

2 **1 Title**

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

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12 **Author contributions:** RG led sampling, laboratory work, data analysis, and writing. RPK  
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14 the work. EJP and KC assisted in sampling, laboratory work, and analysis.

15 The authors declare no conflict of interest.

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 more  
26 acidified conditions, with overall reduced richness in diatom assemblages and increased richness  
27 in dinoflagellates. Individual taxa finding greater suitable habitat in near-future waters are more  
28 taxonomically varied and include the ubiquitous coccolithophore *Emiliania huxleyi* and the harmful  
29 dinoflagellate *Alexandrium sp.* These results suggest foundational changes for nearshore food webs  
30 under near-future conditions.

31 **3 Keywords**

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

33 **4 Main Text**

34 **4.1 Background**

35 As ocean acidification and warming continue apace, changes in the marine environment are having  
36 an effect on many species' metabolism, development, growth and reproduction success [37, 20, 5,  
37 13], very likely altering food webs [57, 10, 27] and species' interactions in ways that are poorly  
38 understood. Laboratory or mesocosm-based manipulation experiments have documented a wide  
39 variety of biological responses under projected climate scenarios of  $p\text{CO}_2$ , pH, solar radiation,  
40 salinity and temperature [23, 16, 40], showing an array of species-specific responses among particular  
41 taxa of interest. However, information regarding multi-species or community-wide responses to  
42 these stressors is far more limited [38, 32]. The scarcity of such data is likely attributable to the  
43 difficulty of simultaneously tracking the responses of many species in the field, and to the difficulty  
44 of identifying natural systems that adequately reflect the environmental gradients under study.

45 Two natural  $\text{CO}_2$  seeps in nearshore marine habitats – one in Italy and one in Papua New Guinea  
46 – have demonstrated shifts in benthic communities associated with especially acute acidification in  
47 the present day, previewing those we might expect at a more global scale under future conditions  
48 [38, 21]. But beyond these exceptional sites, it is difficult to measure changes in ecological com-  
49 munities associated with the relatively subtle shifts in nearshore ocean chemistry observed to date,  
50 particularly in light of naturally large spatial and temporal variation in these communities. The

51 Puget Sound in Washington, USA, offers a gradient of carbonate chemistry parameters and other  
52 environmental conditions in close geographic proximity. Complex bathymetry, water circulation  
53 patterns, and nearshore landforms create intertidal sites exposed to large variations in tempera-  
54 ture,  $p\text{CO}_2$ , pH, and related parameters [36], creating an opportunity to test the effect of these  
55 measures on marine communities under conditions expected worldwide in the near future [50], and  
56 time-series sampling across the spatial gradient lets us control for site- and season-specific effects.  
57 This study system therefore provides a powerful means of modeling community-level responses to  
58 changing environmental conditions.

59 Even given the appropriate environmental gradients, tracking the biological responses of many  
60 taxa simultaneously remains challenging. Environmental DNA (eDNA) metabarcoding [29, 33]  
61 addresses this problem by amplifying a common gene region out of DNA present in a water sample;  
62 the technique can detect hundreds to thousands of taxa per sample, potentially with species-  
63 level identification. A growing body of evidence supports the efficacy of eDNA metabarcoding for  
64 monitoring biodiversity (see a review in [58]), and this approach has been successfully used to detect  
65 community composition variation across environmental changes in aquatic [19], estuarine [12, 41],  
66 and marine ecosystems [6, 18].

67 Here we use series of metabarcoding samples taken across space and time to track changes in  
68 nearshore ecological communities associated with differences in pH, water temperature, and other  
69 environmental variables. We use broad-spectrum PCR primers [43] to target eukaryotes specifically,  
70 identifying 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 [55]. 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 [56]. 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’ [25].

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 [50], 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 [55]. After reducing inhibition via a 1/10 to 1/100 dilution, the extract was used as  
102 template for a PCR reaction targeting a 313bp fragment of cytochrome oxidase I [43]. PCR  
103 reactions were performed in triplicate and sequenced individually to quantify the stochasticity of  
104 PCR reactions on a mixed template sample, and we attached secondary indexing tags using a two-  
105 step PCR process [51]. PCR conditions and protocols for sample identification followed [34], and  
106 batches of 49 to 178 multiplexed samples were sequenced using MiSeq v2-500 or v3-600 sequencing  
107 kits using manufacturer protocols. On each sequencing run, we added triplicate samples consisting  
108 on DNA obtained from species not present in the marine environment under study (Red Kangaroo

109 (*Macropus rufus*) and Ostrich (*Struthio camelus*)) to establish quality controls of sample assignment  
110 and to quantify levels of 'tag-jumping' or sample-cross-talk [59].

