

2 **1 Title**

3 **Environmental DNA Metabarcoding Reveals Winners and Losers of Global Change  
4 in Coastal Waters**

5 **Ramón Gallego <sup>a,b,1</sup>, Emily Jacobs-Palmer <sup>b</sup>, Kelly Cribari <sup>b</sup>, Ryan P. Kelly<sup>b</sup>**

6 <sup>a</sup> NRC Research Associateship Program, Northwest Fisheries Science Center, National Marine Fisheries  
7 Service, National Oceanic and Atmospheric Administration. 2725 Montlake Boulevard East, Seattle, WA  
8 98112

9 <sup>b</sup> School of Marine and Environmental Affairs. University of Washington. 3707 Brooklyn Ave NE Seattle,  
10 WA 98105

11 <sup>1</sup>To whom correspondence should be addressed. E-mail: rgallego@uw.edu

12 **Author contributions:** RG led sampling, laboratory work, data analysis, and writing. RPK  
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16 **2 Abstract**

17 Studies of the ecological effects of global change often focus on one or few species at a time.  
18 Consequently, we know relatively little about the changes underway at real-world scales of biological  
19 communities, which typically have hundreds or thousands of interacting species. Here, we use  
20 monthly samples of environmental DNA to survey over 222 planktonic taxa along a gradient of  
21 temperature, salinity, dissolved oxygen, and carbonate chemistry in nearshore marine habitat. The  
22 result is a high-resolution picture of changes in ecological communities using a technique replicable  
23 across a wide variety of ecosystems. We estimate community-level differences associated with  
24 time, space and environmental variables, and use these results to forecast near-term community  
25 changes due to warming and ocean acidification. We find distinct communities in warmer and  
26 more acidified conditions, and a strong reduction in richness of current diatom assemblages. Taxa  
27 finding greater suitable habitat in near-future waters are more taxonomically varied and include  
28 the ubiquitous coccolithophore *Emiliania huxleyi* and the harmful *Alexandrium sp.* These results  
29 suggest foundational changes for nearshore food webs under near-future conditions.

30 **3 Keywords**

31 Ocean Acidification | environmental DNA | metabarcoding | ecosystem response | climate change

32 **4 Main Text**

33 **4.1 Background**

34 As ocean acidification and warming continue apace, changes in the marine environment are having  
35 an effect on many species' metabolism, development, growth and reproduction success [35, 18, 4,  
36 12], very likely altering food webs [51, 9, 25] and species' interactions in ways that are poorly  
37 understood. Laboratory or mesocosm-based manipulation experiments have documented a wide  
38 variety of biological responses under projected climate scenarios of  $p\text{CO}_2$ , pH, solar radiation,  
39 salinity and temperature [21, 14, 38], showing an array of species-specific responses among particular  
40 taxa of interest. However, information regarding multi-species or community-wide responses to  
41 these stressors is far more limited [36, 30]. The scarcity of such data is likely attributable to the  
42 difficulty of simultaneously tracking the responses of many species in the field, and to the difficulty  
43 of identifying natural systems that adequately reflect the environmental gradients under study.

44 Two natural  $\text{CO}_2$  seeps in nearshore marine habitats – one in Italy and one in Papua New Guinea  
45 – have demonstrated shifts in benthic communities associated with especially acute acidification in  
46 the present day, previewing those we might expect at a more global scale under future conditions  
47 [36, 19]. But beyond these exceptional sites, it is difficult to measure changes in ecological com-  
48 munities associated with the relatively subtle shifts in nearshore ocean chemistry observed to date,  
49 particularly in light of naturally large spatial and temporal variation in these communities. The  
50 Puget Sound in Washington, USA, offers a gradient of carbonate chemistry parameters and other

51 environmental conditions in close geographic proximity. Complex bathymetry, water circulation  
52 patterns, and nearshore landforms create intertidal sites exposed to large variations in tempera-  
53 ture,  $p\text{CO}_2$ , pH, and related parameters [34], creating an opportunity to test the effect of these  
54 measures on marine communities under conditions expected worldwide in the near future [46], and  
55 time-series sampling across the spatial gradient lets us control for site- and season-specific effects.  
56 This study system therefore provides a powerful means of modeling community-level responses to  
57 changing environmental conditions.

58 Even given the appropriate environmental gradients, tracking the biological responses of many  
59 taxa simultaneously remains challenging. Environmental DNA (eDNA) metabarcoding [27, 31]  
60 addresses this problem by amplifying a common gene region out of DNA present in a water sample;  
61 the technique can detect hundreds to thousands of taxa per sample, potentially with species-level  
62 identification.

