Title:

Biogeography masks diet-induced shifts in the bacterial community associated with larvae of the sea urchin *Strongylocentrotus droebachiensis*

Running title:

Population-specific microbiome of urchin larvae

Authors:

Tyler J. Carrier[1,*], Sam Dupont[2], and Adam M. Reitzel[1]

Affiliations:

[1] Department of Biological Sciences, University of North Carolina at Charlotte, 9201 University City Blvd., Charlotte, NC 28223 USA

[2] Department of Biological and Environmental Sciences, University of Gothenburg, The Sven Lovén Centre for Marine Infrastructure, Kristineberg, Sweden

[*] Corresponding author: tcarrie1@uncc.edu

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Abstract

Animals acclimate to changes in their environment through diverse responses, including phenotypic plasticity and shifts in their microbiome. These microbial communities are also taxonomically distinct across the geographical distribution of the host. It is less known, however, whether taxonomic differences in host-associated bacterial communities between geographically distinct populations mask shifts due to environmental changes within a population. We tested for potential ecological masking using larvae of the echinoid *Strongylocentrotus droebachiensis* from three coastal locations in the Pacific and Atlantic Oceans that were exposed to four feeding regimes. When considering OTU membership and the relative proportion of those taxa, the composition of the larval-associated bacterial communities were best explained by location, not feeding regime. Similarly, predicted metagenomic gene profiles from these bacterial communities were congruent with population specificity and may suggest a role in metabolism. We hypothesize that, while much of the differences in the bacterial communities is related to the large geographic distances between these locations, the predicted overlapping functions of the microbiome may relate to responding to ecological variation experienced by these larvae. Taken together, these results suggest that differences in community composition between populations masks local variation, and that scaling should be considered in when studying microbiome dynamics.

Introduction

Acclimating to environmental variability through morphological, developmental, and/or physiological plasticity is a common trait of animals (Boidron-Metairon 1988, Bradshaw 1965, DeWitt et al 1998, Miner et al 2005, Schlichting and Smith 2002, Sterns 1989, West-Eberhard 2003). Over the past decade, the appreciation for the role that animal-associated microbial communities play in ameliorating environment-induced stress has grown profoundly (Apprill 2017, Carrier and Reitzel 2017, Carrier and Reitzel 2018, Kohl and Carey 2016, Macke et al 2016, Shapira 2016, Theis et al 2016). When experiencing a heterogeneous environment, the animal host may recruit, expel, and/or shuffle the relative proportion of associated microbiota (Bordenstein and Theis 2015, Zilber-Rosenberg and Rosenberg 2008), to assemble a community with particular molecular pathways for the environmental conditions (Burke et al 2011, Louca et al 2016, Roth-Schulze et al 2018).

Microbial communities associated with animals often vary in response to diverse abiotic and biotic factors, including temperature, salinity, diet quality and quantity, season, and habitat-type (see reviews by Carrier and Reitzel 2017, Kohl and Carey 2016). Of these, dietary responses are best studied and have a major impact on the composition of and potential mutualistic functions for this community (David et al 2014, Kohl and Dearing 2012, Rosenberg and Zilber-Rosenberg 2016, Sonnenburg et al 2016). When faced with prolonged food deprivation, for example, the community composition and diversity of microbiota associated with both invertebrate and vertebrate hosts shift considerably (Carrier and Reitzel 2018, Kohl et al 2014), a response hypothesized to buffer against reduced exogenous nutrients.

Microbial communities associated with animals are also species-specific (Carrier and Reitzel 2018, Fraune and Bosch 2007, Schmitt et al 2012) and taxonomically variable across the geographical distribution of the host species (Dishaw et al 2014, Huang et al 2018, Marino et al 2017, Marzinelli et al 2015, Mortzfeld et al 2015). Habitat-specific microbial communities are primarily controlled by environmental conditions (Pantos et al 2015) and diverge with respect to dispersal limitations (Moeller et al 2017). Despite this taxonomic variation, microbial communities can remain functionally similar due to shared genes across bacterial species (Roth-Schulze et al 2018). The bacterial communities of the green alga *Ulva* spp., for example, are too variable to define a 'core' community; however, nearly 70% of the microbial genes are biogeographically consistent (Roth-Schulze et al 2018). How components of host ecology attribute to the taxonomic variation in these bacterial communities is less understood and are needed to identify the relative strength and importance of each abiotic or biotic factor.

Planktotrophic (feeding) larvae are one biological system to compare the components of host ecology and their dynamics on animal microbial communities. At a local scale, many planktotrophic larvae (*e.g.*, the pluteus of sea urchins) inhabit heterogeneous feeding environments and are morphologically and physiologically plastic to food availability (Adams et al 2011, Boidron-Metairon 1988, Byrne et al 2008, Carrier et al 2015, Hart and Strathmann 1994, McAlister and Miner 2018, Miner 2004, Miner 2011, Soars et al 2009). Feeding-induced plasticity in the echinoid *Strongylocentrotus droebachiensis*, specifically, is correlated with phenotype-, diet-, and development-specific bacterial communities (Carrier and Reitzel 2018). At a regional scale, adult *S. droebachiensis* have an Arctic-boreal distribution (Scheibling and Hatcher 2013) and can

be divided into genetically distinct populations across multiple oceans (Addison and Hart 2004, Addison and Hart 2005). Attributes of the reproductive biology of *S. droebachiensis* (*e.g.*, sperm morphology) have significant phenotypic variation between populations, suggesting potential directional selection (Manier and Palumbi 2008, Marks et al 2008). How these differences in reproductive characteristics relate to the variation in other phenotypic traits, such as larval morphological plasticity or symbioses with bacteria have not been studied, although population-specific variation and differential selection would not be surprising given the differences experienced in their natural environments.

The ability of *S. droebachiensis* larval holobionts to acclimate to local feeding variation across its broad geographic distribution was used as a biological system to evaluate local versus regional effects. Specially, using *S. droebachiensis* larvae, we tested the hypothesis that host geographical origin better correlates with community composition than does local variations on food availability, and that the predicted functional gene profiles converge between host habitats. To test these hypotheses, *S. droebachiensis* larvae from three sites (Figure 1) were differentially fed, and the associated bacterial communities were assayed and used to coarsely predict functions of the metagenome.

Experimental Procedures

Adult urchin collection and larval rearing

Adult *S. droebachiensis* were collected from populations in the North Sea in March 2015, the Salish Sea in April 2016, and the Gulf of Maine in February 2017 (Figure 1). Individuals from the North Sea were collected by divers in Droebak, Norway

113 (59°39’ N, 10°37’ E) and transported in cold and aerated seawater to the Sven Lovén
114 Centre for Marine Infrastructure (Kristineberg, Sweden). Urchins were maintained in
115 natural seawater and fed *ad libitum* on a live mix of *Ulva lactuca* and *Laminaria* spp.
116 collected from the Kristineberg shoreline. Urchins from the Salish Sea were hand-
117 collected at low tide at Cattle Point, San Juan Island, USA (48°27' N, 122°57' W),
118 transferred to the Friday Harbor Laboratories within one hour, suspended in sub-tidal
119 cages off the dock at FHL, and fed *Nereocystis* spp. *ad libitum* until spawning two weeks
120 later. Lastly, individuals from the Gulf of Maine were collected from Frenchman Bay,
121 Maine (44°25' N 68°12' W), shipped overnight to the Darling Marine Center, and were
122 maintained in flow-through aquaria and fed *Saccharina latissima ad libitum* until
123 spawning within one week.

