glass pipette, and pelleted larvae were then preserved in RNAlater (Thermo Scientific,
332 Massachusetts, USA) and stored at -20 °C before DNA extractions.
333 Complementary to sampling *S. droebachiensis* larvae, the environmental microbiota
334 from the seawater was also sampled. When larval cultures were sampled, triplicate ~1-L
335 of 5.0-µm FSW was filtered onto a 0.22-µm Millipore filter to retain the environmental
336 microbiota. Full filter disks were then preserved in RNAlater and stored at -20 °C before
337 DNA extractions.
338 In addition to sampling larvae to assay the associated bacterial communities, twenty
339 larvae (n=20) from a single replicate of each dietary treatment were sampled for
340 morphometric analysis. Larvae were imaged using a compound microscope (Salish Sea:
341 Nikon Eclipse E600; camera: QImaging MicroPublisher 5.0 RTV; Gulf of Maine: Zeiss
342 Primo Star HD digital microscope; North Sea: Leica stereomicroscope) and morphometrics
343 (length of larval body, post-oral arms, and stomach area; Figs. S1-2) were performed using

344 ImageJ, v. 1.9.2 (108). Statistical differences in larval size and stomach volume were
345 compared using a two-way analysis of variance (ANOVA; JMP Pro v. 13) to test for
346 differences over time and across feeding conditions. Moreover, differences between sites
347 were compared with a one-way ANOVA. Where statistical differences were observed
348 ($p<0.05$), a Tukey's post-hoc test were used to determine the affect at each time point and
349 for each diet.
350
351 *Assaying microbial communities*
352 Total DNA was extracted from larval samples using the GeneJet Genomic DNA
353 Purification Kit (Thermo Scientific, Massachusetts, USA). For FSW samples, eDNA was
354 extracted using the FastDNA Spin Kit for Soil (MP Biomedical, Illkirch, France). DNA
355 was then quantified using the NanoDrop 2000 UV-Vis Spectrophotometer (Thermo
356 Scientific, Massachusetts, USA) and diluted to 5 ng•μL^{-1} using RNase/DNase-free water.
357 Bacterial sequences were amplified using universal primers for the V3/V4 regions of
358 the 16S rRNA gene (Forward: 5′ CTACGGGNGGCWGCAG, Reverse: 5′
359 GACTACHVGGGTATCTAATCC; 109). Products were purified using the Axygen
360 AxyPrep Mag PCR Clean-up Kit (Axygen Scientific, New York, USA), indexed via PCR
361 using the Nextera XT Index Kit V2 (Illumina, California, USA), and then purified again.
362 At each of these three steps, fluorometric quantitation was performed using a Qubit (Life
363 Technologies, California, USA) and libraries were validated using a Bioanalyzer High
364 Sensitivity DNA chip (Agilent Technologies, California, USA). Illumina MiSeq
365 sequencing (v3, 2x300 bp paired-end reads) was performed at the University of North
366 Carolina at Charlotte. See, Table S2 for PCR recipe and thermal profiles.

367 Forward and reverse sequences were paired and trimmed using PEAR (110) and
368 Trimmomatic (111), respectively, and converted from fastq to fasta using custom script.
369 Chimeric sequences were then detected using USEARCH (112) and removed using
370 filter_fasta.py prior to analysis of bacterial 16S rRNA sequences. Using QIIME 1.9.1 (113)
371 and Greengenes (v. 13.5), bacterial 16S rRNA sequences were analyzed and grouped into
372 operational taxonomic units (OTUs) based on a minimum 97% similarity. The biom table
373 generated by pick_open_reference_otus.py was filtered of OTUs with ten or less sequences
374 as well as sequences matching the chloroplast of cryptophytes (*i.e.*, *R. lens*; as per 13).
375 Using the filtered biom table and "biom summarize-table" to count total sequences per
376 sample, the rarefaction depth of 3,193 reads was determined and applied to all subsequent
377 analyses (Fig. S3). Alpha diversity (*i.e.*, Fisher's alpha, Shannon equitability, Faith's
378 phylogenetic distance, and observed OTUs) was calculated using alpha_diversity.py and
379 compared statistically using one-way ANOVAs in JMP. Beta diversity was calculated
380 using the unweighted and weighted UniFrac (114) as part of jackknifed_beta_diversity.py.
381 These values were compared using principal coordinate analyses (PCoA) and recreated
382 using make_2d_plots.py and stylized for presentation in Adobe Illustrator CS6.
383 Community similarity across phenotypes, dietary states, developmental stages, and
384 biogeography were compared statistically using an analysis of similarity (ANOSIM),
385 permutational multivariate analysis of variance using distance matrices (ADONIS), and
386 permutational multivariate analysis of variance (PERMANOVA) using
387 compare_categories.py. The shared OTUs between urchin populations was determined
388 using compute_core_microbiome.py and shared_phylotypes.py. Average bacterial
389 community for each urchin population as well as across populations and the shared

390 community was generated using summarize_taxa_through_plots.py, visualized with Prism
391 7 (GraphPad Software), and stylized for presentation in Adobe Illustrator CS6.
392 A step-by-step listing of QIIME scripts used to convert raw reads to OTUs for
393 visualization of these data is located in Note S1.
394
395 *Functional predictions using PICRUSt*
396 For the QIIME-generated OTU (*i.e.*, biom) table to be compatible with PICRUSt (115),
397 all *de novo* OTUs were filtered according to Greengenes (v. 13.5). This 'closed' OTU table
398 (that retained 50.9% of original OTUs) was normalized by copy number, upon which
399 metagenomic gene profiles were predicted and categorized by biological function (*i.e.*,
400 KEGG categories). The PICRUSt output was made compatible for Statistical Analysis of
401 Metagenomic Profiles (STAMP; 116) using biom_to_stamp.py in Microbiome Helper
402 (117) and analyzed for population-level differences. A STAMP-generated principle
403 coordinate analysis was compared statistically with an ANOVA within the STAMP
404 program. Functional profiles were visualized with Prism 7 (GraphPad Software) and
405 stylized for presentation in Adobe Illustrator CS6. Lastly, metagenome_contributions.py
406 and custom scripts to extract taxonomic information were used to determine which
407 bacterial taxa contributed to predicted genes categories of interest.
408 A step-by-step listing of PICRUSt scripts used to convert from QIIME and the
409 subsequent data analysis is located in Note S1.
410
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425 **Fig. Legends**

426

427 **Fig. 1.** Location of *Strongylocentrotus droebachiensis* experiments. Cartoon representation
428 of adult populations and the geographical distribution for where differential feeding
429 experiments were conducted.

430

431 **Fig. 2.** Dietary and temporal shifts in the bacterial communities associated with
432 *Strongylocentrotus droebachiensis* larvae. Community similarity based on *S.*
433 *droebachiensis* larval-associated bacterial communities based on food availability (A-C)
434 over a multi-week exposure (D-F) for populations from the Salish Sea (A, D), Gulf of
435 Maine (B, E), and North Sea (C, F). Comparisons between food availability (A-C) and over
436 time (D-F) are based on weighted UniFrac comparisons.

437

438 **Fig. 3.** Similarity of the bacterial communities associated with *Strongylocentrotus*
439 *droebachiensis* larvae from three populations. Community similarity of *S. droebachiensis*
440 larval-associated bacterial communities between three geographic locations, with variation
441 due to diet, time and development, and phenotype collapsed by site. Comparisons between
442 populations are based on (A) unweighted and (B) weighted UniFrac comparisons.

443

444 **Fig. 4.** Bacterial taxa associated with *Strongylocentrotus droebachiensis* larvae. (A) Class-
445 level profiles of the bacterial communities associated with *S. droebachiensis* larvae from
446 the Salish Sea, Gulf of Maine, and North Sea as well as the mean community across
447 populations and the shared taxa between each population. (B) Distribution of OTUs