63 A growing body of evidence supports the efficacy of eDNA metabarcoding for monitoring bio-  
64 diversity (see a review in [52]), and this approach has been successfully used to detect community  
65 composition variation across environmental changes in aquatic [17], estuarine [11, 39], and marine  
66 ecosystems [5, 16].

67 Here we use a temporal and spatial series of metabarcoding samples to track changes in nearshore  
68 ecological communities associated with differences in pH, water temperature, and other environmen-  
69 tal variables. We use broad-spectrum PCR primers [40] to target eukaryotes specifically, identifying  
70 the likely effects of future climate scenarios on suites of planktonic taxa.

## 71 5 Methods

### 72 Sampling

73 We collected water samples to assess eDNA communities in two regions of the Salish Sea (Wash-  
74 ington, USA): San Juan Island and the Hood Canal. These sites experience substantial variation  
75 in water chemistry and other environmental conditions despite geographic proximity (ca. 300km;  
76 Fig. 1). We sampled eight sites monthly for approximately 1.5 years (March 2017 to August 2018),  
77 taking three 1L samples (biological replicates; ca. 10m apart) each month at each site (261 bottle  
78 samples total). Each sample was filtered through a 0.45  $\mu\text{m}$  cellulose filter, and the filter preserved  
79 in Longmire buffer until DNA extraction [49]. Concurrently, we collected one 120 ml water sample  
80 from each site and poisoned it with 0.1 ml of saturated  $\text{HgCl}_2$  for carbonate chemistry analysis,  
81 following [50]. We also collected *in situ* measurements of temperature, salinity and dissolved oxy-  
82 gen using a handheld multiprobe (Hanna Instruments, USA) and a portable refractometer. We  
83 note that many unmeasured variables influence planktonic communities (e.g., nutrients, sunlight,  
84 wave energy), but that our set of measured parameters clearly distinguished communities and was  
85 adequate for our purposes.

86 We characterized sample carbonate chemistry by measuring Total Alkalinity (TA; open-cell  
87 automated titration based on a 876 Dosimat plus (Metrohm AG) as part of a custom system  
88 assembled by Andrew Dickson (UCSD) and used in the laboratory of Alex Gagnon at UW) and  
89 Dissolved Inorganic Carbon (DIC; Apollo Instruments, USA;  $\text{CO}_2$  extraction system with 10%  
90 (v/v) phosphoric acid). Both measurements were calibrated and validated with certified reference  
91 material from the Scripps Oceanographic Institute. Using DIC and TA, we calculated pH and the  
92 remaining carbonate system parameters using the R package ‘seacarb’ [23].

93 Our sampled areas differed in the environmental variables driving changes in carbonate chem-  
94 istry. San Juan Island was less seasonally variable than the Hood Canal in every measured param-  
95 eter (Figure 1B); the island is more directly affected by summer coastal upwelling as a function  
96 of bathymetry and circulation patterns [46], and this appears to be the dominant influence on  
97 carbonate chemistry there. By contrast, photosynthesis and respiration likely drive much of the  
98 carbonate chemistry variation in the Hood Canal (See Supporting Information).

### 99 eDNA sequencing and bioinformatic processes

100 We purified DNA from each filtered sample using a Phenol Chloroform Isoamyl Alcohol protocol,  
101 following [49]. A 1/10 to 1/100 dilution of the extract was used as template for a PCR reac-  
102 tion targeting a 313bp fragment of cytochrome oxidase I [40]. PCR reactions were performed in  
103 triplicate and sequenced individually to quantify the stochasticity of PCR reactions on a mixed  
104 template sample, and we attached secondary indexing tags using a two-step PCR process [47].  
105 PCR conditions and protocols for sample identification followed [32], and batches of 49 to 178 mul-  
106 tiplexed samples were sequenced using MiSeq v2-500 or v3-600 sequencing kits using manufacturer  
107 protocols. On each sequencing run, we added triplicate samples consisting on DNA obtained from  
108 species not present in the marine environment under study (Red Kangaroo (*Macropus rufus*) and

109 Ostrich (*Struthio camelus*) to establish quality controls of sample assignment and quantify levels  
110 of 'tag-jumping' or sample-cross-talk [53].

111 Code for all quality-screening and bioinformatics is available in the Supporting Information,  
112 implemented in Unix and R [48]. Briefly, we used a Unix script that calls secondary programs  
113 for primer-trimming and preliminary quality-control [42, 10] we estimated the likely composition  
114 of each sample using DADA2. This approach avoids clustering, such that we retained all of the  
115 amplicon sequence variants (ASVs, *i.e.*, unique sequences); we subsequently carried out secondary  
116 quality-control and decontamination following [32]. We then assigned sequences to known taxa  
117 using phylogenetic tree placement with insect v1.1 [59]; where insect could not place individual  
118 taxa, we supplemented assignment with a COI database (CITE CRUX). We conservatively kept  
119 only taxa annotated at the level of taxonomic family, genus, or species, so we could reliably infer  
120 taxon natural history under the assuming that taxa within the same family shared broad natural-  
121 history characteristics. Using published literature and online databases, we placed every recovered  
122 taxon into a benthic/planktonic category and focused our analysis on the planktonic community  
123 (see Supporting Information).