124 Adult urchins were spawned with a one- to two-mL intracoelomic injection of
125 0.50 M KCl. For each population, gametes from three males and three females were
126 separately pooled. Fertilization of eggs and larval rearing followed Strathmann (1987),
127 except, to include the environmental microbiota, embryos and larvae were reared using
128 5.0-µm filtered seawater (FSW). Briefly, embryos were incubated in one-liter of FSW at
129 ambient temperature and salinity, and two hours post-fertilization were transferred to
130 three or five-L of FSW, divided into triplicates, and larval density was adjusted to two
131 larvae•mL^{-1} and subsequently diluted as larvae reached the 6- and 8-armed stages. Larval
132 cultures were given 90 to 95% water changes every other day by reverse filtration.

133 Monocultures of *Rhodomonas lens* were grown at room temperature with f/2
134 media and a combination of ambient and artificial lighting (Guillard 1975).
135

Experimental feeding and larval morphometrics

At 48 hours post-fertilization, prism-stage larvae were divided into three replicate jars for each of the four experimental feeding treatments varying in *R. lens* quantity: 10,000, 1,000, 100, or 0 cells•mL^{-1}. For each experiment, larvae fed 10,000 cells•mL^{-1} were reared through metamorphosis while diet-restricted and started larvae were cultured until developmental stasis (Supplemental Table 1). Larvae (n=100) from each replicate for each treatment were sampled weekly. Immediately after sampling, larval samples were concentrated into a pellet using a microcentrifuge and all seawater was removed. Pelleted larvae were then preserved in RNAlater and stored at -20 °C before DNA extractions.

Complementary to sampling *S. droebachiensis* larvae, the environmental microbiota from the seawater was also sampled. When larval cultures were sampled weekly, triplicates of ~1-L of seawater was filtered onto a 0.22-µm Millipore filter to retain the environmental microbiota. Full filter disks were then preserved in RNAlater and stored at -20 °C before DNA extractions.

In addition to sampling larvae to assay the associated bacterial communities, twenty larvae (n=20) from a single replicate from each dietary treatment were sampled for morphometric analysis. Larvae were imaged using a compound microscope (Salish Sea: Nikon Eclipse E600; camera: QImaging MicroPublisher 5.0 RTV; Gulf of Maine: Zeiss Primo Star HD digital microscope; North Sea: Leica stereomicroscope) and morphometrics (length of larval body, post-oral arms, and stomach area; Supplementary Figures 1-2) were measured using ImageJ, v. 1.9.2 (Schneider et al 2012). We tested whether larval morphology and stomach volume were influenced by differential feeding

159 over time using a two-way ANOVA (JMP Pro v. 13), and a whether this pattern was site-
160 specific using a one-way ANOVA. Where statistical differences were observed ($p<0.05$),
161 we used a post-hoc test to determine the affect at each time point and for each diet.
162
163 *Assaying microbial communities*
164 We extracted total DNA from larval samples using the GeneJet Genomic DNA
165 Purification Kit (Thermo Scientific). For filtered seawater samples, we extracted eDNA
166 using the FastDNA Spin Kit for Soil (MP Biomedical). DNA was then quantified using
167 the NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific) and diluted to 5
168 $ng \bullet \mu L^{-1}$ using RNase/DNase-free water.
169 Bacterial sequences were amplified using 'universal' primers for the V3/V4
170 regions of the 16S rRNA gene (Forward: 5′ CTACGGGNGGCWGCAG, Reverse: 5′
171 GACTACHVGGGTATCTAATCC) developed by (Klindworth et al 2013). Products
172 were purified using the Axygen AxyPrep Mag PCR Clean-up Kit (Axygen Scientific),
173 indexed via PCR using the Nextera XT Index Kit V2 (Illumina Inc.), and then purified
174 again. At each of these steps, fluorometric quantitation was performed using a Qubit
175 (Life Technologies) and libraries were validated using a Bioanalyzer High Sensitivity
176 DNA chip (Agilent Technologies). Illumina MiSeq sequencing (v3, 600 cycles) was
177 performed at the University of North Carolina at Charlotte.
178 Forward and reverse sequences were paired and trimmed using PEAR (Zhang et
179 al 2014) and Trimmomatic (Bolger et al 2014), respectively, converted from fastq to fasta
180 using custom script (Supplemental Note 1), and, prior to analysis of bacterial 16S rRNA
181 sequences, chimeric sequences were detected using USEARCH (Edgar et al 2011) and

removed using filter_fasta.py. Using QIIME 1.9.1 (Caporaso et al 2010), bacterial 16S rRNA sequences were analyzed and grouped into operational taxonomic units (OTUs) based on a minimum 97% similarity. The biom table generated by the pick_open_reference_otus.py script was filtered of OTUs with less than ten reads as well as sequences matching chloroplast for cryptophytes (*i.e.*, *R. lens*; after (Carrier and Reitzel 2018).

Using the filtered biom table and "biom summarize-table" function to count total sequences per sample, the rarefaction depth of 3,193 was determined and applied to all subsequent analyses (Supplemental Figure 1). Beta diversity was calculated using the weighted UniFrac (Lozupone and Knight 2005), and principal coordinate analyses (PCoA) were visualized in EMPeror (Vazquez-Baeza et al 2013) and recreated using PhyloToAST (Dabdoub et al 2016) or stylized for presentation in Adobe Illustrator CS6. Community composition was generated using summarize_taxa_through_plots.py script and visualized using Prism 7 (GraphPad Software). Community similarity across phenotypes, dietary states, and developmental stages were compared statistically using an ANOSIM as part of the compare_categories.py script.

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

Functional predictions using PICTUSt

For the QIIME-generated OTU (*i.e.*, biom) table to be compatible with PICTUSt (Langille et al 2013), all *de novo* OTUs were filtered according to the Greengenes (v. 13.5) database. Closed OTU tables (that retained 57.8% and 88.2% of OTUs from full

and 'shared' communities, respectively) were normalized by copy number, upon which metagenomic gene profiles were predicted and categorized by biological function. The PICTUSt output was made compatible for STAMP using biom_to_stamp.py from Microbiome Helper (Comeau et al 2017). These metadata were subsequently analyzed using STAMP (Parks et al 2014) to test for a population-specific predicted functional profile. The principle coordinate from STAMP were compared statistically using a one-way ANOVA (JMP Pro, ver. 13), as part of the STAMP package. We then generated taxa plots for Gene Ontology groups of interest using metagenome_contributions.py and custom scripts.

A step-by-step listing of PICTUSt scripts used to convert from QIIME and the subsequent data analysis is located in Supplementary Note 1.