111 Code for all quality-screening and bioinformatics is available in the Supporting Information,  
112 implemented in Unix and R [54]. Briefly, we used a Unix script that calls secondary programs  
113 for primer-trimming and preliminary quality-control [46, 11] 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 [34]. We then assigned sequences to known taxa  
117 using phylogenetic tree placement with *insect* v1.1 [66]; where *insect* could not place individual  
118 taxa, we supplemented assignment by classification against a custom COI database using *anacapa*  
119 [15] and *bowtie2* [42]. We conservatively kept only taxa annotated at the level of taxonomic family,  
120 genus, or species, so we could reliably infer taxon natural history under the assumption that taxa  
121 within the same family shared broad natural-history characteristics. Using published literature and  
122 online databases, we placed every recovered taxon into a benthic/planktonic category and focused  
123 our analysis on the planktonic community (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 [35], using pooled data from technical replicates and  
126 mean proportions across biological replicates. We used this index of abundance in the multivariate  
127 community analysis, and used binary (presence/absence) data to capture species-level responses to  
128 environmental conditions.

## 129 Present scenario community analyses

130 We measured community changes across environmental space using multivariate analyses. We  
131 used the index of eDNA abundance to calculate Bray-Curtis dissimilarities between samples, and  
132 estimated the effects of temperature, pH, and salinity on community composition using Constrained  
133 Analysis of Principal Components (CAP, [3]; 'capscale' function in the R package *vegan* [52]).

134 Independent of environmental parameters, we separately clustered samples by pairwise Bray-  
135 Curtis dissimilarities (k-means; N = 3) to identify groups of samples that were similar to one  
136 another with respect to biological community. The SIMPER procedure in *vegan* revealed the taxa  
137 most contributing to between-cluster differences.

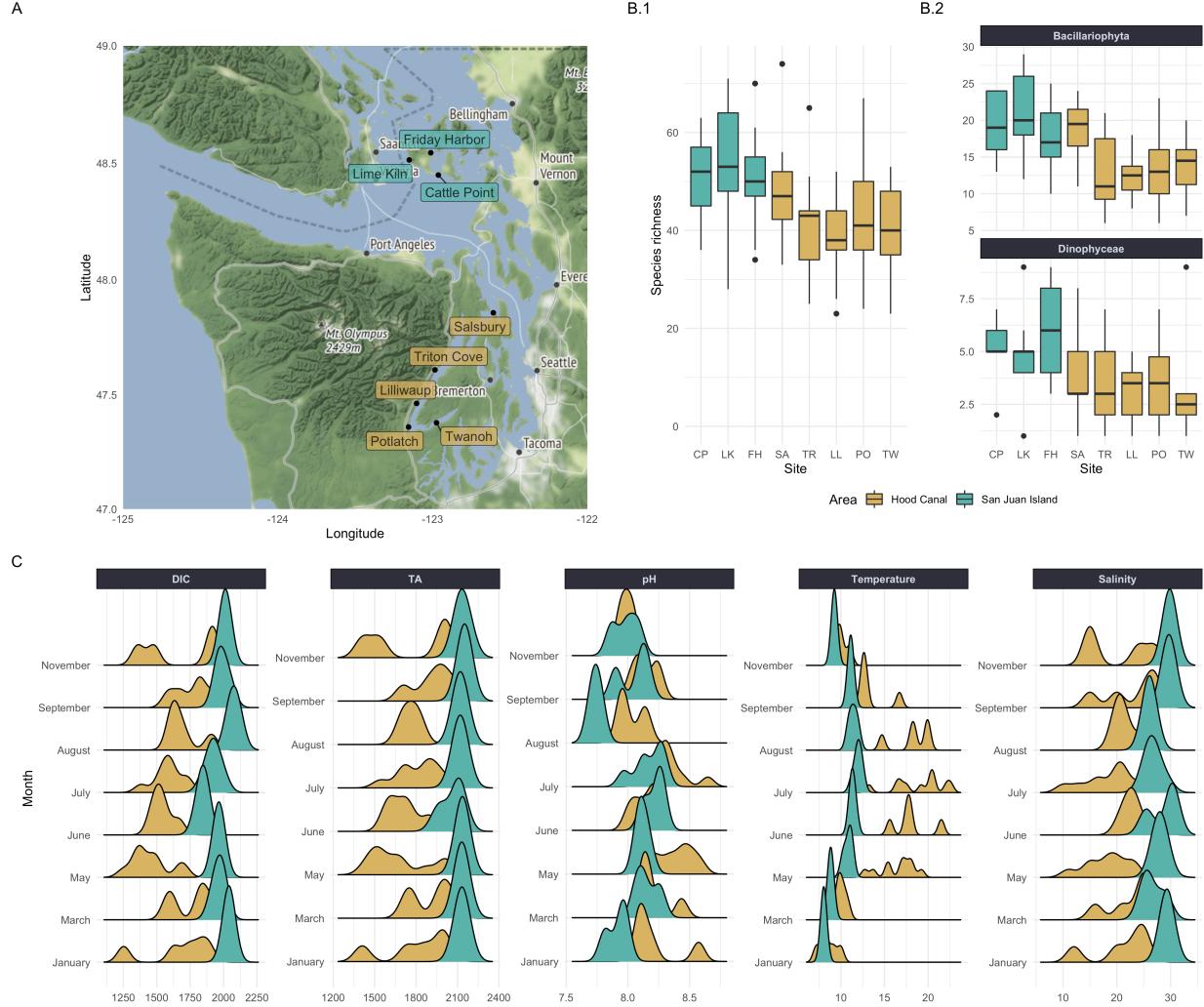
138 For community-level projections, we coded community-cluster identity (Figure 2) as an un-  
139 ordered response variable in a multinomial logistic model, with temperature, pH, and area (Hood  
140 Canal vs. San Juan Island), as predictor variables. Salinity is predicted to remain largely unchanged  
141 in future scenarios [36], and because salinity was correlated with temperature in our dataset, it was  
142 not an important predictor variable and we subsequently dropped it from our models. We calculated  
143 the probability of each community, given these predictors, using the R package *nnet* [62].