124 By treating amplification efficiency as consistent within a given taxon, we created an index of  
125 abundance for each taxon across space and time [33], using pooled data from technical replicates  
126 and mean proportions across biological replicates. The multivariate tests (PERMANOVA, CAP,  
127 k-means clustering) used these index values as the species abundances and Bray-Curtis as the  
128 dissimilarity measurement between samples.

## 129 Year-2095 Environmental Scenario and Biological Responses

130 We estimated the distribution of environmental parameters for the overall Salish Sea in 2095 from  
131 the results of [34], which estimated an annual mean increase in temperature of 1.51 °C and mean  
132 pH decrease of 0.18 for the Salish Sea as a whole. **[ADD DETAIL HERE]**

133 For community-level projections, we coded community-cluster identity (Figure 2) as an un-  
134 ordered response variable in a multinomial logistic model, with temperature, pH, and area (Hood  
135 Canal vs. San Juan Island), as predictor variables. **Salinity is predicted to remain largely unchanged**  
136 **in future scenarios [34], and because salinity was correlated with temperature in our dataset, it was**  
137 **not an important predictor variable and we subsequently dropped it from our models.** We cal-  
138 culated the probability of each community, given these predictors, using the package 'nnet' [56].  
139 Most-common combinations of T and pH were estimated from 2-dimensional density plots for each  
140 year.

141 Within a geographic area, temperature and pH were most strongly associated with community-  
142 level changes, although these conditions varied substantially between the Hood Canal and San Juan  
143 Island sites. We therefore developed a hierarchical logistical regression model relating the presence  
144 of each taxon to temperature and pH, in which the slopes of temperature and pH effects varied by  
145 taxon, and each taxon had a unique intercept that was allowed to vary by geographic area. For  
146 each taxon, we fit these models using the Bayesian generalized linear mixed effects functions in R  
147 package rstanarm [24] for R. Model selection using WAIC supported this as the preferred model  
148 over several similar ones (see Supporting Information).

149 Given the estimated sea-surface temperatures and pH values for 2095 and observed data from  
150 2017, we then used taxon-specific logistic regression models to estimate the suitability of habitat  
151 for each taxon in the future scenario. For each point in the pH - temperature grid, we calculated  
152 species richness as the mean result of 100 independent draws of the posterior distributions of all  
153 modeled taxa.

154 We can only model responses of taxa present in our data set. That is, we may predict that the  
155 number of (for example) diatom species present will decline relative to those present today, but our  
156 data do not allow us to predict whether new species will immigrate from elsewhere or how species  
157 might evolutionarily adapt to future conditions. It is beyond the scope of our work to account  
158 for the latter, and furthermore, because of the extreme uncertainty of evolutionary responses, the  
159 predictions of species distribution models are often interpreted without considering adaptation or  
160 phenotypic plasticity [44].

## 161 Results

### 162 Variation in Carbonate Chemistry and in Ecological Communities

163 We collected intertidal environmental and genetic data from bottle samples each month between  
164 March 2017 and August 2018 (Figure 1A). Despite geographic proximity and similar overall species  
165 composition (127 of the 222 planktonic taxa were found in both regions and accounted for 98%  
166 of the sequences), the areas under study – San Juan Island and the Hood Canal – varied widely  
167 in pH, temperature, and other environmental parameters (Figure 1C), with a smooth gradient  
168 in conditions along the Hood Canal, and San Juan Island more closely resembling full marine