Results

Larval holobionts and the feeding environment

Diet-induced morphological plasticity was recorded for *S. droebachiensis* larvae from each population, with the pattern of expression being location-specific (ANOVA, $p<0.0001$; Supplemental Figures 2-3). Diet-restricted larvae from the Salish Sea and Gulf of Maine exhibited a higher post-oral arm to mid-body line ratio than *ad libitum* counterparts (ANOVA, $p<0.0001$; Supplemental Figure 2; Supplemental Table 2), even though analyses of Gulf of Maine larvae were confounded by developmental stage (Supplemental Table 1). 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-restriction (ANOVA, $p<0.0001$; Supplemental Figure 2; Supplemental Table 2).

228 *S. droebachiensis* larvae from each population associated with a diet-specific
229 bacterial community (Supplemental Figures 4-6; ANOSIM, Supplemental Table 4).
230 Larvae from the Salish Sea and Gulf of Maine exhibited similar diet-specific community-
231 level patterns (Supplemental Figures 4-5; ANOSIM, Supplemental Table 4), where the
232 bacterial consortium of food-restricted individuals generally were more similar to each
233 other than to well-fed counterparts. Larvae from the North Sea, on the other hand,
234 exhibited the opposite response (Supplemental Figure 6), where diet-specific bacterial
235 communities were still observed (ANOSIM, Supplemental Table 4) except that all food
236 rations were more similar to each other than to starved individuals (Supplemental Figure
237 6). In addition to diet-specificity, larvae from each population associated with bacterial
238 communities that were specific to phenotype (Supplemental Figure 7; ANOSIM,
239 Supplemental Table 4) as well as varied with developmental stage and/or
240 ecological/stochastic drift (Supplemental Figure 8-10; ANOSIM, Supplemental Table 4)
241 and were distinct from the environmental bacterial community (Supplemental Figure 11;
242 ANOSIM, Supplemental Table 4).
243
244 *Location-specific bacterial communities*
245 Variation in OTU diversity and the relative proportions of those taxa associated
246 with *S. droebachiensis* larvae were best correlated with geography (ANOSIM, $p<0.001$;
247 Figure 2), where larvae from the Western and Eastern Atlantic Ocean were more similar
248 to each other than to those from the Pacific Ocean (Figure 2; Supplemental Figure 12A).
249 Site-specific bacterial communities of *S. droebachiensis* larvae were independent of
250 plasticity state, developmental stage, and feeding regime (Figure 2; Supplemental Figures

4-10, 13-14; Supplemental Table 2), even though larvae at each site associated with phenotype- (Supplemental Figure 7), diet- (Supplemental Figure 8-10), and development- (Supplemental Figure 4-6) specific bacterial communities (Supplemental Table 2). Moreover, the structure of the bacterial community associated with Gulf of Maine larvae are taxonomically richer and the most diverse while larvae from the Salish Sea were the least rich and diverse, leaving those from the North Sea as intermediate (Table 1).

Of the thousands of OTUs associated with *S. droebachiensis* larvae across sites, phenotypes, developmental stages, and diets, ~32.7% were found in at least one sample at each of the three sites (Supplemental Figure 15). Moreover, ~8.1% to ~13.0% of all OTUs were shared between two sites, and ~10.8% to ~12.7% were unique to a single site (Supplemental Figure 15). When clustered by bacterial classes, *S. droebachiensis* larvae from the North Sea primarily associate with γ-proteobacteria (34.2%; Phylum: Proteobacteria), α-proteobacteria (26.8%; Phylum: Proteobacteria), Flavobacteriia (19.5%; Phylum: Bacteroidetes), and Saprospirae (12.3%; Phylum: Bacteroidetes), while larvae from the Gulf of Maine primarily associated with γ-proteobacteria (49.1%;), α-proteobacteria (17.8%), Flavobacteriia (17.7%), and, lastly, larvae from the Salish Sea primarily associate with Flavobacteriia (44.3%), α-proteobacteria (23.2%), and γ-proteobacteria (20.0%) (Supplemental Figure 15).

Dynamics of shared taxa

For balanced inter-population comparisons, only OTUs in at least one sample from each population were retained. This restriction yielded 4,502 shared OTUs (Supplemental Figure 15), which were divided by population and subsequently filtered to

only include 'core' taxa (*i.e.*, those found in all samples for a given urchin population). Inclusion of the 'core' OTUs for each population totaled 178 OTUs (Supplemental Figure 16). An unweighted and weighted comparison of these OTUs suggest that these 'core' communities associated with *S. droebachiensis* larvae is, again, best correlated with geography (ANOSIM, $p<0.001$; Figure 3), with larvae from the Atlantic Ocean being more similar to each other than larvae from the Pacific Ocean (Figure 3; Supplemental Figure 12B).

Of the combined 'core' bacterial communities associated with *S. droebachiensis* larvae, three OTUs (~1.8%) were found in all samples within and between populations: an unclassified species in the class γ-proteobacteria (OTU number: 1106577), an unclassified species in the family Flavobacteriaceae (OTU number: 1105269), and an unclassified species in the genus *Polaribacter* (OTU number: 586650). Of the 178 OTUs, 27 OTUs were shared between North Sea and Gulf of Maine samples, four between all Gulf of Maine and Salish Sea samples, and three between all Salish Sea and North Sea samples (Supplemental Figure 16). Furthermore, 6, 52, and 78 OTUs were specific to *S. droebachiensis* larvae from the Salish Sea, Gulf of Maine, and North Sea, respectively (Supplemental Figure 16). When clustered by bacterial classes, these 'core' communities associated with *S. droebachiensis* larvae, individuals from the North Sea primarily included α-proteobacteria (41.8%; Phylum: Proteobacteria), γ-proteobacteria (34.8%; Phylum: Proteobacteria), and Flavobacteriia (20.9%; Phylum: Bacteroidetes), while larvae from the Gulf of Maine primarily associated with γ-proteobacteria (57.0%), α-proteobacteria (17.4%), and Flavobacteriia (17.1%), and larvae from the Salish Sea