## 144 Year-2095 Environmental Scenario and Biological Responses

145 We estimated the distribution of environmental parameters for the overall Salish Sea in 2095 from  
146 the results of [36], which estimated an annual mean increase in temperature of 1.51 °C and mean pH  
147 decrease of 0.18 for the Salish Sea as a whole. We fit a normal distribution to our 2017 environmental  
148 observations to create baseline conditions, and modeled the change in mean parameter values  
149 between 2017 and 2095 as a linear function of time. We then used the modeled distribution of  
150 environmental parameters to generate 1000 simulations of each year scenario. The scenario labeled  
151 as 2095 is the set of parameters falling within the 95% percentile in simulations for the years  
152 2091-2095. See the R code in the supplementary information (lines 98-135).

153 To model biological responses to present and future scenarios, we used a hierarchical logistical  
154 regression model relating the presence of each taxon to temperature and pH, in which the slopes of  
155 temperature and pH effects varied by taxon, and each taxon had a unique intercept that was allowed  
156 to vary by geographic area. For each taxon, we fit these models using the Bayesian generalized  
157 linear mixed effects functions in R package *rstanarm* [26] for R. Model selection using WAIC [64]  
158 supported this as the preferred model over several similar ones (see Supporting Information for  
159 model comparison information and code) and helped to avoid model overfitting and maintain out-  
160 of-sample predictive power.

161 Given the sea-surface temperatures and pH values for 2017 (observed) and 2095 (estimated)  
162 and taxon-specific logistic regression models, we then evaluated the suitability of habitat for each  
163 taxon in the future scenario. For each point in the pH - temperature grid, we calculated a species'  
164 probability of presence as the mean of 100 independent draws of the posterior model response. For  
165 richness estimations in future scenarios, we used each of the 100 draws of the posteriors in each  
166 environmental point as a sample and tallied the number of species detected on each draw. The  
167 mean value across the 100 draws was the input for a Wilcoxon test of differences in species richness



**Figure 1:** **A** Sampling locations in intertidal areas of the Hood Canal (dark gold) and San Juan Island (turquoise). **B** Planktonic richness (unique taxa) per sampling locality, as reflected by the eDNA COI assay. Boxplots represent the variability in richness across all time points for samples taken at the indicated site. **B.1** Taxa from all phyla; **B.2** Diatoms (above) and dinoflagellates (below) shown separately; note change in the scale of the y-axis. **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,  $\mu\text{moles/kg}$ ); Temperature ( $^{\circ}\text{C}$ ) and Salinity (PSU). Individual sampling sites shown as vertical facets.

168 between the 2017 and 2095 scenarios in each region. We performed the Wilcoxon test globally  
169 (total species richness) and on each phylum independently.

170 Taxa surveyed are a function of our metabarcoding PCR primers [43] and reflect the current  
171 status of genetic databases, rather than a complete sampling of the planktonic community; we view  
172 these results as a cross-section of common taxa useful for understanding the biological effects of  
173 ocean conditions.