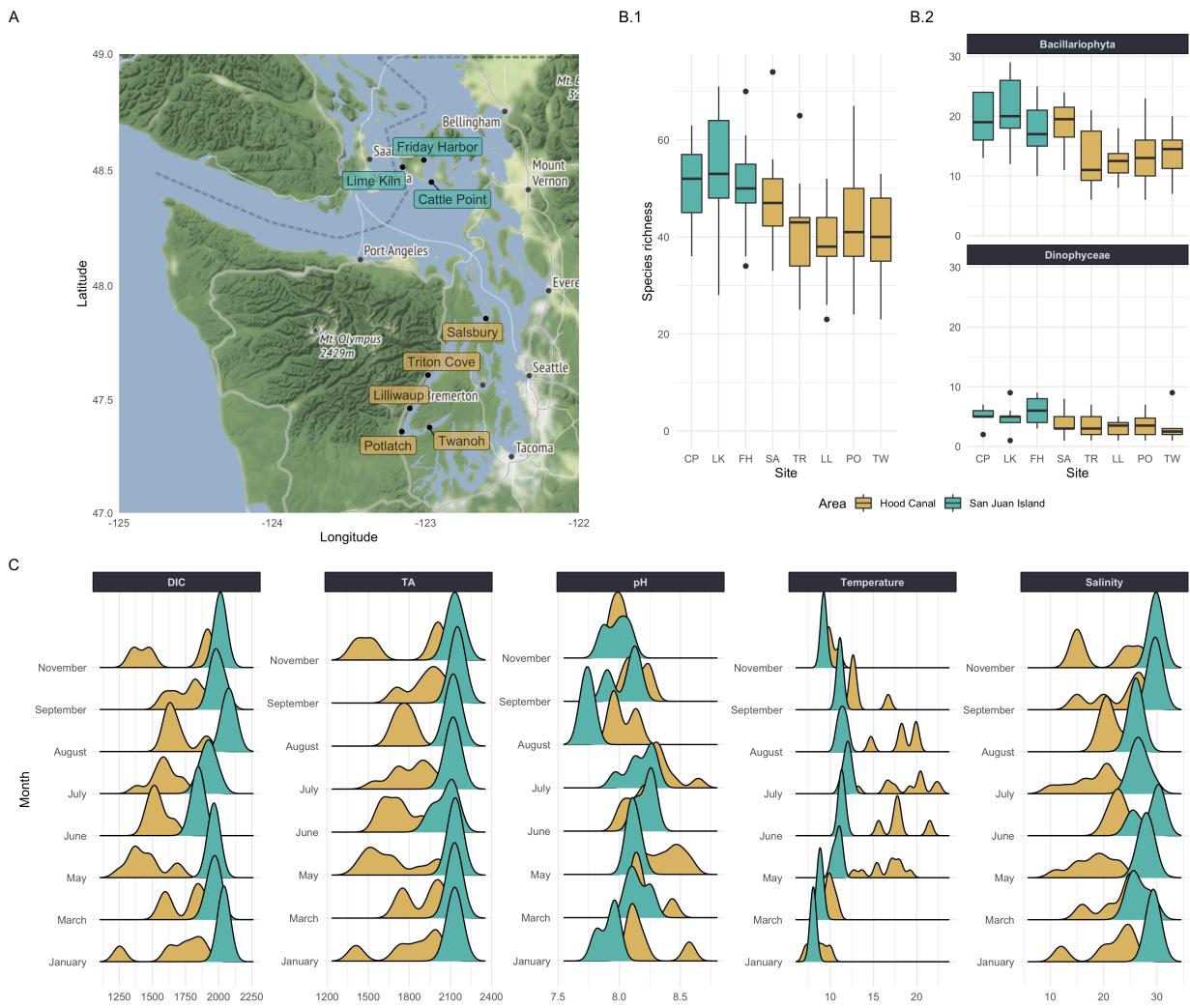


Figure 1: **A** Sampling locations in intertidal areas of the Hood Canal (Red) and San Juan Island (Blue). **B** Planktonic richness (unique taxa) per sampling locality, as reflected by the eDNA COI assay. **C** The observed environmental profiles of these two regions reflect a broad range of environmental conditions, with the Hood Canal resembling future conditions in temperate areas worldwide. Shown: Dissolved Inorganic Carbon (DIC,  $\mu\text{moles/kg}$ ); Total Alkalinity (TA); Temperature ( $^{\circ}\text{C}$ ) and Salinity (PSU). Individual sampling sites shown as facets. **CHANGE y-scale for Dinophyceae**

169 conditions. Different points along the environmental continuum simultaneously showed differences  
170 equivalent to those predicted between present-day and future oceans [8].

171 Metabarcoding analysis of eDNA samples generated more than 52.5M sequences from 745 samples.  
172 These samples represented biological and technical replicates from 86 unique sampling events.  
173 After bioinformatics quality-control (See Methods) the dataset included ~41M sequences, from  
174 4782 unique amplicon sequence variants (ASVs). Of these, 1374 ASVs (22.8M reads) could be  
175 annotated to a taxonomic level of Family or lower. These ~ 500 taxa from 43 phyla were split ac-  
176 cording to their natural history and habitat (benthic vs. planktonic; see Supporting Information).  
177 Because we expect planktonic taxa to vary with water mass [32] and therefore with bottle-sampled  
178 carbonate chemistry, here we focus on only the planktonic taxa (N = 222). These taxa showed a  
179 seasonal richness gradient between study areas, consistent with documented biodiversity clines in  
180 the area (Fig. 1B.1) [15].