448 associated with *S. droebachiensis* larvae uniquely found in each population, common
449 between two populations, and shared between all populations.
450
451 **Fig. 5.** Predicted *Strongylocentrotus droebachiensis* metagenome. (A) Community
452 similarity of level-1 KEGG categories for PICRUSt-generated *S. droebachiensis* larval
453 metagenomes. (B) Profiles of level-1 KEGG categories from the Salish Sea, Gulf of Maine,
454 and North Sea as well as the mean community across populations and the shared taxa
455 between each population. (C) Relative proportions of bacterial classes linked to the
456 predicted metabolic functionality of the PICRUSt-generated *S. droebachiensis* larval
457 metagenome.
458
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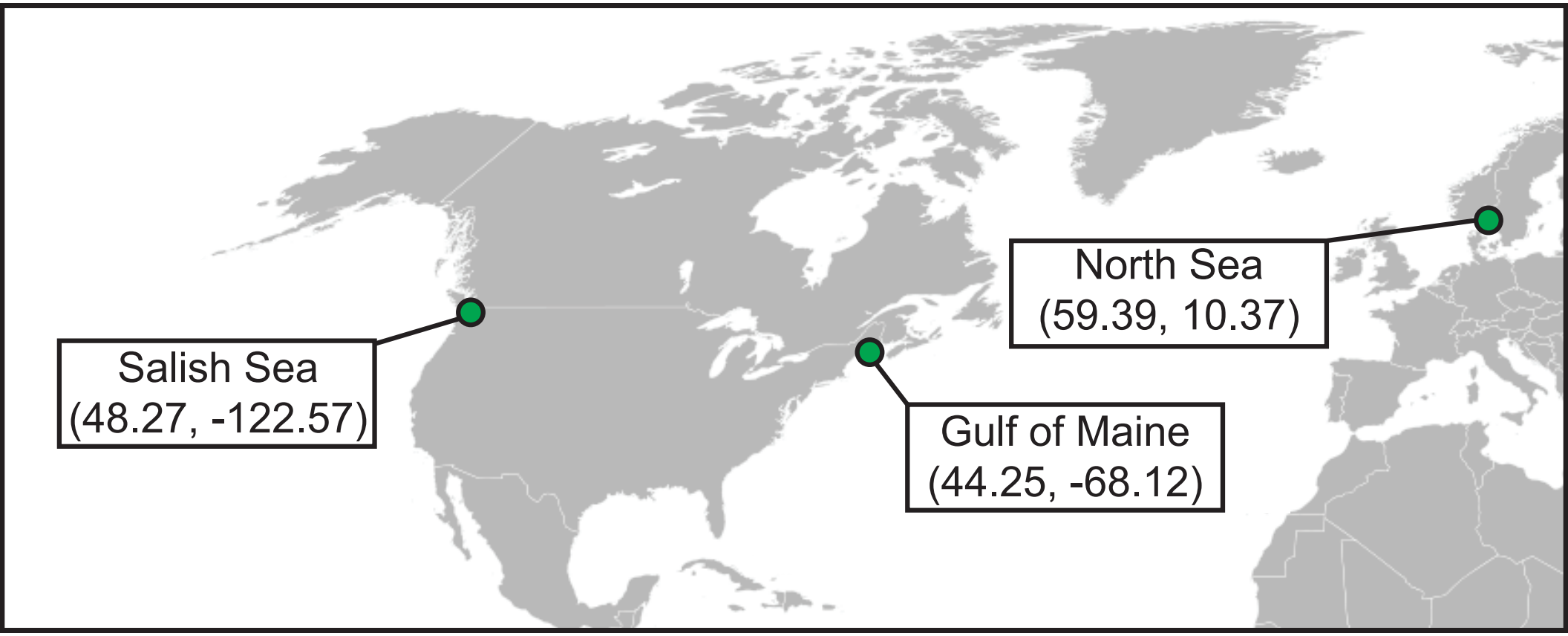
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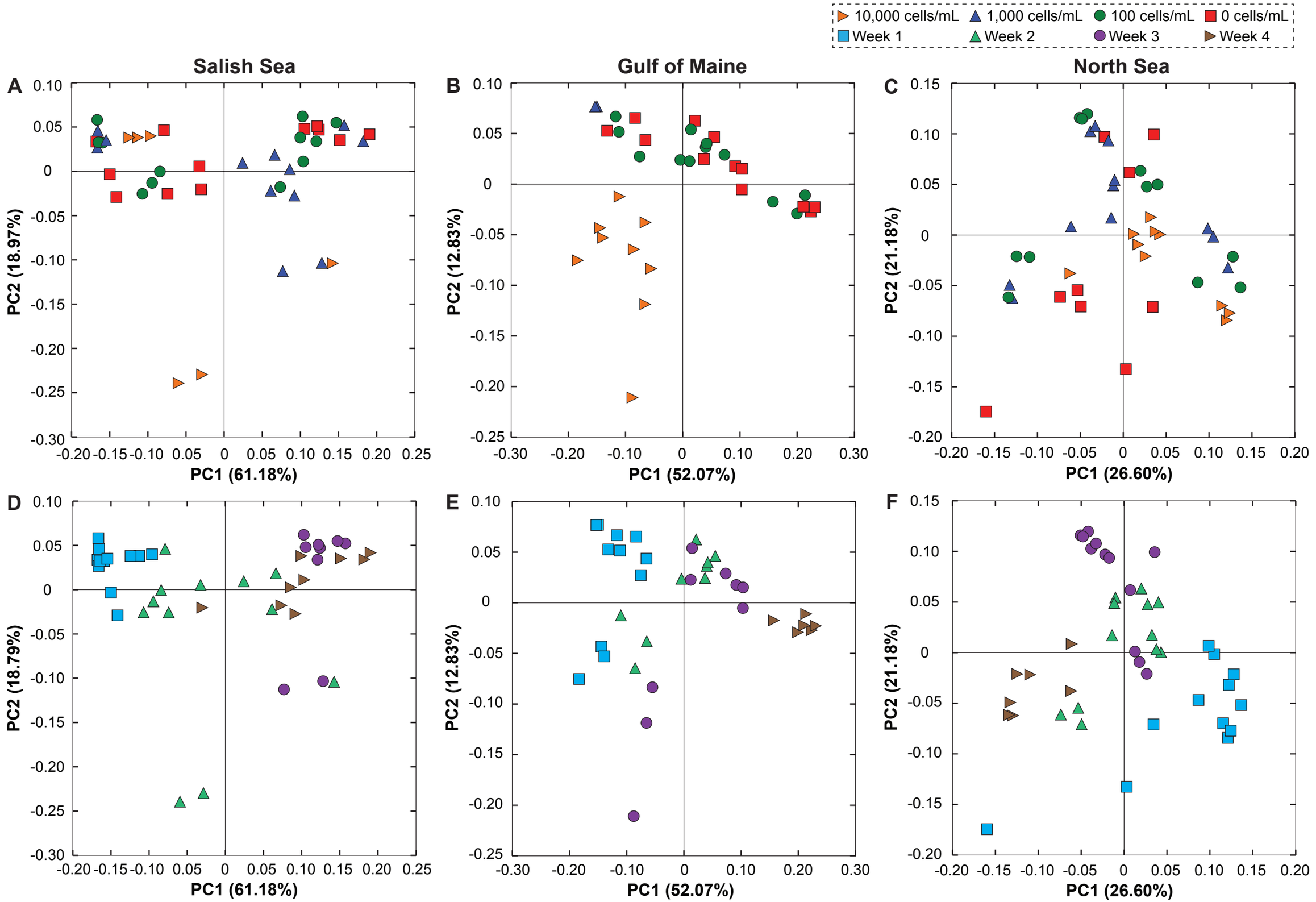
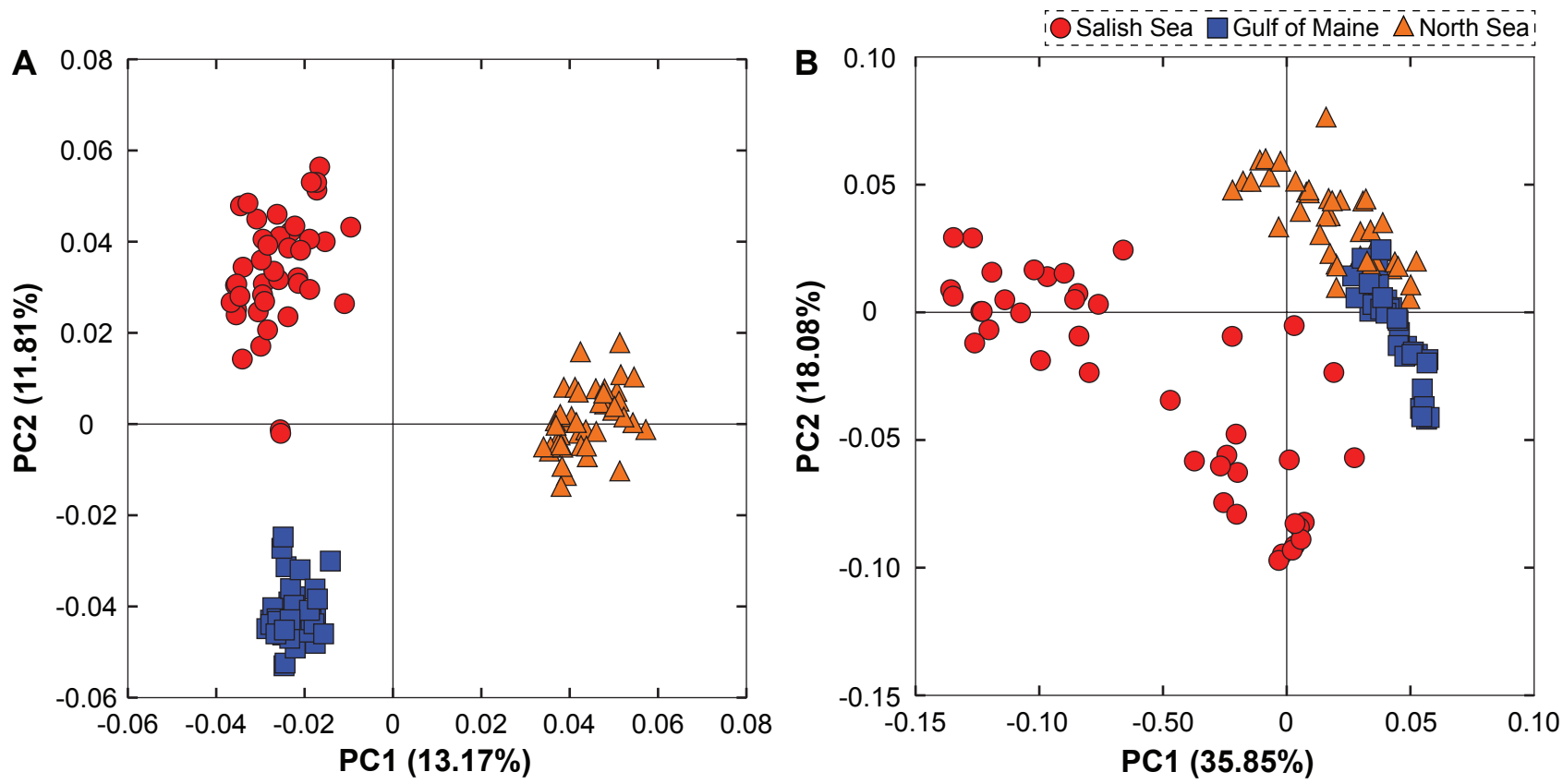
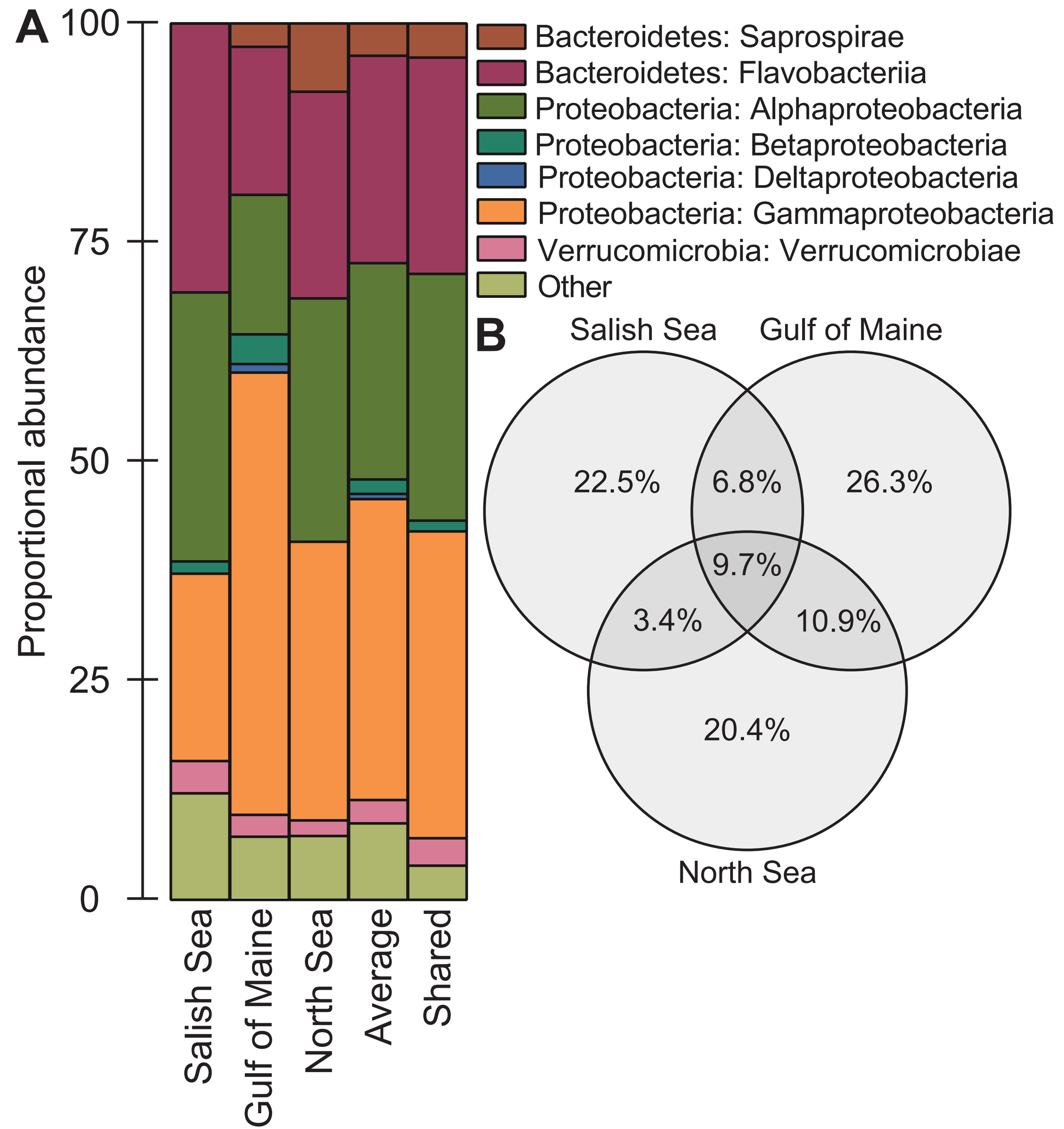
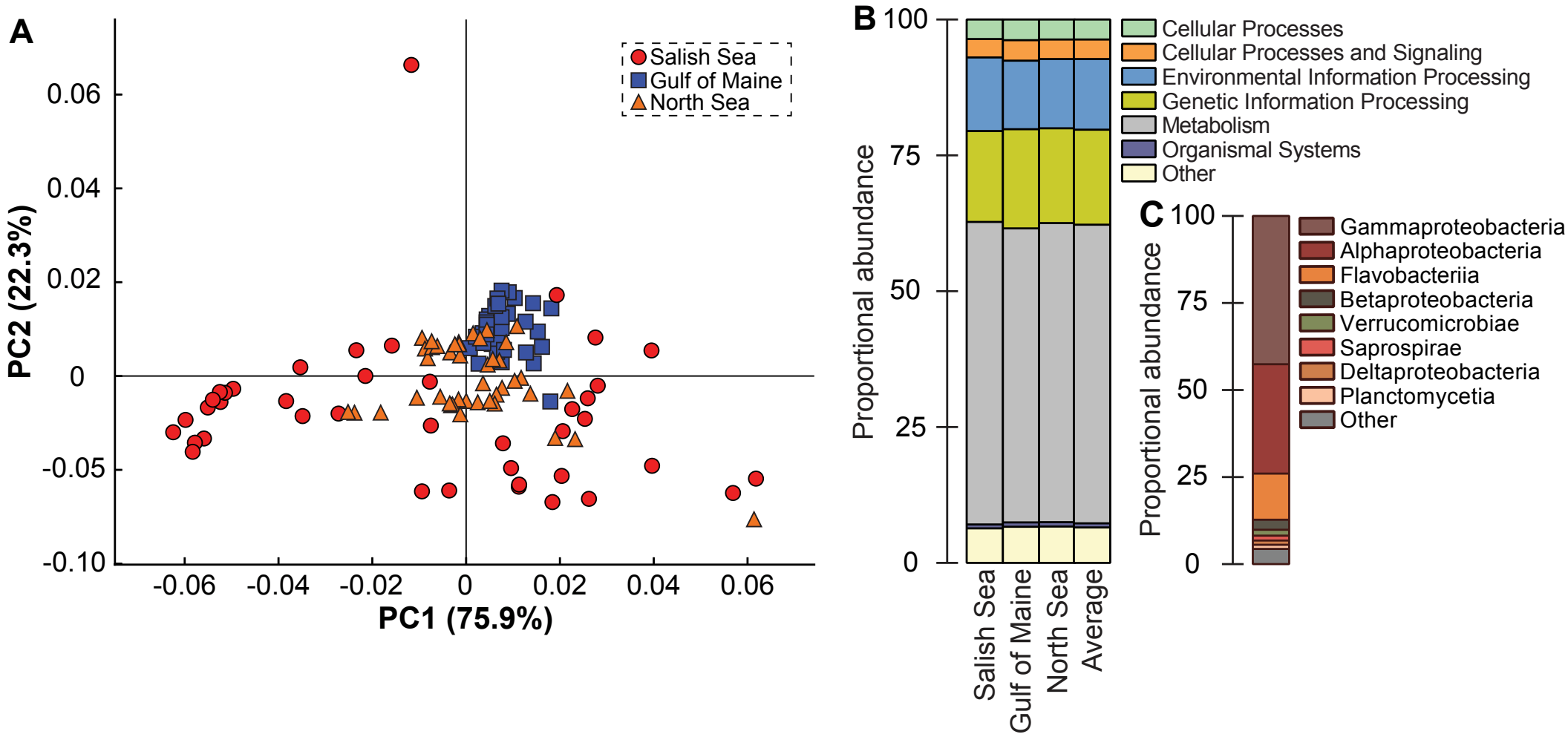
Gulf of Maine
(44.25, -68.12)