174 We can only model responses of taxa present in our data set. That is, we may predict that the  
175 number of (for example) diatom species present will decline relative to those present today, but our  
176 data do not allow us to predict whether new species will immigrate from elsewhere or how species  
177 might evolutionarily adapt to future conditions. It is beyond the scope of our work to account  
178 for the latter, and furthermore, because of the extreme uncertainty of evolutionary responses, the  
179 predictions of species distribution models are often interpreted without considering adaptation or  
180 phenotypic plasticity [48].

## 181 Results

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

183 Despite geographic proximity and similar overall species composition (127 of the 222 planktonic  
184 taxa were found in both regions and accounted for 98% of the sequences), the areas under study –  
185 San Juan Island and the Hood Canal – varied widely in pH, temperature, and other environmental  
186 parameters (Figure 1C), with a smooth gradient in conditions along the Hood Canal, and San Juan  
187 Island more closely resembling full marine conditions. Different points along the environmental  
188 continuum simultaneously showed differences equivalent to those predicted between present-day  
189 and future oceans [9].

190 Metabarcoding analysis of eDNA samples generated more than 52.5M sequences from 745 sam-  
191 ples. These samples represented biological and technical replicates from 86 unique sampling events.

192 After bioinformatics quality-control (See Methods) the dataset included ~41M sequences, from  
193 4782 unique amplicon sequence variants (ASVs). Of these, 1374 ASVs (22.8M reads) could be  
194 annotated to a taxonomic level of Family or lower. These ~ 500 taxa from 43 phyla were split ac-  
195 cording to their natural history and habitat (benthic vs. planktonic; see Supporting Information).  
196 Because we expect planktonic taxa to vary with water mass [34] and therefore with bottle-sampled  
197 carbonate chemistry, here we focus on only the planktonic taxa (N = 222). These taxa showed a  
198 seasonal richness gradient between study areas, consistent with documented biodiversity clines in  
199 the area (Fig. 1B.1) [17].

200 Bray-Curtis dissimilarities among samples revealed large differences in metabarcoding commu-  
201 nities due to geographic Area (Hood Canal vs. San Juan Island; F-model = 5.4; p < 0.0001). We  
202 therefore performed a constrained analysis of principal components (CAP) for samples within each  
203 Area, showing the differences among communities as a function of temperature, pH, and salinity  
204 (Fig. 2; for clarity, results for Hood Canal shown; full analysis in the Supporting Information).

205 Each biological cluster (colored hulls, Fig. 2A) occupied a unique area of environmental param-  
206 eter space. Planktonic communities therefore varied predictably with water temperature, salinity,  
207 and pH, across a range of those parameters likely to be encountered in many near-term future-ocean  
208 scenarios [50]. Multinomial logistic regression yielded predictions of the most-likely community for  
209 any combination of environmental parameters (Fig. 2B).

210 These communities were distinguished by changes in the relative abundances of a wide variety  
211 of taxa. In the Hood Canal for example, the colder water and higher-pH community (community  
212 1 in Fig. 2C) showed higher eDNA indexes of diatoms *Thalassiosira nordenskioeldii*, *Nitzschia*  
213 *cf. promare*, and *Ditylum brightwellii* – among other species – relative to a higher-temperature  
214 community (community 3 in Fig. 2C). Communities 2 and 3 had relatively higher values for taxa  
215 from a mix of groups including *Emiliania huxleyi*, *Minutocellus polymorphus*, *Micromonas pusilla*,  
216 and *Bathycoccus prasinus*. Community 3 (Fig. 2A,B) and similar planktonic communities occupy  
217 the spectrum of environmental conditions most likely to be encountered in near-future climate  
218 scenarios as temperature rises and pH falls. For example, we expect the Hood Canal ~~in 2095 to~~  
219 ~~present the conditions in which community 3 is the most likely community 75% of the time, an~~  
220 ~~increase of 22% compared to 2017(see Supplemental Material). T > 12°C and pH < 7.9 where~~  
221 ~~community 3 is the most likely community – more than four times as often in 2095 than in 2017~~  
222 ~~(4.9 % vs 20.7%). I couldn't find where you were doing this calculation, so I made one up – see~~  
223 ~~lines 1134 and following in the Rmd. BUT ALSO : community 3 isn't sensitive to pH, so what are~~  
224 ~~we even saying here?~~

#### 225 Climate envelopes and future distributions

226 To explore the suitability of different environmental conditions for each taxon, we modeled the  
227 likelihood of taxon presence as a function of temperature, pH, and geographic Area as described in  
228 the Methods. Salinity was not informative in our models, as it is highly correlated with temperature  
229 in our dataset and is moreover predicted to remain largely unchanged in future scenarios [36]. Model  
230 projections let us show the change in the probability of presence of each individual taxon for 2095  
231 vs 2017 (Fig. 3 A.1, B.1, C.1), estimate richness for larger taxonomic grounds as a whole for these  
232 two climates (Fig. 3 A.2, B.2, C.2), and estimate richness within taxonomic groups across the  
233 pH-temperature continuum (Fig. 3 A.3, B.3, C.3).