181 Bray-Curtis dissimilarities among samples revealed large differences in metabarcoding commu-  
182 nities due to geographic Area (Hood Canal vs. San Juan Island; F-model = 15.4; p < 0.001).  
183 We therefore performed a constrained analysis of principal components (CAP) for samples within  
184 each combination of Season and Area, showing the differences among communities as a function  
185 of temperature, pH, and salinity (Fig. 2 shows results for Hood Canal in summer as an example;  
186 full analysis in the Supporting Information). Independent of environmental parameters, we sepa-  
187 rately clustered samples by pairwise Bray-Curtis dissimilarities among metabarcoding communities  
188 (k-means; N = 3 or 4) to identify groups of samples that were similar to one another with respect  
189 to biological community. Each biological cluster (colored hulls, Fig. 2) occupied a unique area of  
190 environmental parameter space. Planktonic communities therefore varied predictably with water  
191 temperature, salinity, and pH, across a range of those parameters likely to be encountered in many  
192 near-term future-ocean scenarios [46]. Multinomial logistic regression yielded predictions of the  
193 most-likely community for any combination of environmental parameters (Fig. 2B).

194 These communities were distinguished by changes in the relative abundances of many taxa: in  
195 the Hood Canal in summer for example (Fig. 2C), the colder water communities showed higher  
196 values of *Centropages* sp., *Nitzschia longissima*, *Prorocentrum* sp., *Chrysochromulina* sp., *Navicula*  
197 sp. and *Chaetoceros* sp. Warmer waters were dominated by *Bathycoccus prasinus* and *Cymbomonas*  
198 sp., while at lower pH values, marine communities showed *Litchtheimia ramosa*, *Micromonas pusilla*  
199 at higher relative abundances.

200 Climate envelopes and future distributions

201 The low-pH planktonic communities (cluster 3, Fig. 2) occupy the spectrum of environmental  
202 conditions most likely to be encountered in near-future climate scenarios as temperature rises and  
203 pH falls. For example, by 2095 we expect summer in the Hood Canal to be T > 12 and pH < 8.2  
204 – where cluster 3 is the most likely community – to occur more than two-thirds of the time (68%).

205 To explore the suitability of different environmental conditions for each taxon, we modeled the  
206 likelihood of taxon presence as a function of area and season (random effects) and temperature and  
207 pH (fixed effects). Salinity is predicted to remain largely unchanged in future scenarios [34]. Using  
208 predicted conditions for Puget Sound for the year 2095 [34], we then projected the probability of  
209 presence of each taxon across these conditions over 100 draws of the posterior probabilities of all  
210 taxa. We used these draws to 1) show the change in the probability of presence of each taxon for  
211 each scenario (i.e. 2017 vs 2095, Fig.3 top panel); 2) calculate the taxon richness within taxonomic  
212 groups across the pH-temperature continuum (Fig. 3 center panel),and 3) visualized the change in  
213 suitability for each taxon in our dataset individually (selected taxa in Fig. 3 bottom panel).

214 Diatoms show the steepest richness decline under future conditions (Figure 3); the probability of  
215 occurrence decreases markedly for diverse diatom taxa including both pennate (e.g., *Thalassionema*,  
216 *Navicula*, *Nitzschia*) and centric (e.g., *Coscinodiscus*, *Ditylum*) body forms. These declines in  
217 diatom richness were more accentuated at lower pH values, both in the warm and colder seasons.  
218 Other taxa likely to find less-suitable habitat in the future include *Karlodinium* (dinoflagellate; San  
219 Juan Island), and *Phaeocystis* and red-tide-associated *Chattonella* (haptophyte and raphidophyte,  
220 respectively; Hood Canal).

221 Likely winners under future conditions are more widely scattered among higher taxonomic  
222 groups. The coccolithophore *E. huxleyi* and the dinoflagellate *Alexandrium* sp. each finds more  
223 suitable habitat in both of our study areas. Among others, *Chaetoceros* (diatom) and two small  
224 hydrozoans (Cnidaria) likely increase in San Juan Island, and the potentially fish-killing heterokont  
225 flagellate *Pseudochattonella* increases in the warmer Hood Canal. See Supporting Information for  
226 a complete list of taxon-specific projections.

227 Given such heterogeneity in projections, gains and losses tend to balance one another out when  
228 looking at richness variation within phyla; we find no change in median richness for the year 2095  
229 relative to the present (ANOVA; taxon richness within phyla by year, p = 0.3). Diatoms, however,  
230 show consistent and significant declines in richness (Wilcoxon p < 10<sup>-15</sup>).