296 associate with Flavobacteriia (67.3%) and α-proteobacteria (30.0%) (Supplemental
297 Figure 16).
298
299 *Predicted community function and representative taxa*
300 Similar to the 16S rRNA assays, PICRUSt-generated metagenomic gene profiles
301 of the full (ANOVA, $p<0.0001$; Figure 4A) and shared (ANOVA, $p<0.0001$; Figure 4B)
302 bacterial community associated with *S. droebachiensis* larvae were specific to urchin
303 biogeography. Predicted gene content of PICRUSt-generated metagenomes were, on
304 average, ~55.1% metabolism, ~17.9% genetic information processing, ~12.5%
305 environmental information processing, and ~7.2% cellular processes and signaling
306 (Supplemental Figures 17-18). Of these, total gene content was significantly different
307 between locations for metabolism (ANOVA, $p<0.0001$), cellular processes (ANOVA,
308 $p<0.0001$), and cellular processes and signaling (ANOVA, $p<0.0001$) (Supplemental
309 Figure 17-18). Moreover, of only the shared or 'core' community ~55.8% metabolism,
310 ~17.3% genetic information processing, ~12.8% environmental information processing,
311 and ~6.8% cellular processes and signaling (Supplemental Figure 19-20), with total gene
312 content being significantly different between locations for metabolism (ANOVA,
313 $p<0.0001$), cellular processes (ANOVA, $p<0.0001$), and cellular processes and signaling
314 (ANOVA, $p<0.0001$).
315 More than half of the gene content of the predicted *S. droebachiensis* larval
316 metagenome was related to metabolism (Supplemental Figures 17-18). Several of the
317 bacterial classes from the Salish Sea, Gulf of Maine, and North Sea are predicted to
318 contribute to metabolism. For *S. droebachiensis* larvae from the Salish Sea this group of

bacteria primarily included the α-proteobacteria (41.5%), γ-proteobacteria (29.7%), and Flavobacteriia (21.8%); larvae from the Gulf of Maine primarily included α-proteobacteria (41.9%), γ-proteobacteria (39.1%), Flavobacteriia (16.5%); and larvae from the North Sea primarily included γ-proteobacteria (40.5%), α-proteobacteria (29.8%), and Flavobacteriia (25.8%) (Figure 6).

Discussion

Comparisons of the bacterial communities associated with *S. droebachiensis* larvae across dietary treatments and host biogeography suggests three primary findings. First, the composition of the bacterial community associated with *S. droebachiensis* larvae is best correlated with location. Second, for each of the geographical regions, urchin larvae associated with a microbial community specific to phenotype, developmental stage, and dietary state. Lastly, the predicted metagenomic profiles are site-specific and primarily related to metabolism.

Marine invertebrate larvae experience a feeding environment that varies in space, time, and composition (Bidigare and Ondrusek 1996, Chevez et al 1996, Cloern and Jassby 2010, Milici et al 2016, Needham and Fuhrman 2016). In response to this variation, planktonic larvae can arrest their development and/or increase the frequency of encounter rates by enlarging their feeding structure (Boidron-Metairon 1988, Byrne et al 2008, Carrier et al 2015, Carrier and Reitzel 2018, Hart and Strathmann 1994, McAlister and Miner 2018, Miner 2004, Miner 2011, Soars et al 2009). Plasticity in development and morphology have historically been viewed as a means for the host to acclimate (Bradshaw 1965, Hart and Strathmann 1994, McAlister and Miner 2018, Miner 2011,

342 Soars et al 2009, West-Eberhard 2003). Recent work suggests this response is also linked
343 to the associated bacterial communities (Carrier and Reitzel 2018).

344 Inter-population comparisons of the dynamics of the bacterial community
345 associated with *S. droebachiensis* larvae suggest three additional, not mutually exclusive
346 inferences. First, composition of the bacterial communities is seemingly a product of the
347 host feeding environment. Second, while both community composition and predicted
348 functional profiles are dynamic across and specific to host feeding environment, the
349 functional profiles are more informative for understanding hologenomic acclimation.
350 Third, urchin larval holobionts may be locally adapted. Of these, the data presented here
351 largely support the first inference, while the latter two are supported but require specific
352 validation (*e.g.*, using molecular, genomic, and physiological assays and manipulations
353 (Williams and Carrier 2018).

354 Population-specific bacterial communities is an emerging theme of animal-
355 microbiome ecology (Dishaw et al 2014, Huang et al 2018, Marino et al 2017, Marzinelli
356 et al 2015, Mortzfeld et al 2015). For the three populations of *S. droebachiensis* used
357 here, the bacterial communities were region specific, with Gulf of Maine and North Sea
358 individuals being more similar to each other than to Salish Sea larvae. The environmental
359 conditions of these regions are different, and the selective pressures on the microbial
360 partners, larva, and holobiont likely vary (Bordenstein and Theis 2015). Differential
361 selection on multiple components of the *S. droebachiensis* larval holobiont may result in
362 local adaption (Pespeni et al 2013, Sanford and Kelly 2011). The feeding environment-
363 specific differences in the microbial community and predicted metagenomic gene profiles
364 are suggestive of potential adaptation, where the functional microbial community may aid

365 in acclimating to unique oceanographic conditions of these three *S. droebachiensis* larval
366 populations face.

367 Previous studies on the populations of *S. droebachiensis* larvae documented that
368 phenotypic traits varied across host geography. Manier and Palumbi (2008), for example,
369 reported significant differences in sperm morphology between urchins across this spatial
370 scale and found evidence of strong directional selection for sperm traits between
371 locations, particularly between *S. droebachiensis* in the Pacific and Western Atlantic.
372 Population genetic studies of *S. droebachiensis*, on the other hand, show significantly
373 higher F_{ST} values between the Eastern and Western Atlantic than the Pacific and Western
374 Atlantic due to more frequent genetic exchange through the Bering Strait (Addison and
375 Hart 2004, Biermann et al 2003, Manier and Palumbi 2008, Palumbi and Wilson 1990).
376 Consistent with sperm morphology, the population-specific differences in bacterial
377 communities and predicted metagenome is suggestive of environmental influence
378 shaping this variation over, perhaps, the last few hundred thousand years. Future
379 population genomic studies of *S. droebachiensis* should identify outlier loci that correlate
380 with specific differences in OTUs and characterize the larval metagenome to provide a
381 window into how animal genetic variation and the environmental conditions may shape
382 the associated microbial community.

383 A growing body of literature suggests that planktotrophic larvae utilize diverse
384 'alternative' nutritional resources (Feehan et al 2018, Manahan et al 1993, Rivkin et al
385 1986). Based on our predicted metagenomic gene profiles, we propose that planktotropic
386 larvae are aided by metabolites derived from their bacterial symbionts. This is of
387 particular importance because larvae often inhabit food-limited environments (Fenaux et

388 al 1994, Olson and Olson 1989, Pauley et al 1985). To decrease mortality due to
389 starvation (Morgan 1995, Rumrill 1990, Young and Chia 1987), a metabolic input from
390 the symbiont community may serve as a physiological buffer and complement metabolic
391 depression induced by diet-restriction (Carrier et al 2015).

392 Our comparisons of the bacterial communities associated with *S. droebachiensis*
393 larvae suggest that geographic location better correlated with community composition
394 than local biological (*e.g.*, phenotype) and ecological variation (*e.g.*, diet quantity). This
395 type of specific comparison suggests that in studying the dynamics of animal—and
396 perhaps plant—interactions with their associated bacterial community, location may drive
397 the taxonomic profiles, and that in transitions towards functional or predicted functional
398 comparisons, the potential for local adaptation should be considered (Kelly et al 2014,
399 Pespeni et al 2013, Sanford and Kelly 2011).

400

401 **Conflict of Interest**

402 The authors declare that they have no conflict of interest.

403

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417

418 **Figure and Table Legends**

419

420 **Figure 1.** Location of *Strongylocentrotus droebachiensis* experiments. Cartoon
421 representation of adult populations and the geographical distribution for where
422 differential feeding experiments were conducted.