234 Diatoms (Bacillariophyta) show the steepest richness decline under future conditions (Figure  
235 3 B.2); the probability of occurrence decreases markedly for diverse diatom taxa including both  
236 pennate (e.g., *Nitzschia*) and centric (e.g., *Coscinodiscus*, *Stephanodiscus*) body forms. These  
237 declines in diatom richness were more accentuated at lower pH values and higher temperatures.  
238 Other taxa likely to find less-suitable habitat in the future include dinoflagellates *Karlodinium* and  
239 *Gymnodiniaceae* sp.

240 Likely winners under future conditions are more widely scattered among higher taxonomic  
241 groups. The haptophytes *E. huxleyi* and *Chrysochromulina* sp. the dinoflagellate *Alexandrium* sp.  
242 each finds more suitable habitat in both of our study areas. Among others not shown in Fig 3,  
243 *Chaetoceros* (diatom) and many hydrozoans (Cnidaria) likely increase in San Juan Island, and the  
244 potentially fish-killing heterokont flagellate *Pseudochattonella* increases in both study areas. See  
245 Supporting Information for a complete list of taxon-specific projections.

246 Given such heterogeneity in projections, gains and losses tend to balance one another out  
247 when looking at overall richness variation; ~~we find no change in median richness for the year 2095~~  
248 ~~relative to the present in the Hood Canal (overall taxon richness by year, 95% confidence interval~~  
249 ~~in median species richness -0.08, +0.04; Wilcoxon p = 0.5); while higher diversity is expected in~~  
250 ~~the San Juan Island in 2095 (increase in median species richness of 1.84-2.2, Wilcoxon p < 10<sup>-16</sup>).~~  
251 Diatoms, however, show small but significant declines in richness in the Hood Canal (0.47-0.57  
252 ~~species, Wilcoxon p < 10<sup>-16</sup>), while the changes on the San Juan Island are undetectable (median~~  
253 ~~change 0-0.08 p = 0.04). Dinoflagellates see their richness increase in both regions with the future~~  
254 ~~scenario(Wilcoxon p < 10<sup>-16</sup> ). I re-did some of these in the Rmd file (starting at line 1129, doing~~