231 Taxa surveyed are a function of our metabarcoding PCR primers [40] and reflect the current

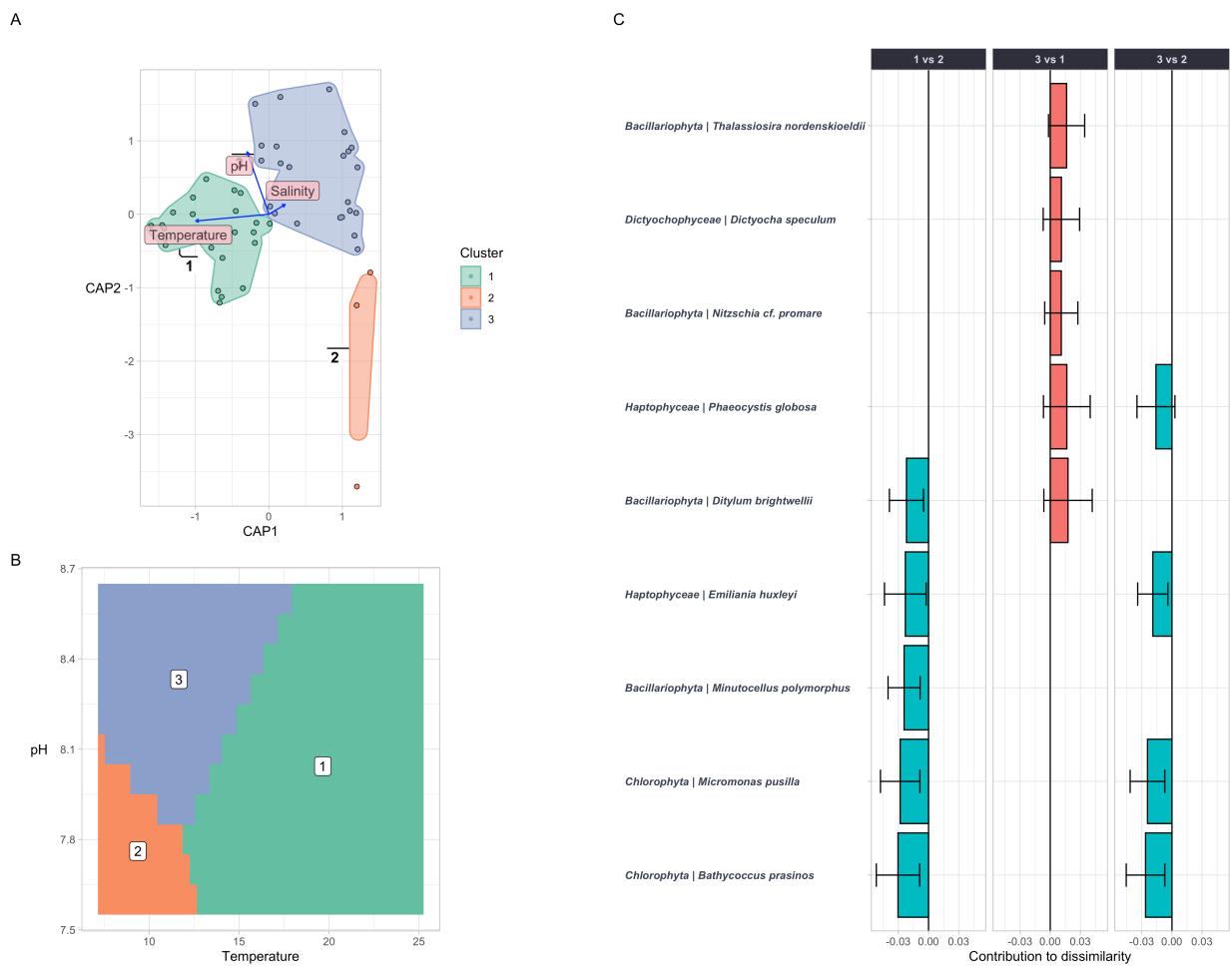
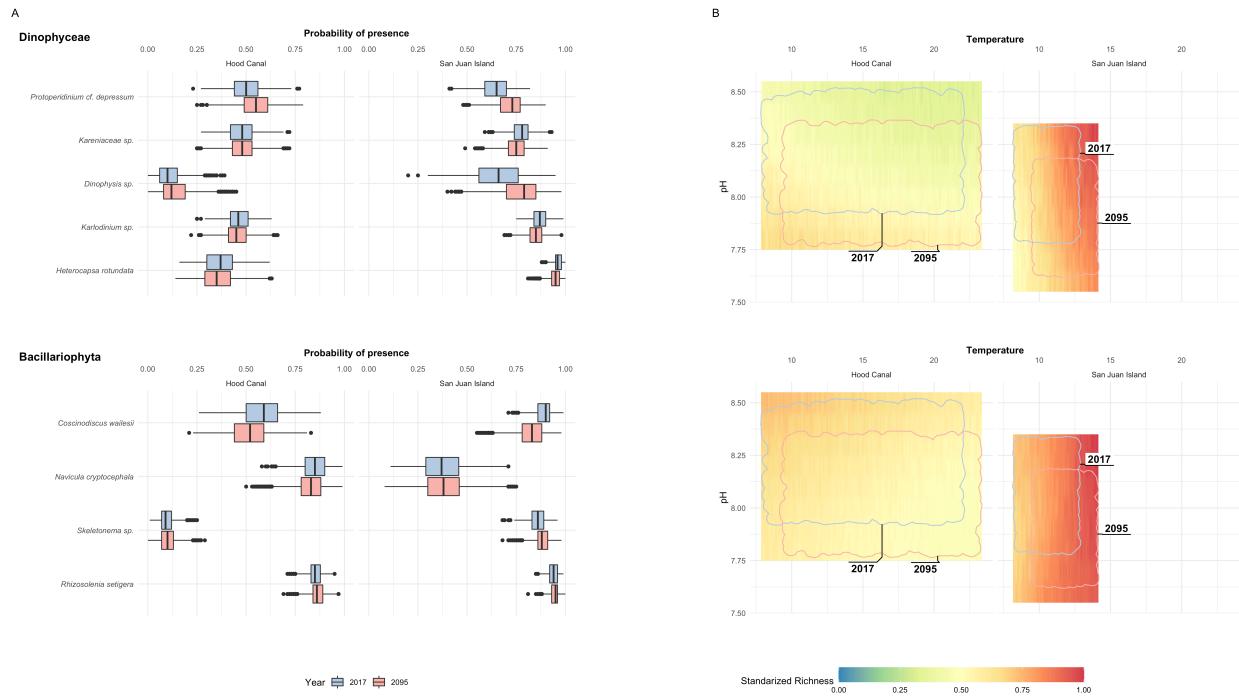