423

424 **Figure 2.** Similarity of the microbial communities associated with *Strongylocentrotus*
425 *droebachiensis* larvae between sites. Community similarity of the *S. droebachiensis*
426 larval microbiome between three geographic locations, independent of phenotype,
427 developmental stage, and dietary state when considering only taxa (A) and taxa and their
428 relative abundance (B).

429

430 **Figure 3.** Similarity of the shared bacterial OTUs associated with *Strongylocentrotus*
431 *droebachiensis* larvae between sites. Community similarity of the bacterial OTUs found
432 in at least a single sample of *S. droebachiensis* larvae from each of the three sites of
433 interest. Comparisons were made between three geographic locations, and independent of
434 phenotype, developmental stage, and dietary state when considering only taxa (A) and
435 taxa and their relative abundance (B).

436

437 **Figure 4.** Similarity of the predicted *Strongylocentrotus droebachiensis* larval
438 metagenome at each site. Community similarity of the gene profiles of the
439 *Strongylocentrotus droebachiensis* larval metagenome as predicted by PICTUSt.

440

441 **Figure 5.** Bacterial taxa of predicted metabolic function. Relative proportions of bacterial
442 classes linked to the predicted metabolic functionality of the *Strongylocentrotus*
443 *droebachiensis* larval metagenome, as predicted by PICTUSt.

444

445 **Table 1.** Alpha diversity indices of the microbial communities associated with
446 *Strongylocentrotus droebachiensis* larvae from three geographical locational.

447

448

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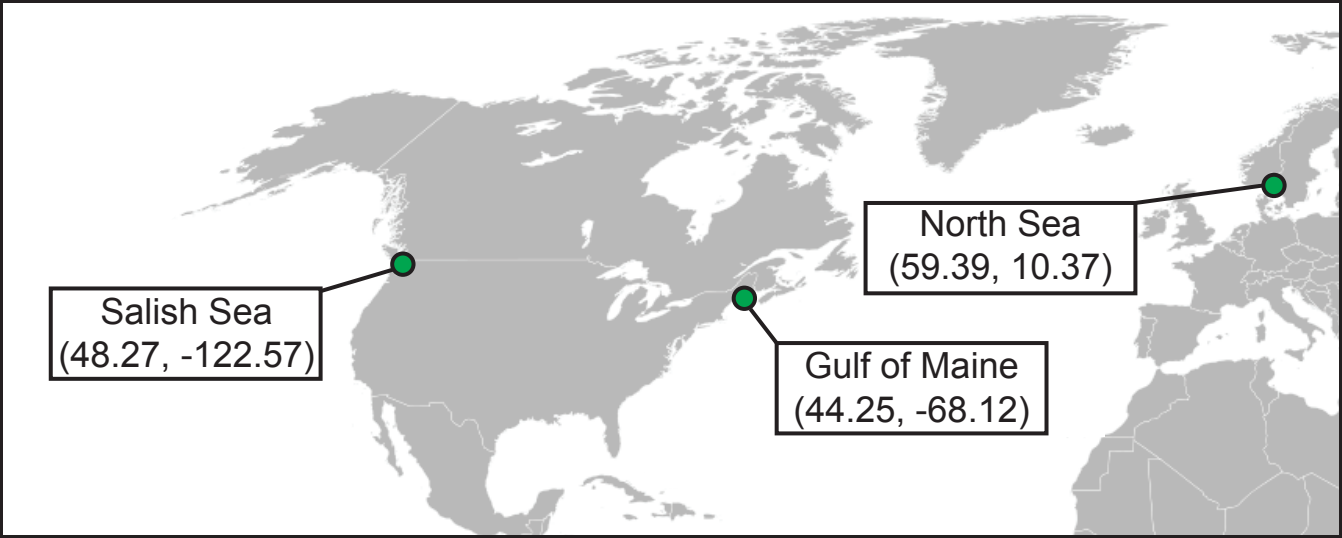
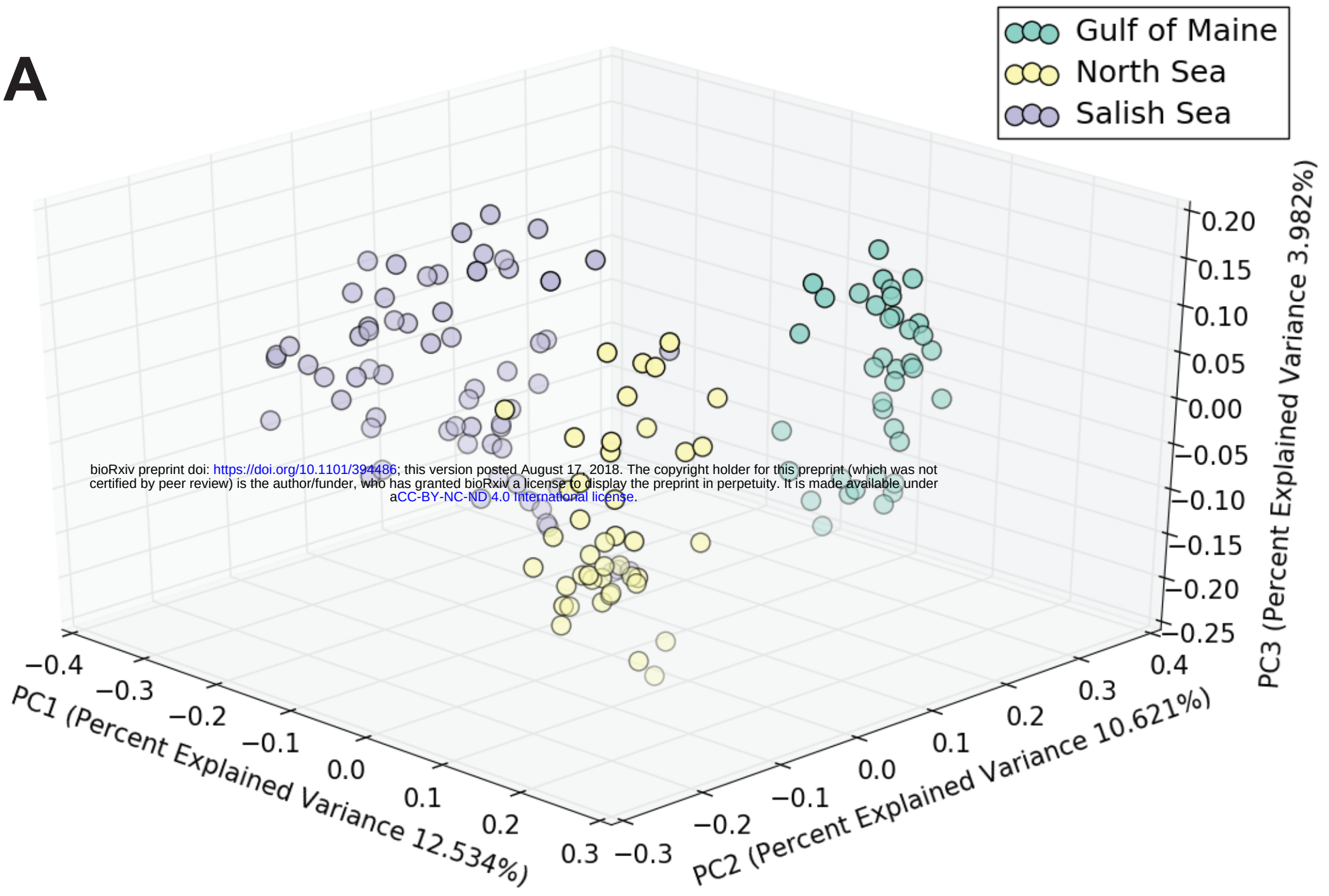
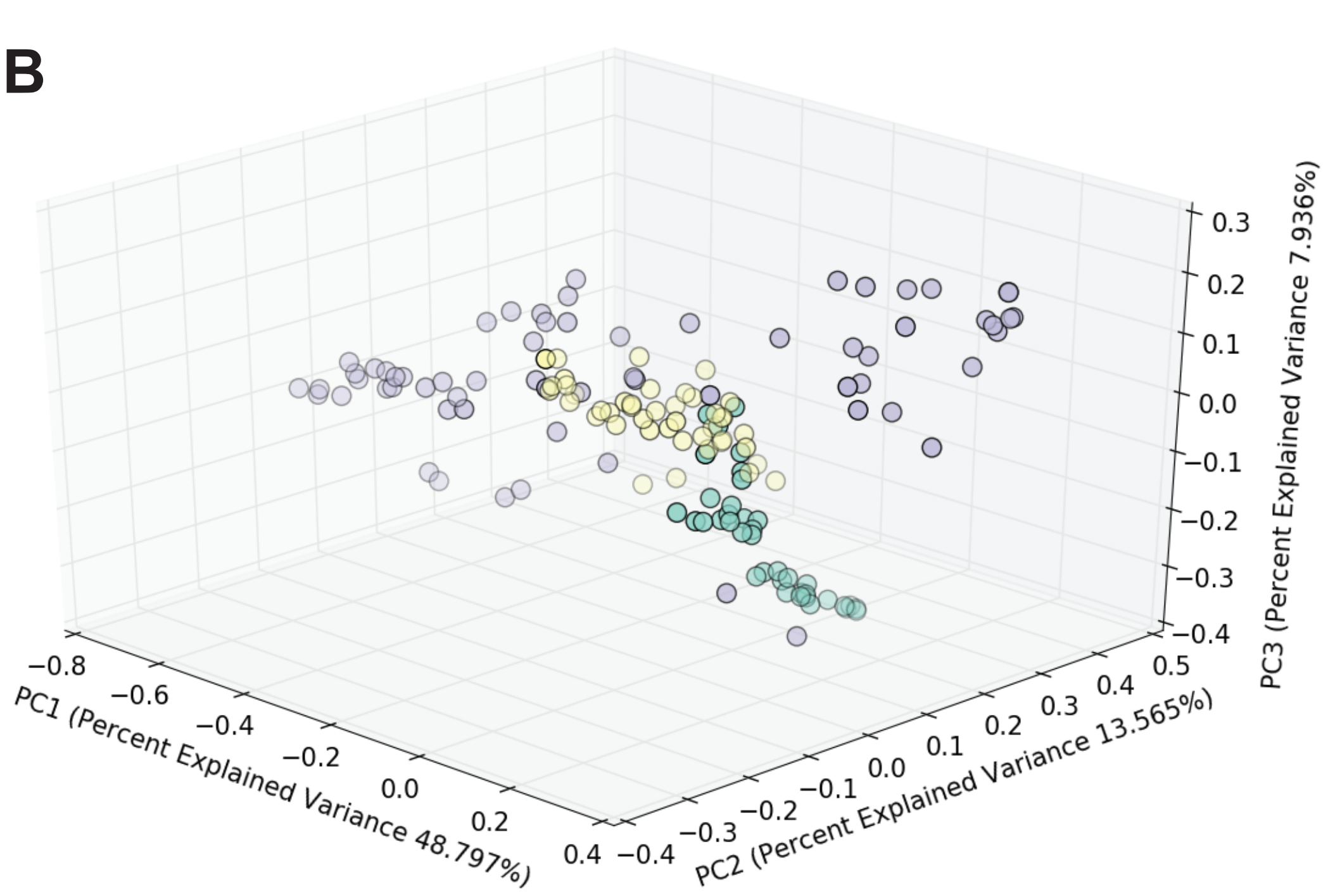