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
B
PC2 (11.81%)
PC1 (13.17%)
PC2 (18.08%)
PC1 (35.85%)
0.08
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
0.10
0.05
-0.05
-0.10
-0.15
-0.50


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%


A
Salish Sea
Gulf of Maine
North Sea
PC2 (22.3%)
PC1 (75.9%)
0.06
0.04
0.02
0
-0.05
-0.10
-0.06
-0.04
-0.02
0
0.02
0.04
0.06
B
Proportional abundance
100
75
50
25
0
Salish Sea
Gulf of Maine
North Sea
Average
Cellular Processes
Cellular Processes and Signaling
Environmental Information Processing
Genetic Information Processing
Metabolism
Organismal Systems
Other
C
Proportional abundance
100
75
50
25
0
Gammaproteobacteria
Alphaproteobacteria
Flavobacteriia
Betaproteobacteria
Verrucomicrobiae
Saprospirae
Deltaproteobacteria
Planctomycetia
Other

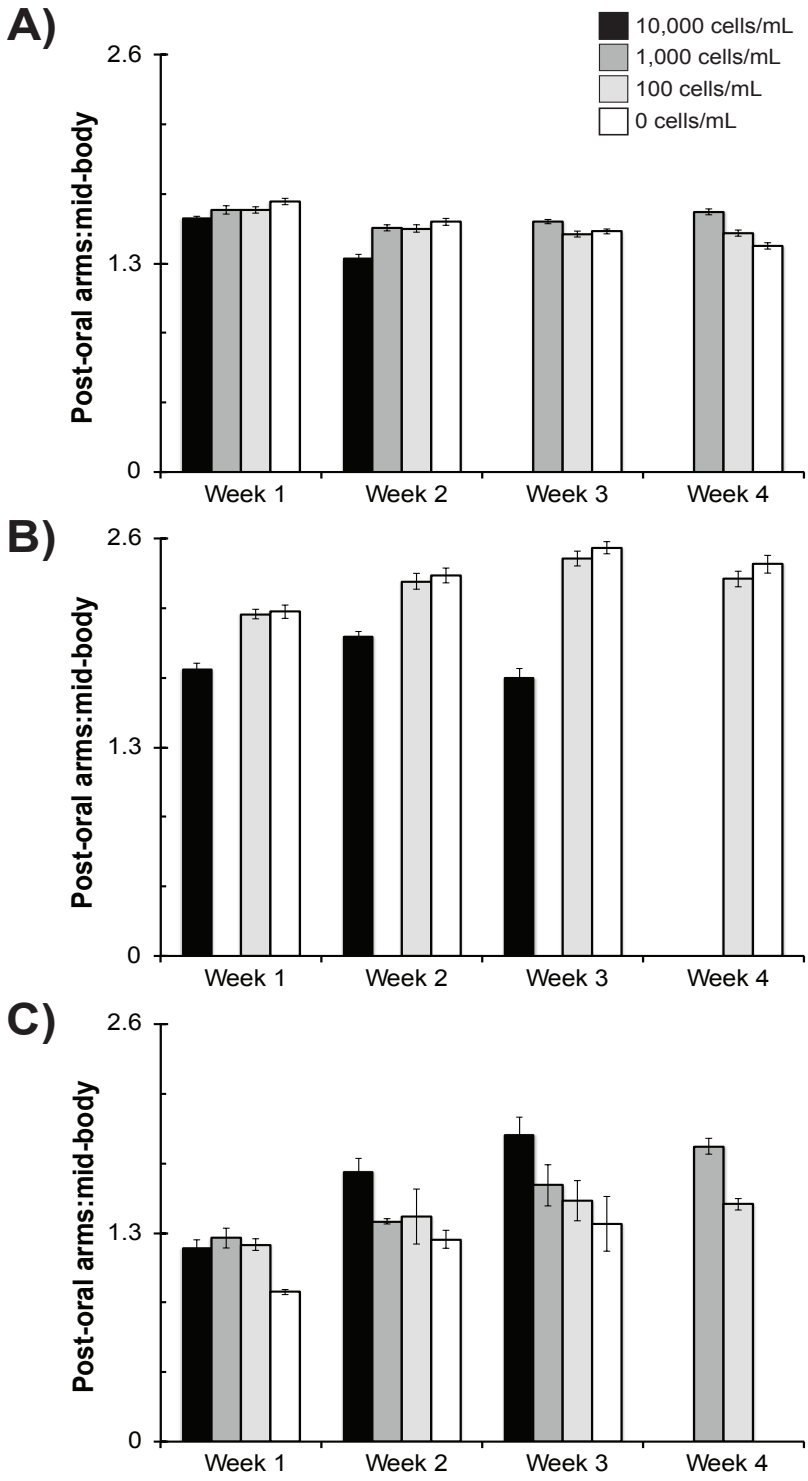


Figure S1. *Strongylocentrotus droebachiensis* larvae alter phenotype to feeding environment. Ratio between the post-oral arm and mid body line (mean ± standard error; n=20) for *S. droebachiensis* from the Salish Sea (A), Gulf of Maine (B), and North Sea (C) larvae having been fed either 10,000 (black),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

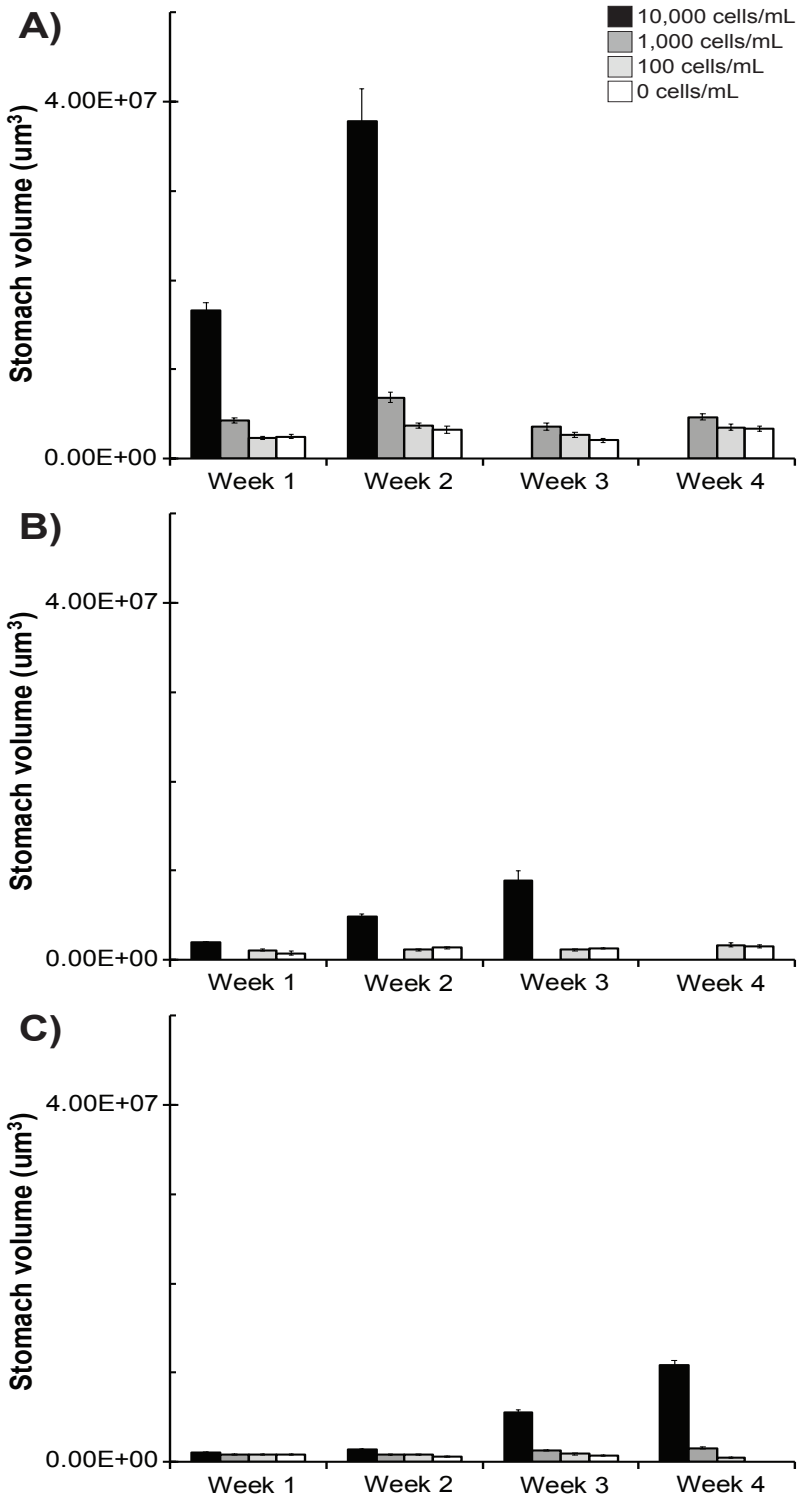


Figure S2. *Strongylocentrotus droebachiensis* larvae alter gut volume to feeding environment. Gut volume (mean ± standard error; n=20) for larval *S. droebachiensis* from the Salish Sea (A), Gulf of Maine (B), and North Sea (C) having been fed either 10,000 (black), 1,000 (dark grey), 100 (grey), and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