Figure 2: Biological communities and their relationship with environmental variables in the summer months of the Hood Canal. **A** Constrained Analysis of Principal Coordinates (CAP) of Bray-Curtis dissimilarities among biological communities, as constrained by pH, temperature and salinity (arrows). **B** Most-likely cluster as a function of temperature and pH given a multinomial logistic model. **C** Relative abundance (eDNA index; see [33]) of the taxa best distinguishing the three communities illustrated (SIMPER analysis). See Supporting Information for full analysis.



**Figure 3:** Forecasted changes in plankton in the Salish Sea. **A** Probability densities for the occurrence of individual taxa (species- and genus-level) for 2017 (blue) and 2095 (red); data are mean probabilities over 100 model draws, and variance in probability is due to differences in underlying environmental conditions. Four prominent higher-taxa are shown: Arthropods, Dinoflagellates (Dinophyceae), Diatoms (Bacillariophyta), and Haptophytes. **B** Individual taxa with the largest changes in probabilities of occurrence between 2017 and 2095, color-coded by higher taxonomic group, for Hood Canal (left) and San Juan Island (right). Median probabilities plotted. **C** Relative taxon richness (raster color, warmer colors are more taxon-rich) for each of these same higher-taxa, for plausible ranges of pH and Temperature. Envelopes of observed (2017, blue) and modeled (2095, red) annual conditions in the Salish Sea shown for reference. Hood Canal and San Juan Island plotted separately to illustrate environmental differences between them.

status of genetic databases, rather than a complete sampling of the planktonic community; we view these results as a cross-section of common taxa useful for understanding the biological effects of ocean conditions.

The bulk of our projected community changes result from now-rare conditions occurring more frequently in the future. For example, in summer in the Hood Canal at present, we expect surface waters to have  $\text{pH} < 7.9$  and  $T > 19^\circ\text{C}$  only 1% of the time. In 2095, we expect these conditions 18 times more frequently (i.e., 18% of the time). At these values of T and pH, our model predicts the harmful *Alexandrium* sp. to occur more often than not (mean frequency of occurrence = 0.7). By contrast, the large centric diatom *Coscinodiscus* – a key source of carbon for zooplankton and small fishes – occurs only one-third of the time under these same conditions (mean frequency = 0.34).

## Discussion

Temperate surface oceans worldwide average approximately  $14^\circ\text{C}$  and  $\text{pH}$  of 8.1 [8], and will change substantially in this century [mean  $\Delta T$   $2.5^\circ\text{C}$ ,  $\Delta \text{pH}$  -0.35 globally; RCP 8.5; 22]. Here we document communities exposed to this same range of projected conditions in the present day, along an environmental gradient only ca. 200km wide, allowing us to project future ocean communities from a robust set of underlying observations. Our results reflect patterns in a diverse selection of species from nearshore marine communities in the Salish Sea, consisting of 222 planktonic taxa obtained from the metabarcoding analysis of 227 discrete samples across 77 space-time points (eight sites, 1.5 years). We find that changes in the composition of biological communities closely mirrored the variation in pH and temperature, with clear winners (e.g., *Emiliania huxleyi*, *Alexandrium*, and others) and losers (many, but not all, diatoms) likely to shift the structure and function of future marine communities.