North Sea
(59.39, 10.37)
Salish Sea
(48.27, -122.57)
Gulf of Maine
(44.25, -68.12)

A



B



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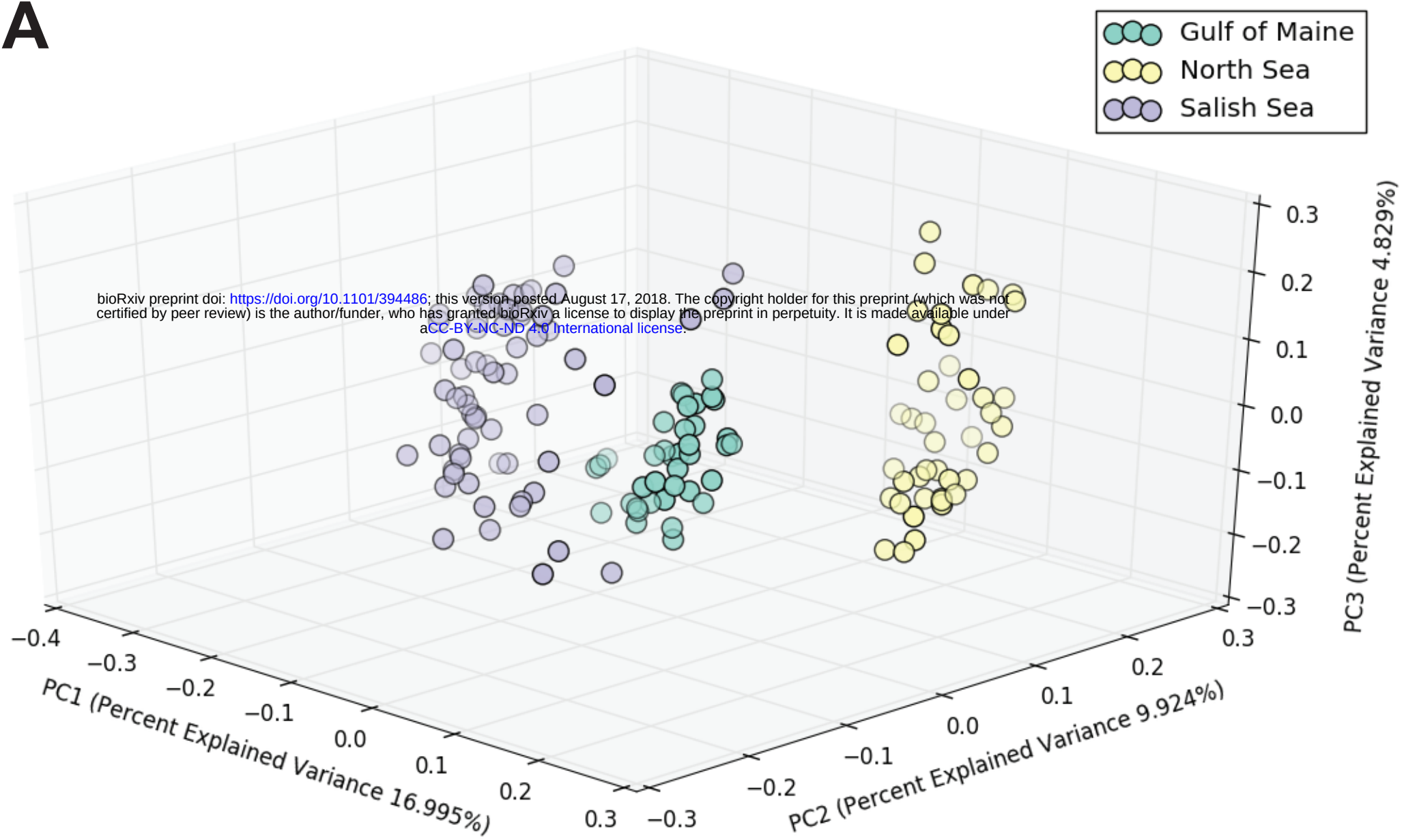