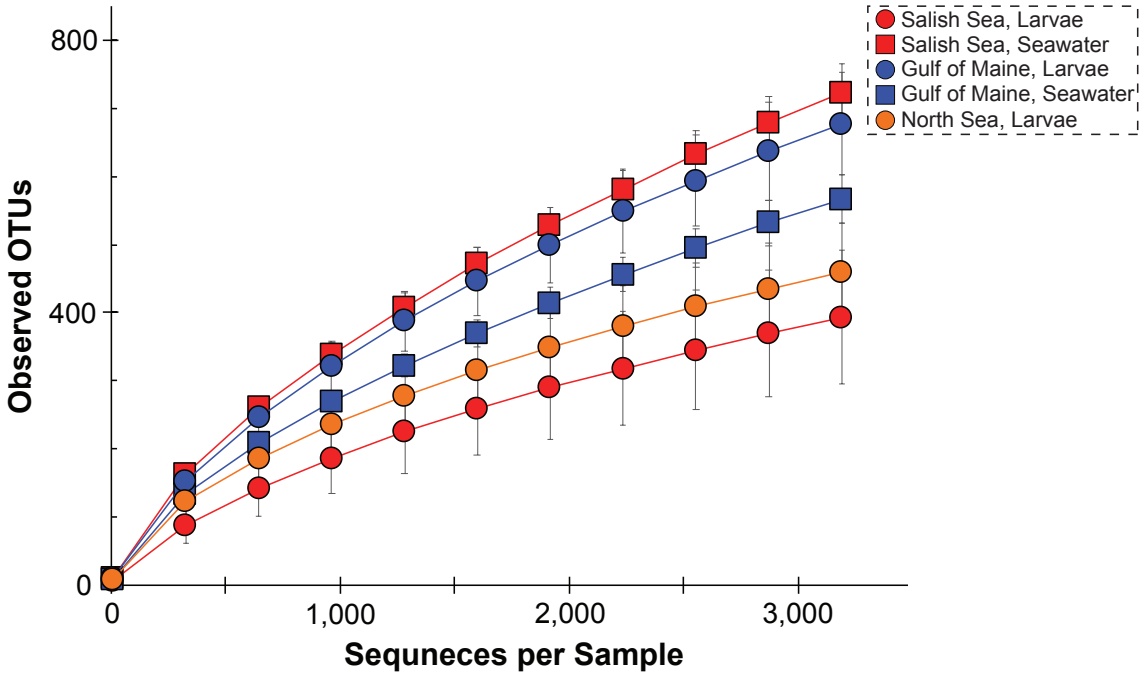


Figure S3. Alpha rarefaction curves for *Strongylocentrotus droebachiensis* larvae from three geographic locations and of the seawater. Alpha rarefaction curves for the associated microbiota for *S. droebachiensis* larvae (circles) from the Salish Sea (blue), Gulf of Maine (red), and North Sea (yellow)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

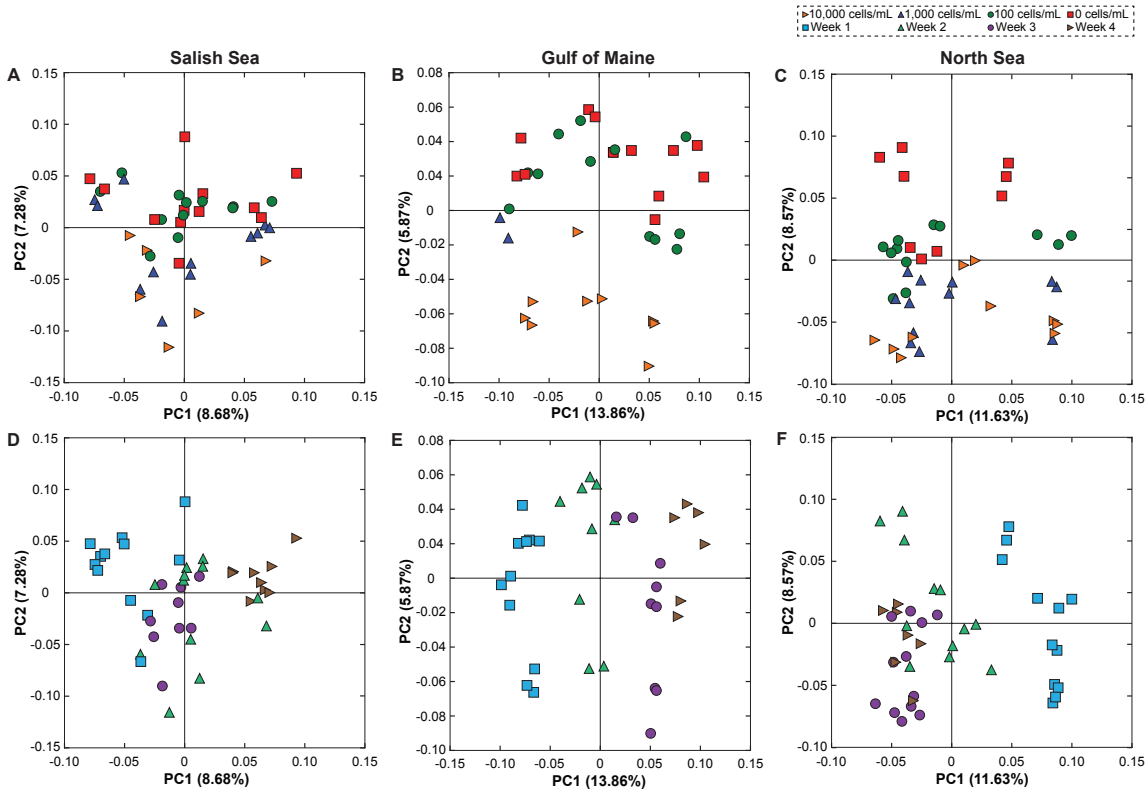


Figure S4. Dietary and temporal shifts in the bacterial communities associated with *Strongylocentrotus droebachiensis* larvae. Community similarity based on *S. droebachiensis* larval-associated bacterial communities based on food availability (A-C) over a multi-week exposure (D-F) for populations from 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.)

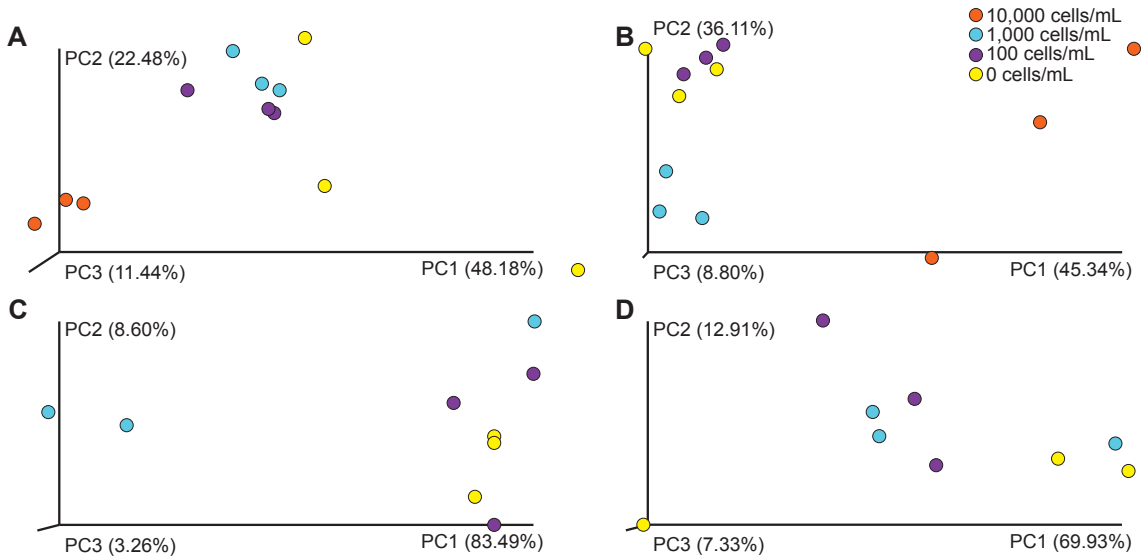


Figure S5. Community similarity of *Strongylocentrotus droebachiensis* larvae from the Salish Sea across time. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea for one (A; $p < 0.002$), two (B; $p < 0.001$), three (C; $p < 0.001$), or four (D; $p < 0.001$)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

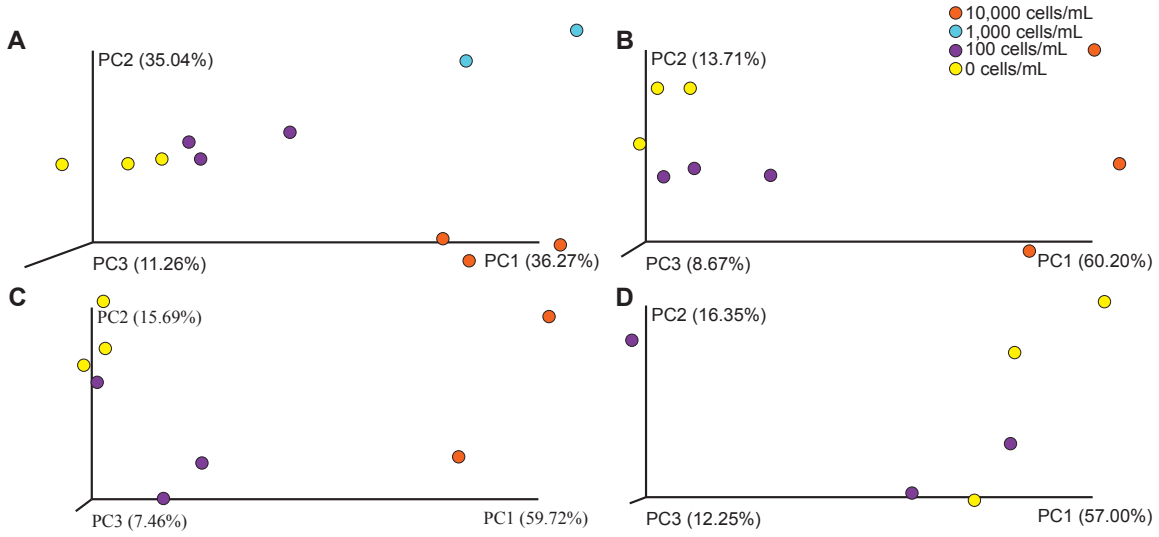


Figure S6. Community similarity of *Strongylocentrotus droebachiensis* larvae from the Gulf of Maine across time. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Gulf of Maine for one (A; $p < 0.001$), two (B; $p < 0.005$), three (C; $p < 0.007$), or four (D; $p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