A vast amount of evidence suggests climate-associated effects on marine species, and broad patterns of sensitivity are discernible within major taxonomic groups [22, 54, among many others]. However, because the strength and direction of these effects are variable and species-specific [37], very little is known about community-level impacts. Our work illustrates the nearshore planktonic communities that can thrive in low pH - high temperature conditions; such communities are therefore likely to become more prevalent under future conditions.

The large number of species and broad set of environmental conditions we sampled yield substantial inferential power despite lacking the degree of experimental control present in a laboratory

263 or mesocosm.

264 Among the taxa surveyed, diatoms are of particular interest for their ubiquity in the world's  
265 oceans and their important roles in marine food webs [3, 57], as well as in ecological and evolutionary  
266 theory [41]. Our model suggests that diatoms will decrease in richness between the present and  
267 2095, particularly in the Hood Canal summer months, when extreme temperatures are more likely.  
268 Although the most prevalent response among diatoms is a decrease in suitability, some substantial  
269 variability in responses exists within the group. For example, the centric diatom *Coscinodiscus*  
270 spp., which is a food source for *Acartia* spp. copepods [29] and many other animal species, will see  
271 future suitable habitat only in colder waters such as those in San Juan Island, while *Skeletonema*  
272 spp. and the HAB-forming species *Pseudo-nitzschia* spp. will see their habitat suitability remain  
273 constant or slightly increased, especially at low pH levels.

274 More strikingly, we see a dramatic increase in suitable environment for the HAB-forming di-  
275 noflagellate *Alexandrium* sp., which can substantially harm local ecosystems [13] and economies [1].  
276 This increase is particularly high in the summer months of the Hood Canal, when pH is low and  
277 temperatures are high. Both archaeological and experimental evidence suggest *Alexandrium* sp.  
278 blooms with warmer temperatures [45], and models [44] also predict an increase in bloom-favorable  
279 conditions for *Alexandrium* sp. in future oceans.

280 Our results therefore suggest a possible change in relative dominance between diatoms and  
281 other phytoplankton species such as dinoflagellates, consistent with those seen at ecological regime  
282 shifts found elsewhere [57, 26]. Such a shift could affect ecosystems in many ways; even under the  
283 assumption that the surviving taxa would maintain the primary production levels, for example,  
284 the smaller cell-size of dinoflagellates and the differential sinking rates of the two groups would  
285 likely alter regional patterns of nutrient cycling[60, 2, 6]. Although the north Atlantic has shown  
286 an increase in diatom abundance[26], the increase in wind stress and associated mixing in the  
287 water column in the open ocean is unlikely to occur in the Hood Canal, where stratification is  
288 the strongest in the Salish Sea [43]. Furthermore, locally focused models support an increase in  
289 dinoflagellate dominance with climate change, particularly during summer months [34].

290 Our model also suggests increased environmental suitability for the coccolithophore *Emiliania*  
291 *huxleyi*. There is evidence supporting increased calcification and respiration rates with higher pCO<sub>2</sub>  
292 levels [28] for this ubiquitous species, although the many strains of this species and its adaptive  
293 capacity make it difficult to predict longer-term effects with confidence [7].

294 Changes in environmental conditions and associated shifts in planktonic communities will likely  
295 reshape ecosystems and food webs, although some environmental processes may be conserved even  
296 as the particular taxa change. A switch from a diatom-dominated ecosystem to one in which  
297 dinoflagellate blooms extend in space and time could provoke cascade effects [60] including fish  
298 mortality, eutrophication [2], and benthic-pelagic coupling [6]. Beyond the phylum-specific patterns,  
299 the increase in suitable habitat for HAB-forming species will alone be an engine for ecosystem change  
300 [55, 58].

301 One general challenge for model-based work is a tendency to extrapolate from observed condi-  
302 tions in ways that are often untestable – by necessity, projections frequently operate outside the  
303 range of parameters on which the model was trained [20]. Our study system lets us avoid this  
304 pitfall, in that our observed conditions encompass much of the environmental range predicted for  
305 future temperate oceans. That is, the changes we predict for the year 2095 do not primarily come  
306 from extreme values of pH and temperature, but rather from presently-rare conditions becoming  
307 more common.

308 Our observations are strong evidence of the kinds of changes likely in future marine communities,  
309 and they offer testable predictions about the magnitude and direction of effects on focal species.

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