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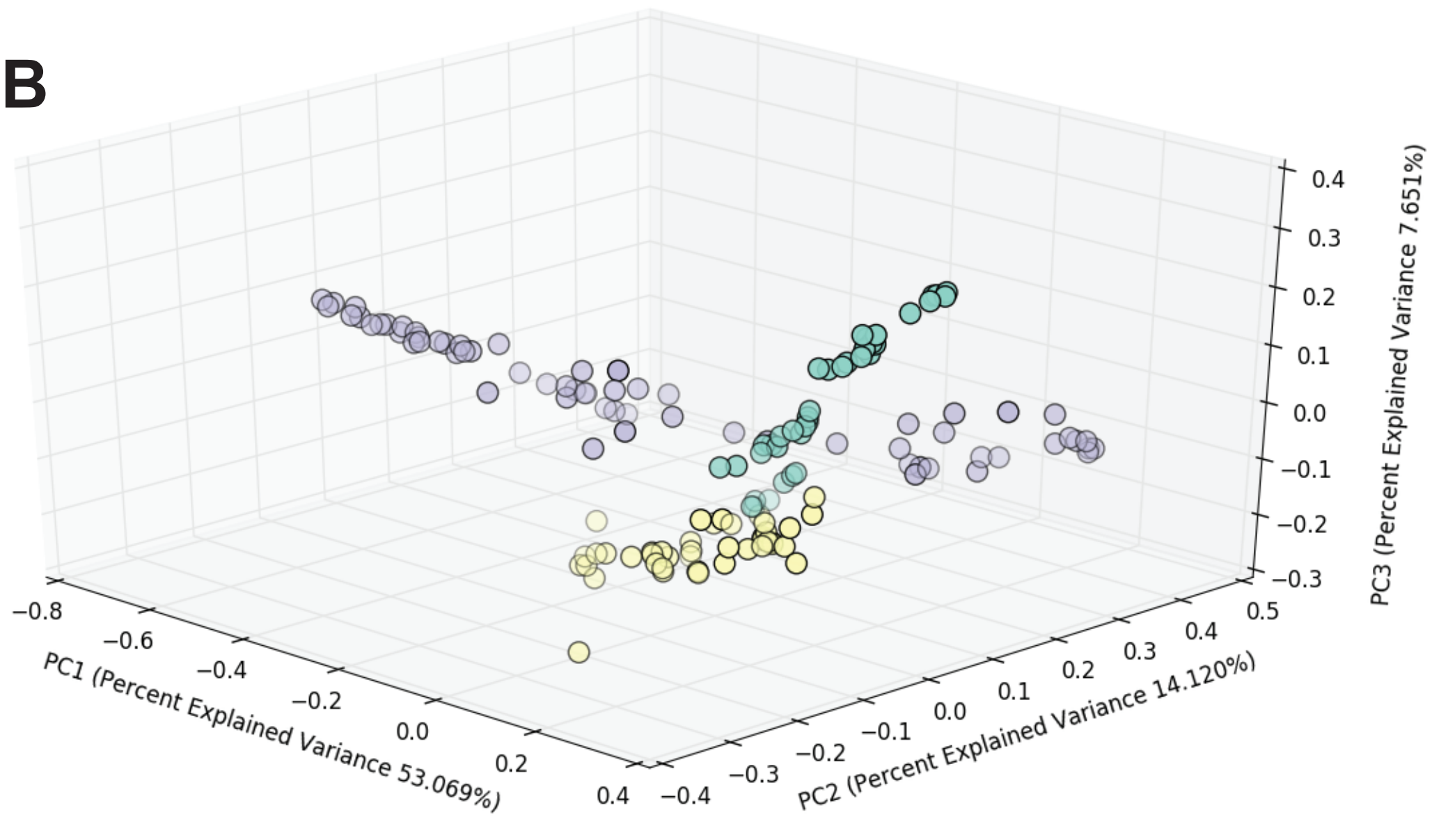