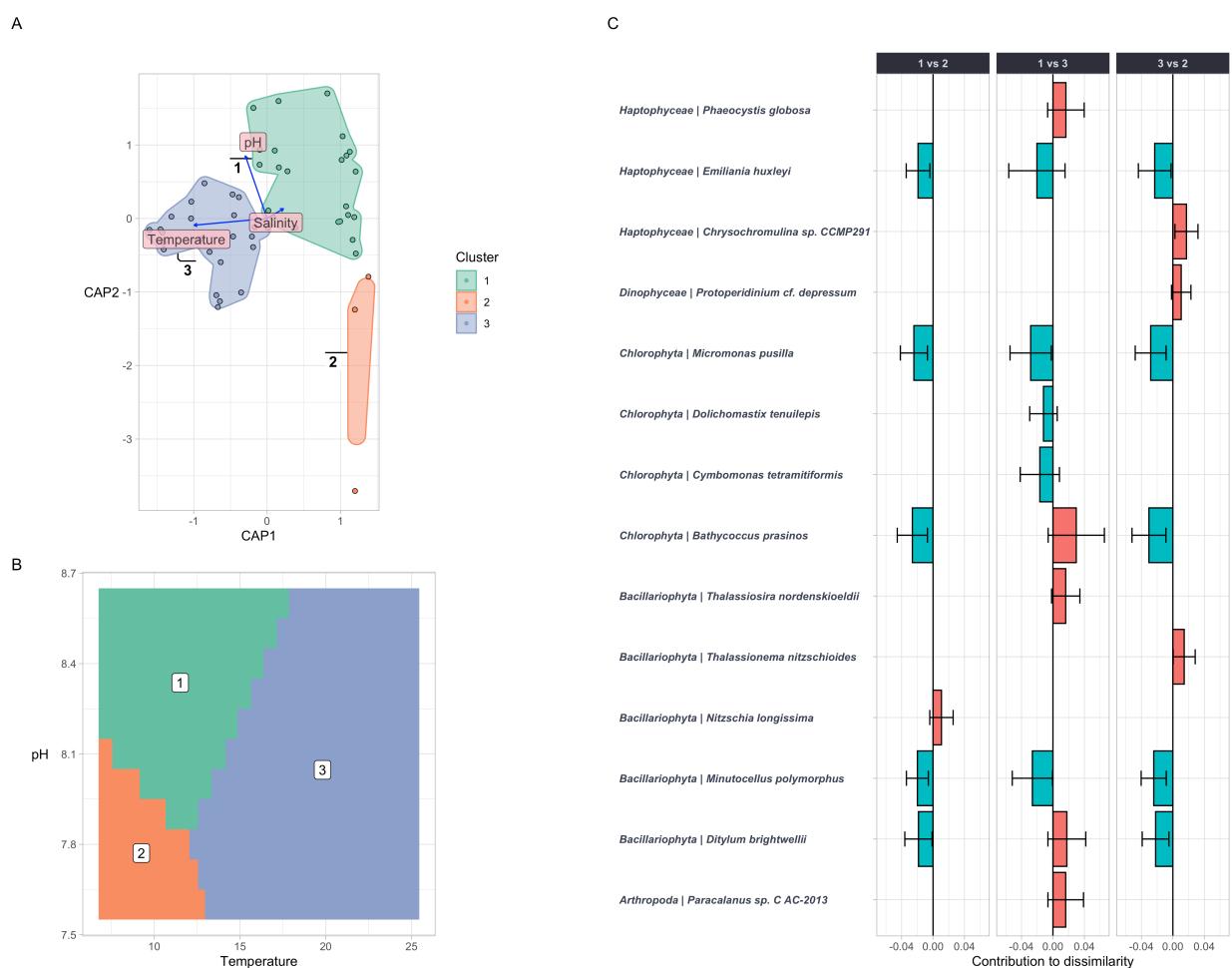
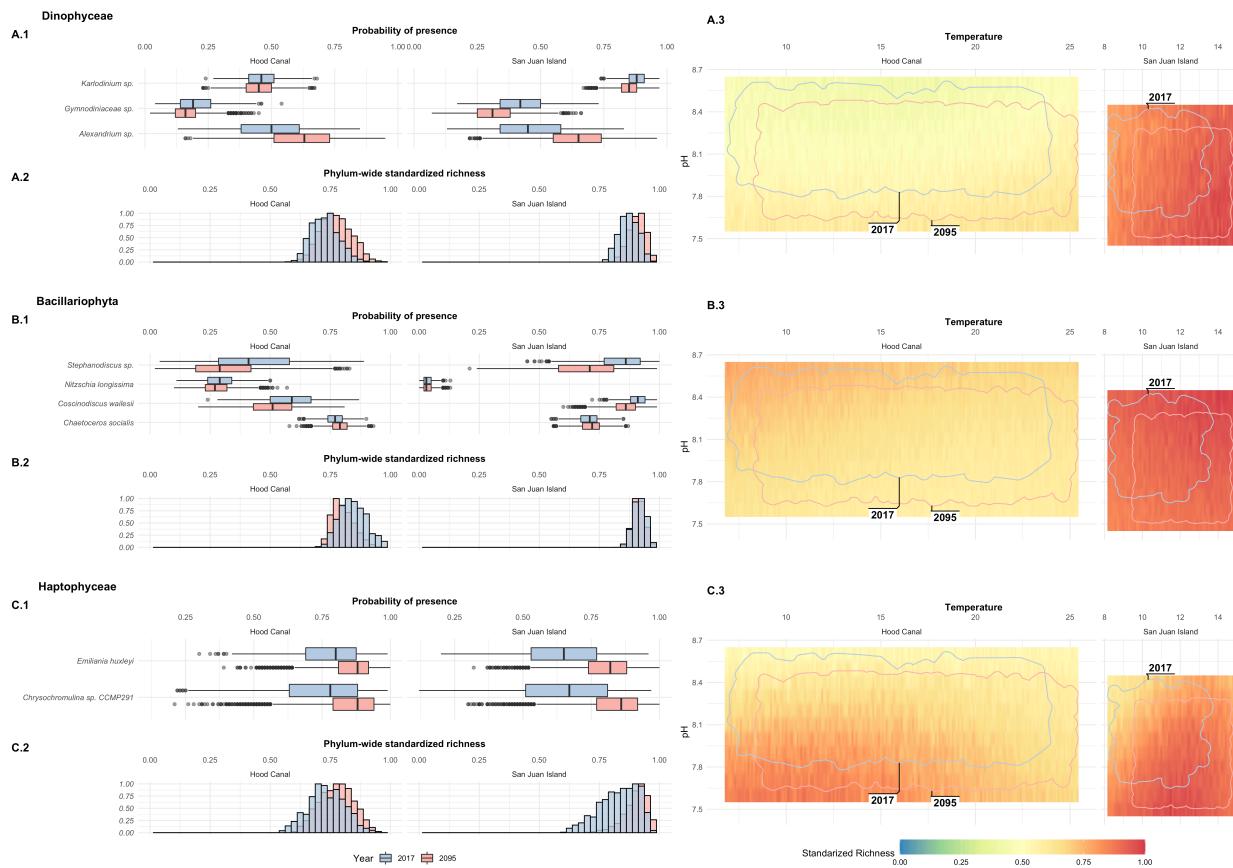