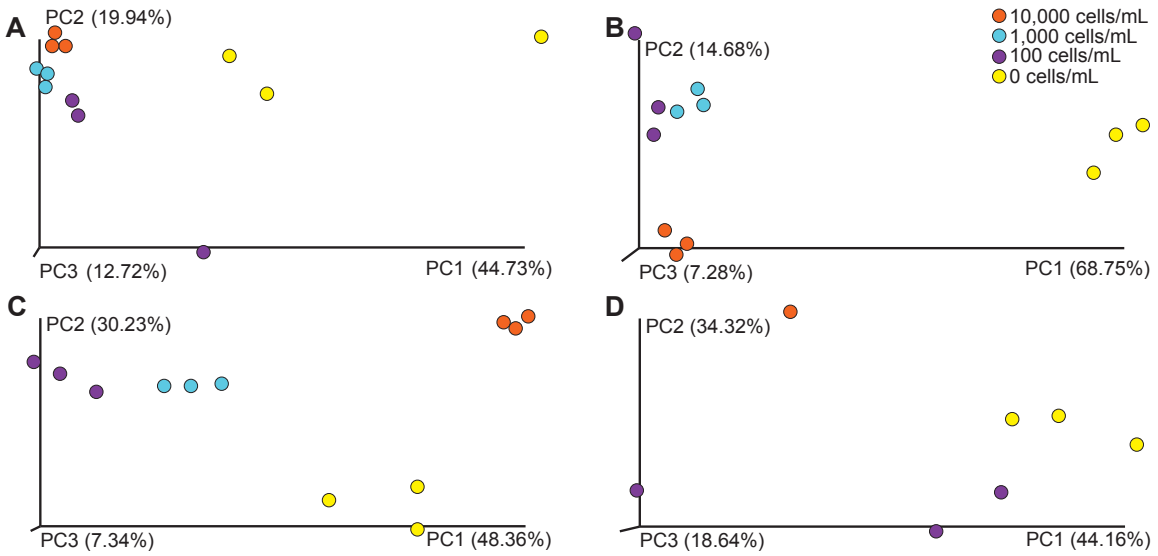


Figure S7. Community similarity of *Strongylocentrotus droebachiensis* larvae from the North Sea across time. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the North Sea for one (A; $p < 0.002$), two (B; $p < 0.001$), three (C; $p < 0.001$), or four (D; $p < 0.020$)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

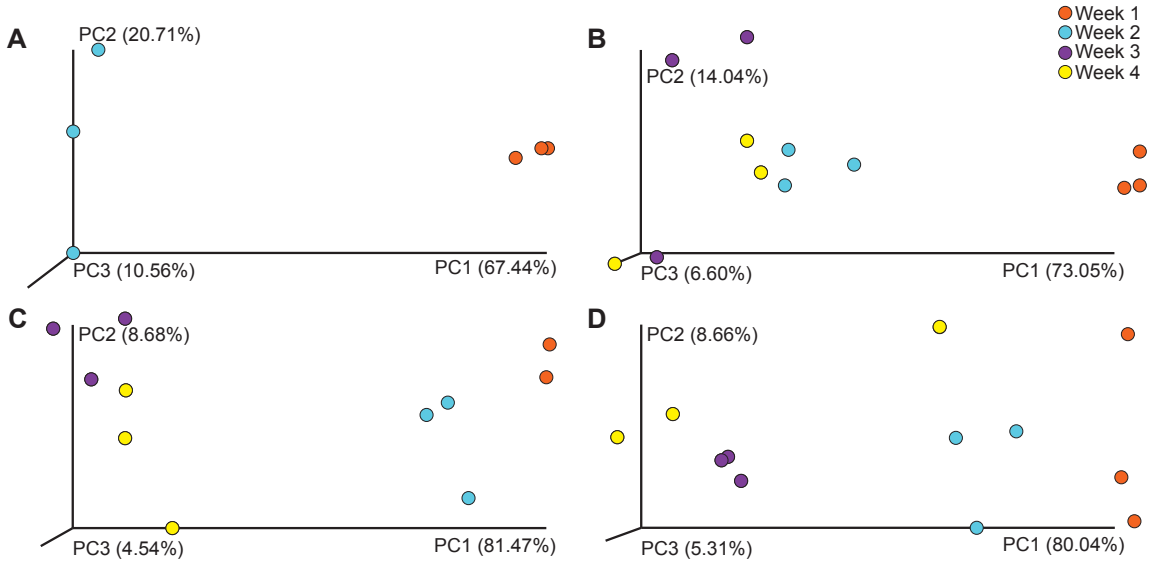


Figure S8. Community similarity of *Strongylocentrotus droebachiensis* larvae from the Salish Sea across diets. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea having been fed 10,000 (A; $p < 0.001$), 1,000 (B; $p < 0.001$), 100 (C; $p < 0.001$), or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

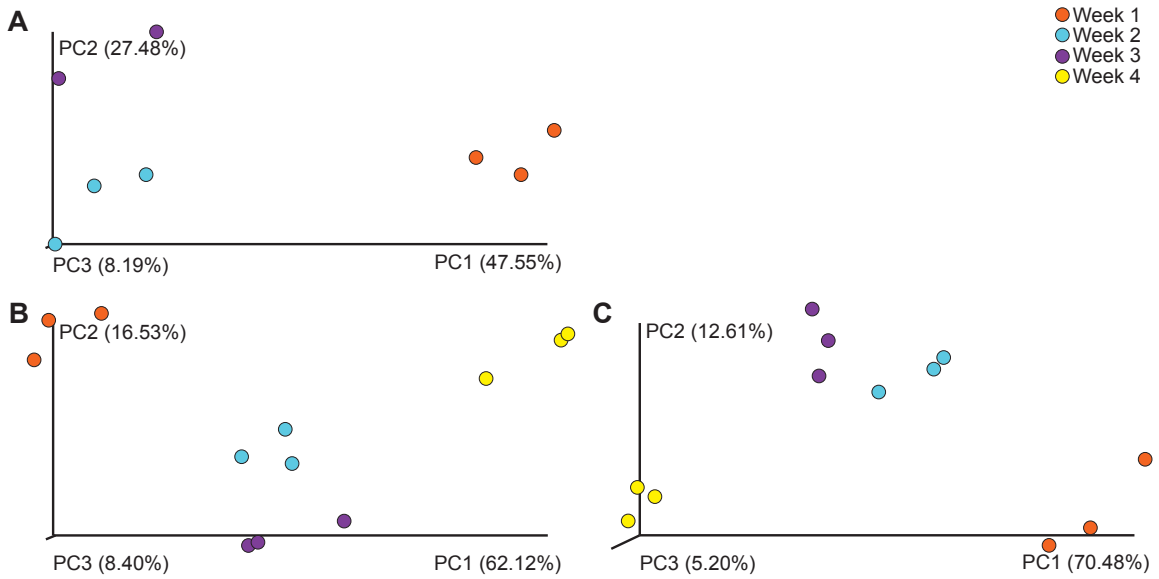


Figure S9. Community similarity of *Strongylocentrotus droebachiensis* larvae from the Gulf of Maine across diets. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Gulf of Maine having been fed 10,000 (A; $p < 0.005$), 100 (B; $p < 0.001$), or 0 (C; $p < 0.002$)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

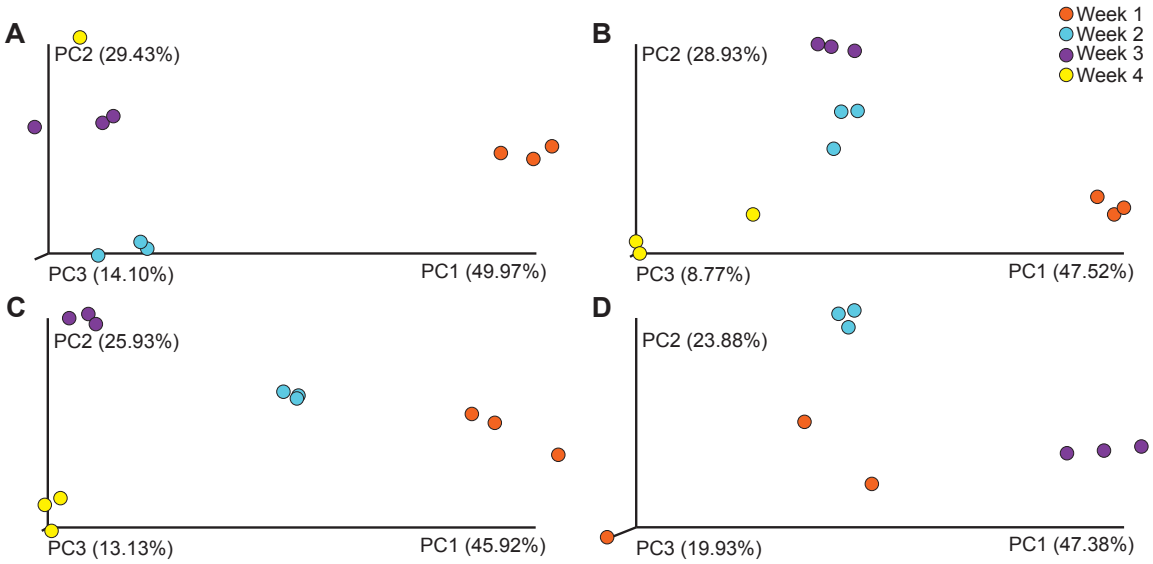


Figure S10. Community similarity of *Strongylocentrotus droebachiensis* larvae from the North Sea across diets. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the North Sea having been fed 10,000 (A; $p < 0.002$), 1,000 (B; $p < 0.002$), 100 (C; $p < 0.002$), or 0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