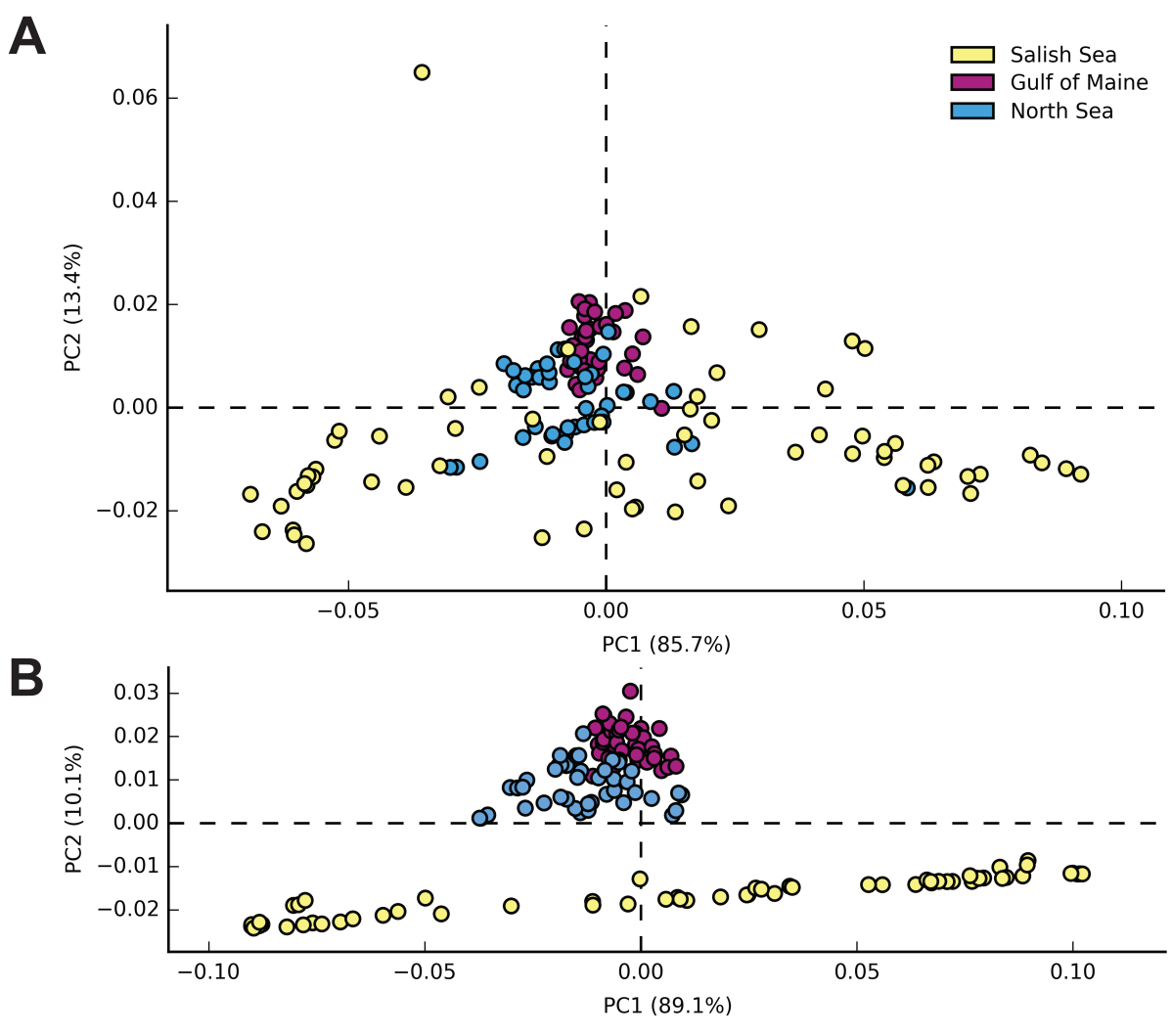
A
Salish Sea
Gulf of Maine
North Sea
PC2 (13.4%)
0.06
0.04
0.02
0.00
−0.02
−0.05
0.00
0.05
0.10
PC1 (85.7%)
B
PC2 (10.1%)
0.03
0.02
0.01
0.00
−0.01
−0.02
−0.10
−0.05
0.00
0.05
0.10
PC1 (89.1%)

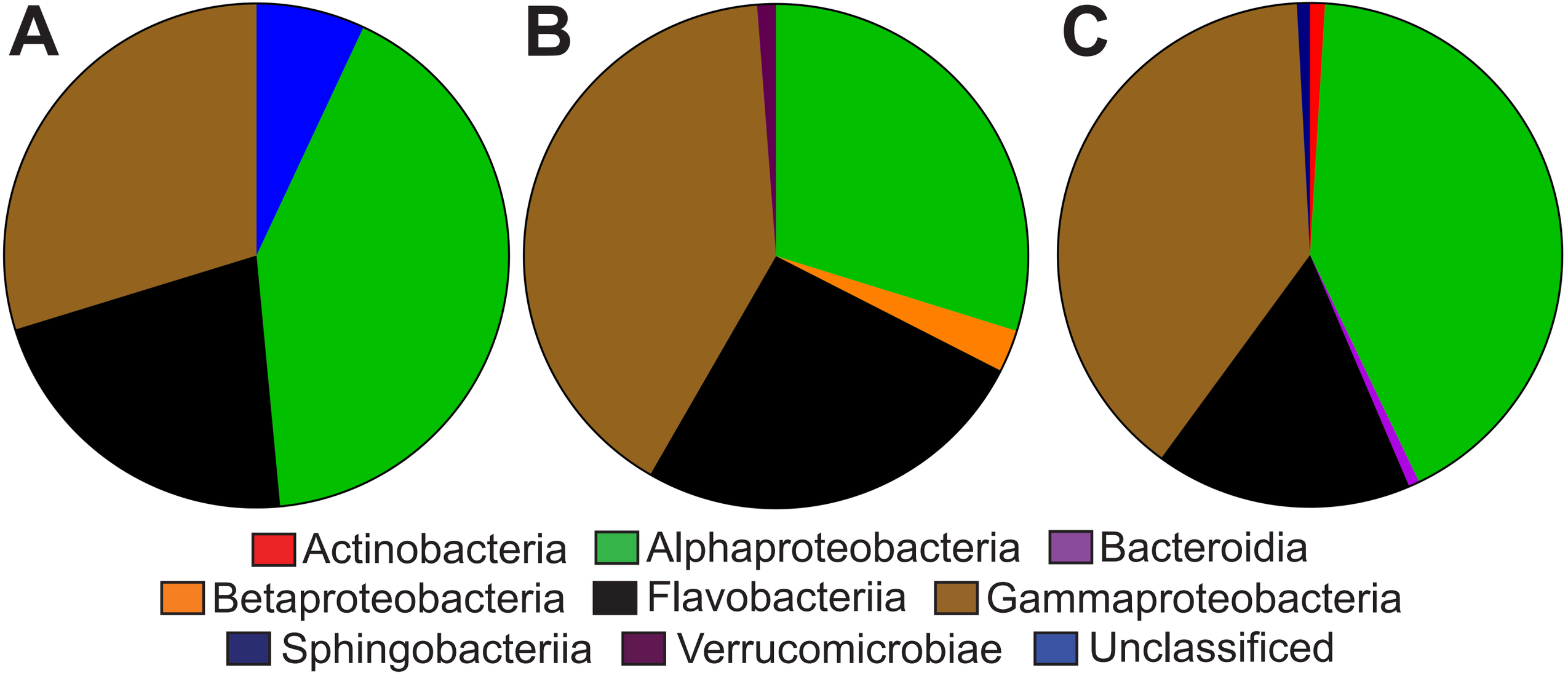
A
B
C
Actinobacteria
Alphaproteobacteria
Bacteroidia
Betaproteobacteria
Flavobacteriia
Gammaproteobacteria
Sphingobacteriia
Verrucomicrobiae
Unclassificed

Table 1. Alpha diversity indices of the microbial communities associated with *Strongylocentrotus droebachiensis* larvae from three geographical locations.

		Salish Sea	**Gulf of Maine**	**North Sea**	SS	*F*-ratio	*P*
Fisher	Mean	252.3	464.69	347.49	1.08E+06	81.67	**<0.0001**
	SE	8.82	13.09	14.65			
		C	A	B			
Shannon	Mean	4.66	7.48	6.71	2.21E+02	108.79	**<0.0001**
	SE	0.02	<0.01	0.01			
		C	A	B			
Simpson	Mean	0.77	0.97	0.95	1.31E+00	61.35	**<0.0001**
	SE	<0.01	<0.01	<0.01			
		B	A	A			