Figure 2: Biological communities and their relationship with environmental variables in the summer months of the Hood Canal. **A** Constrained Analysis of Principal Coordinates (CAP) of Bray-Curtis dissimilarities among biological communities, as constrained by pH, temperature and salinity (arrows). **B** Most-likely cluster as a function of temperature and pH given a multinomial logistic model (salinity was uninformative, being tightly correlated with temperature). **C** Relative abundance (eDNA index; see [35]) 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 for Dinoflagellates (**A**), Diatoms (**B**) and Haptophytes (**C**). Each panels shows: **(.1)** probability densities for the occurrence of selected 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. **(.2)** Changes in relative species richness within each phylum across the simulated scenario. **(.3)** 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.

255 it line-by-line to convince myself what was going on. See line 1179 in particular: for diatoms,  
 256 there is no change between years, but yet the wilcox p value is very low. I can't figure out what's  
 257 happening there.

258 The paragraph below is important, and links to the question I had above: where are we doing  
 259 this calculation in the code? I went through line by line; double check calculations – see lines 1207  
 260 and following in the Rmd. Plus, a reviewer wanted a citation of the *Coscinodiscus* statement, so  
 261 I added some. RYAN- I agree with the statement. Now we do these calculations in the script. I  
 262 will add that the last bits of the prevalence of *Alexandrium* and *Coscinodiscus* are impactful and  
 263 supercool

264 The bulk of our projected community changes result from now-rare conditions occurring more  
 265 frequently in the future. For example, in the Hood Canal at present, we expect surface waters  
 266 to have  $\text{pH} < 7.9$  and  $T > 19^\circ\text{C}$  only 1% of the time. In 2095, we expect these conditions 6 times  
 267 more frequently (i.e., 6% of the time). At these values of T and pH, our model predicts the harmful  
 268 *Alexandrium* sp. to occur more often than not (mean frequency of occurrence = 0.83). By contrast,  
 269 the large centric diatom *Coscinodiscus* – a potentially key source of carbon for zooplankton and  
 270 small fishes [53, 68] with effects on dissolved oxygen and other water-column characteristics [44] –  
 271 occurs only one-third of the time under these same conditions (mean frequency = 0.35).

## 272 Discussion

273 Temperate surface oceans worldwide average approximately  $14^\circ\text{C}$  and pH of 8.1 [9], and will change  
 274 substantially in this century [mean  $\Delta T 2.5^\circ\text{C}$ ,  $\Delta \text{pH } -0.35$  globally; RCP 8.5; 24]. Here we  
 275 document communities exposed to this same range of projected conditions in the present day, along  
 276 an environmental gradient only ca. 200km wide, allowing us to project future ocean communities  
 277 from a robust set of underlying observations. Our results reflect patterns in a diverse selection  
 278 of species from nearshore marine communities in the Salish Sea, consisting of 222 planktonic taxa  
 279 obtained from the metabarcoding analysis of 227 discrete samples across 77 space-time points (eight  
 280 sites, 1.5 years). We find that changes in the composition of biological communities closely mirrored  
 281 the variation in pH and temperature, with clear winners (e.g., *Emiliania huxleyi*, *Alexandrium*, and  
 282 others) and losers (many, but not all, diatoms) likely to shift the structure and function of future  
 283 marine communities.

284 A vast amount of evidence suggests climate-associated effects on marine species, and broad  
285 patterns of sensitivity are discernible within major taxonomic groups [24, 60, among many others].  
286 However, because the strength and direction of these effects are variable and species-specific [39],  
287 very little is known about community-level impacts. Our work illustrates the nearshore plank-  
288 tonic communities that can thrive in low pH - high temperature conditions; such communities are  
289 therefore likely to become more prevalent under future conditions.