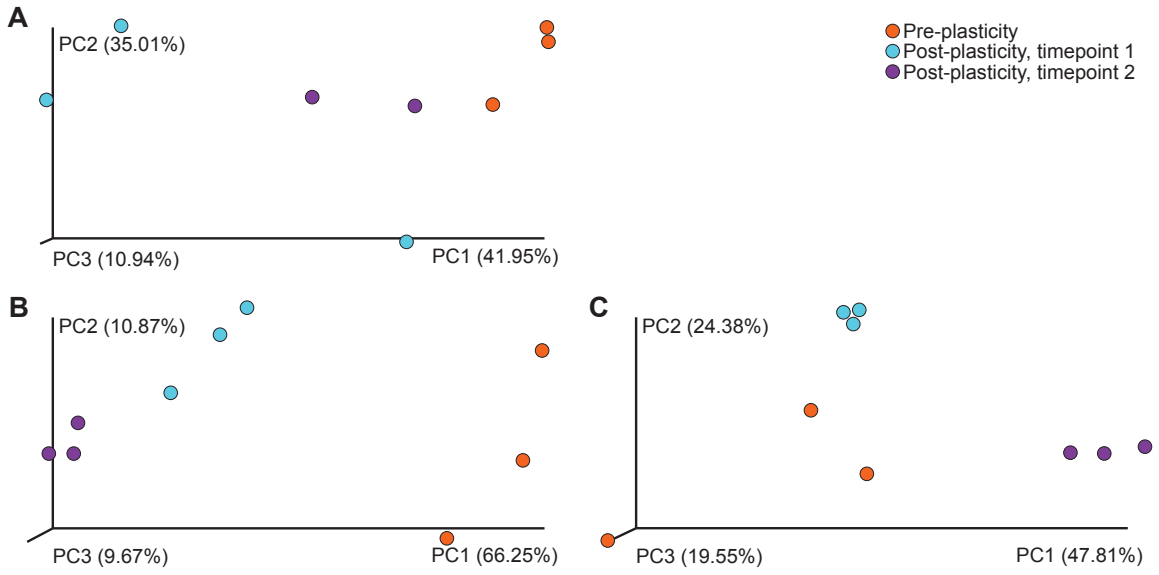


Figure S11. Community similarity of the associated microbial community along a phenotypic continuum for *Strongylocentrotus droebachiensis* from three geographic locations. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea (A; $p = 0.046$), Gulf of Maine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

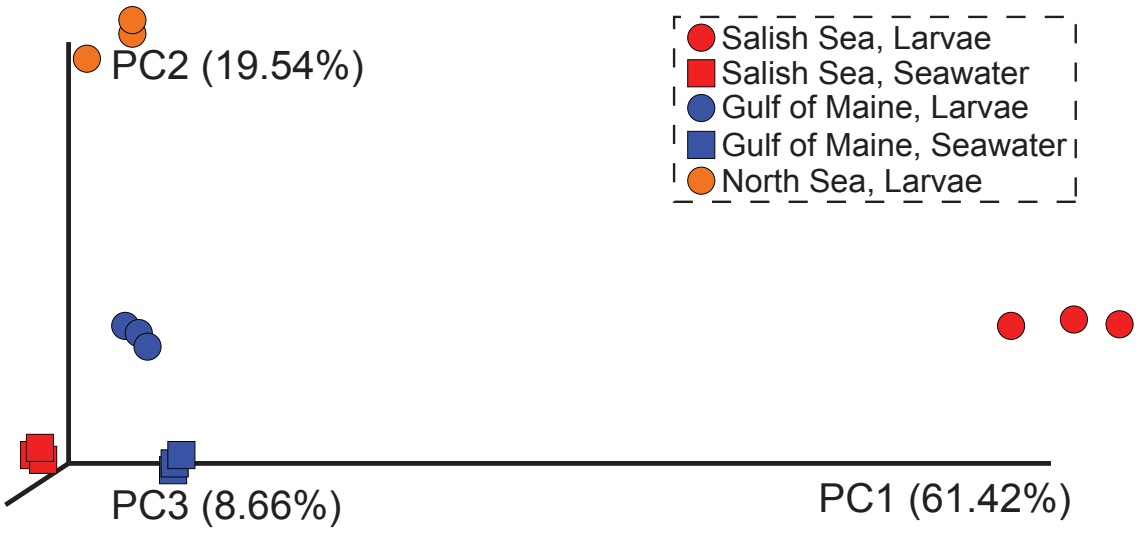


Figure S12. Similarity of the associated microbial community for *Strongylocentrotus droebachiensis* larvae from three geographic locations. Community similarity ($p < 0.001$) of the associated microbiota between *S. droebachiensis* larvae (circles) from the Salish Sea (blue), Gulf of Maine (red), and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

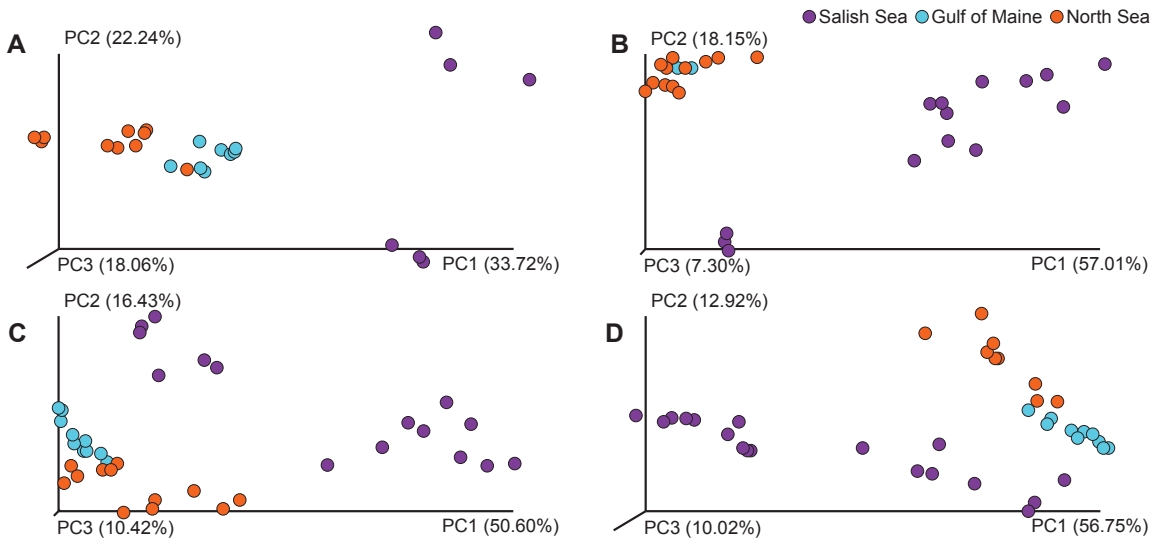


Figure S13. Community similarity of *Strongylocentrotus droebachiensis* larvae across diets. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea (purple), Gulf of Maine (blue), or North Sea (orange) and having been fed 10,000 (A; $p < 0.001$), 1,000 (B;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

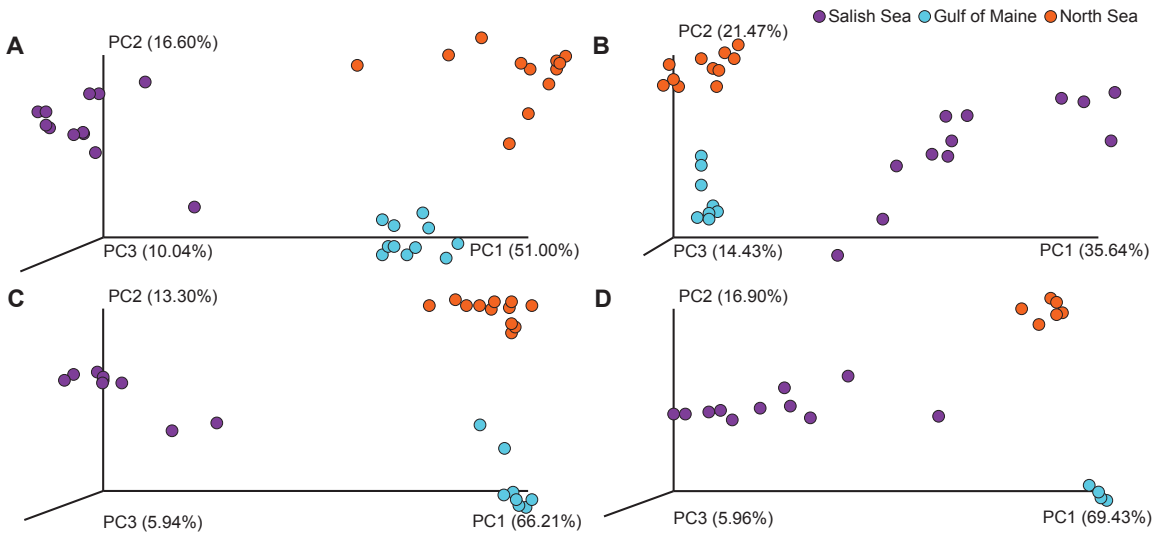


Figure S14. Community similarity of *Strongylocentrotus droebachiensis* larvae across time. Similarity of bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea (purple), Gulf of Maine (blue), or North Sea (orange) and having been differentially fed for one (A; $p < 0.001$),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

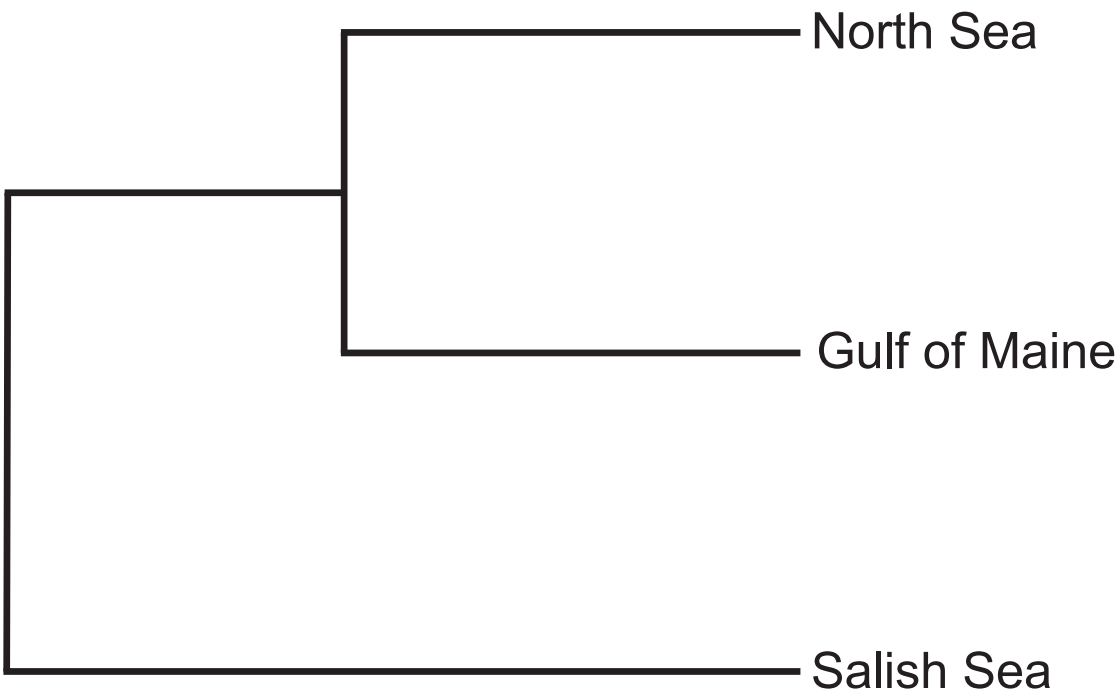


Figure S15. Similarity between the associated bacterial communities of *Strongylocentrotus droebachiensis* larvae from three geographic locations. Dendrograms of *S. droebachiensis* larvae representing the total community for the Salish Sea, Gulf of Maine, and North Sea, with variation due to phenotypes, dietary options, and developmental stages being collapsed. Dendrograms were generated as part of the "jackknifed_beta_diversity.py" command for Figure 3 using weighted UniFrac.

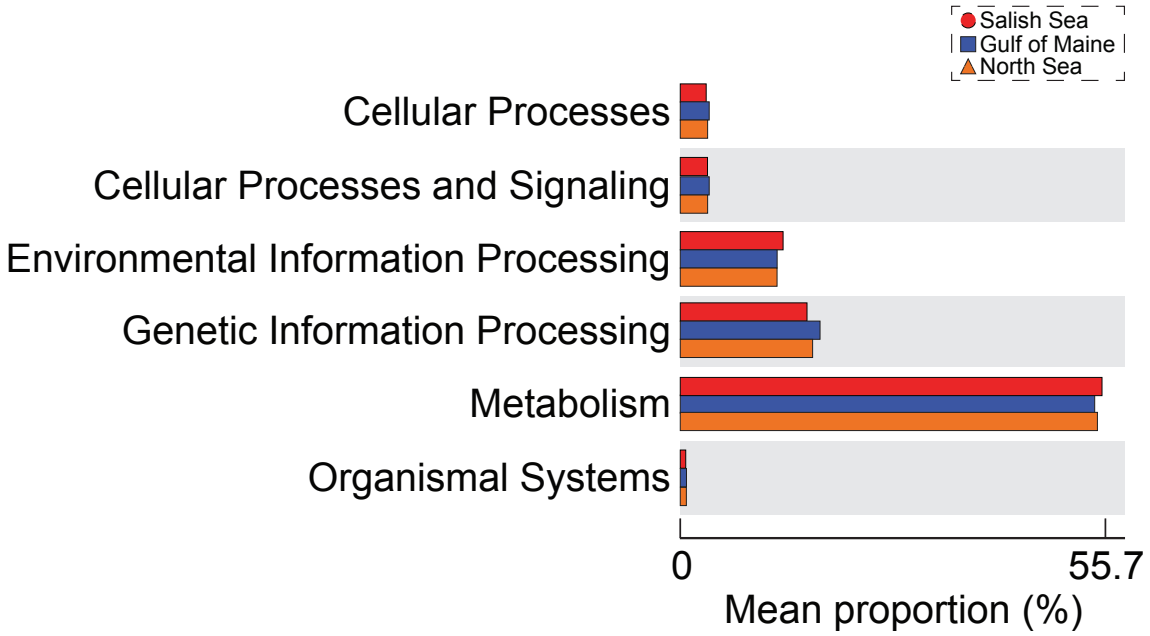


Figure S16. Predicted functional profiles of bacterial communities associated with *Strongylocentrotus droebachiensis* larvae between geographical locations. Bar charts of PICRUSt-generated metagenomics profiles group at level 1 KEGG categories for the bacterial communities associated with *S. droebachiensis* larvae from the Salish Sea (red), Gulf of Maine (blue), and North Sea (orange).

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"

PICRUSt analysis

Starting with the biom table following 'Step 12' from 'QIIME analysis':

1) OTUs picked *de novo* were filtered using "filter_otus_from_otu_table.py" from QIIME
2) The closed OTU table was normalized using "normalized normalize_by_copy_number.py"
3) Metagenomes were predicted using "predict_metagenomes.py"
4) Predicted functions were categorized using "categorize_by_function.py"
5) The output from 'Step 4' was made STAMP-compatible using biom_to_stamp.py from Microbiome Helper
6) Resultant principles coordinates were extracted and analyzed using JMP

Taxa relating to metabolism

Starting with the 'closed' and 'normalized' biom table following 'Step 3' from 'PICRUSt analysis':

1) Determine which taxa contributed to 'Metabolism' using "metagenome_contributions.py" from PICRUSt for all related KEEG ID: "K00010,K00020,K00030,K00040,K00051,K00052,K00053,K00061,K00062,K00071,K00072,K00073,K00100,K00120,K00121,K00130,K00140,K00190,K00195,K00196,K00220,K00230,K00231,K00232,K00240,K00250,K00253,K00254,K00260,K00261,K00270,K00280,K00281,K00290,K00300,K00310,K00311,K00330,K00331,K00332,K00333,K00340,

K00350,K00360,K00361,K00362,K00363,K00364,K00365,K00380,K00400,K00401,K00402,K00403,K00404,K00405,K00410,K00430,K00440,K00450,K00460,K00471,K00472,K00473,K00480,K00500,K00510,K00511,K00512,K00513,K00514,K00515,K00520,K00521,K00522,K00523,K00524,K00525,K00531,K00532,K00533,K00534,K00540,K00550,K00561,K00562,K00563,K00564,K00565,K00571,K00572,K00590,K00591,K00592,K00600,K00601,K00603,K00604,K00620,K00621,K00622,K00623,K00624,K00625,K00626,K00627,K00630,K00633,K00640,K00642,K00643,K00650,K00660,K00670,K00680,K00710,K00720,K00730,K00740,K00750,K00760,K00770,K00780,K00785,K00790,K00791,K00830,K00860,K00900,K00901,K00902,K00903,K00904,K00905,K00906,K00908,K00909,K00910,K00920,K00930,K00940,K00941,K00942,K00943,K00944,K00945,K00950,K00960,K00965,K00966,K00980,K00981,K00982,K00983,K00984,K00999,K01010,K01040,K01051,K01052,K01053,K01054,K01055,K01056,K01057,K01058,K01059,K01060,K01061,K01062,K01063,K01064,K01065,K01066,K01070,K01100,K01110,K01120,K01130,K01200,K01210,K01212,K01220,K01230”

2) Determine class-level composition using custom code: awk '{print $c__*}' [output txt file from ‘1’ of this section]