290 The large number of species and broad set of environmental conditions we sampled yield substan-  
291 tial inferential power despite lacking the the degree of experimental control present in a laboratory  
292 or mesocosm.

293 Among the taxa surveyed, diatoms are of particular interest for their ubiquity in the world's  
294 oceans and their important roles in marine food webs [4, 63], as well as in ecological and evolutionary  
295 theory [45]. Our model suggests that diatoms will decrease in richness between the present and  
296 2095, particularly in the Hood Canal, where extreme temperatures are more common. Although  
297 the most prevalent response among diatoms is a decrease in suitability, some substantial variability  
298 in responses exists within the group. For example, the centric diatom *Coscinodiscus* spp., which is  
299 a food source for *Acartia* spp. copepods [31] and many other animal species, will see future suitable  
300 habitat only in colder waters such as those in San Juan Island, while *Skeletonema* spp. and the  
301 harmful algal bloom (HAB)-forming species *Pseudo-nitzschia* spp. will see their habitat suitability  
302 remain constant or slightly increased, especially at low pH levels (see Supplemental Material).

303 More strikingly, we see a dramatic increase in suitable environment for the HAB-forming di-  
304 noflagellate *Alexandrium* sp., which can substantially harm local ecosystems [14] and economies [1].  
305 This increase is particularly high in the summer months of the Hood Canal, when pH is low and  
306 temperatures are are high. Both archaeological and experimental evidence suggest *Alexandrium* sp.  
307 blooms with warmer temperatures [49], and models [48] also predict an increase in bloom-favorable  
308 conditions for *Alexandrium* sp. in future oceans.

309 Our results therefore suggest a possible change in relative dominance between diatoms and  
310 other phytoplankton species such as dinoflagellates, consistent with those seen at ecological regime  
311 shifts found elsewhere [63, 28]. Such a shift could affect ecosystems in many ways; even under the  
312 assumption that the surviving taxa would maintain the primary production levels, for example,  
313 the smaller cell-size of dinoflagellates and the differential sinking rates of the two groups would  
314 likely alter regional patterns of nutrient cycling[67, 2, 7]. Although the north Atlantic has shown  
315 an increase in diatom abundance[28], the increase in wind stress and associated mixing in the  
316 water column in the open ocean is unlikely to occur in the Hood Canal, where stratification is  
317 the strongest in the Salish Sea [47]. Furthermore, locally focused models support an increase in  
318 dinoflagellate dominance with climate change, particularly during summer months [36].

319 Our model also suggests increased environmental suitability for the coccolithophore *Emiliania*  
320 *huxleyi*. There is evidence supporting increased calcification and respiration rates with higher pCO<sub>2</sub>  
321 levels [30] for this ubiquitous species, although the many strains of this species and its adaptive  
322 capacity make it difficult to predict longer-term effects with confidence [8].

323 Changes in environmental conditions and associated shifts in planktonic communities will likely  
324 reshape ecosystems and food webs, although some environmental processes may be conserved even  
325 as the particular taxa change. A switch from a diatom-dominated ecosystem to one in which  
326 dinoflagellate blooms extend in space and time could provoke cascade effects [67] including fish  
327 mortality, eutrophication [2], and benthic-pelagic coupling [7]. Beyond the phylum-specific patterns,  
328 the increase in suitable habitat for harmful algae species will alone be an engine for ecosystem change  
329 [61, 65].

330 One general challenge for model-based work is a tendency to extrapolate from observed condi-  
331 tions in ways that are often untestable – by necessity, projections frequently operate outside the  
332 range of parameters on which the model was trained [22]. Our study system lets us avoid this  
333 pitfall, in that our observed conditions encompass much of the environmental range predicted for  
334 future temperate oceans. That is, the changes we predict for the year 2095 do not primarily come  
335 from extreme values of pH and temperature, but rather from presently-rare conditions becoming  
336 more common.

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

## 339 6 Acknowledgments

340 We are grateful to the UW Center for Environmental Genomics and Linda Park's lab at NOAA  
341 Fisheries for access to lab equipment and associated expertise and support. Thanks to Micah  
342 Horwith and the Washington State Department of Natural Resources for in-field support, to Terrie  
343 Klinger for feedback on an earlier draft, to Stephanie Moore for advice on phytoplankton generally,  
344 to Ana Ramón-Laca for lab help, to Dan Drinan for field help, and to Alex Gagnon for access to

345 carbonate-chemistry expertise and equipment.

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