

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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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 [58, 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 [59]), 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 [56]. 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 [57]. 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 Institution of Oceanography. Using DIC and TA, we calculated pH and  
92 the 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 1C); 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 [56]. 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 [60].

111 Code for all quality-screening and bioinformatics is available in the Supporting Information,  
112 implemented in Unix and R [55]. 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 [67]; 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. **An implementation in R of this index is provided in the Supplementary  
129 information.**

### 130 Present scenario community analyses

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

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

139 For community-level projections, we coded community-cluster identity (Figure 2) as an un-  
140 ordered response variable in a multinomial logistic model, with temperature, pH, and area (Hood  
141 Canal vs. San Juan Island), as predictor variables. Salinity is predicted to remain largely unchanged  
142 in future scenarios [36], and because salinity was correlated with temperature in our dataset, it was  
143 not an important predictor variable and we subsequently dropped it from our models. We calculated  
144 the probability of each community, given these predictors, using the R package *nnet* [63].

### 145 Year-2095 Environmental Scenario and Biological Responses

146 We estimated the distribution of environmental parameters for the overall Salish Sea in 2095 from  
147 the results of [36], which estimated an annual mean increase in temperature of 1.51 °C and mean pH  
148 decrease of 0.18 for the Salish Sea as a whole. We fit a normal distribution to our 2017 environmental  
149 observations to create baseline conditions, and modeled the change in mean parameter values  
150 between 2017 and 2095 as a linear function of time. We then used the modeled distribution of  
151 environmental parameters to generate 1000 simulations of each year scenario. The scenario labeled  
152 as 2095 is the set of parameters falling within the 95% percentile in simulations for the years  
153 2091-2095. See the R code in the supplementary information (lines 98-135).

154 To model biological responses to present and future scenarios, we used a hierarchical logistical  
155 regression model relating the presence of each taxon to temperature and pH, in which the slopes of  
156 temperature and pH effects varied by taxon, and each taxon had a unique intercept that was allowed  
157 to vary by geographic area. For each taxon, we fit these models using the Bayesian generalized  
158 linear mixed effects functions in R package *rstanarm* [26] for R. Model selection using WAIC [65]  
159 supported this as the preferred model over several similar ones (see Supporting Information for  
160 model comparison information and code) and helped to avoid model overfitting and maintain out-  
161 of-sample predictive power.

162 Given the sea-surface temperatures and pH values for 2017 (observed) and 2095 (estimated)  
163 and taxon-specific logistic regression models, we then evaluated the suitability of habitat for each  
164 taxon in the future scenario. For each point in the pH - temperature grid, we calculated a species'  
165 probability of presence as the mean of 100 independent draws of the posterior model response.  
166 For each point, the sum of mean probabilities across species provided richness estimations. The  
167 mean value across the 100 draws was the input for a Wilcoxon test of differences in species richness  
168 between the 2017 and 2095 scenarios in each region. We performed the Wilcoxon test globally (total

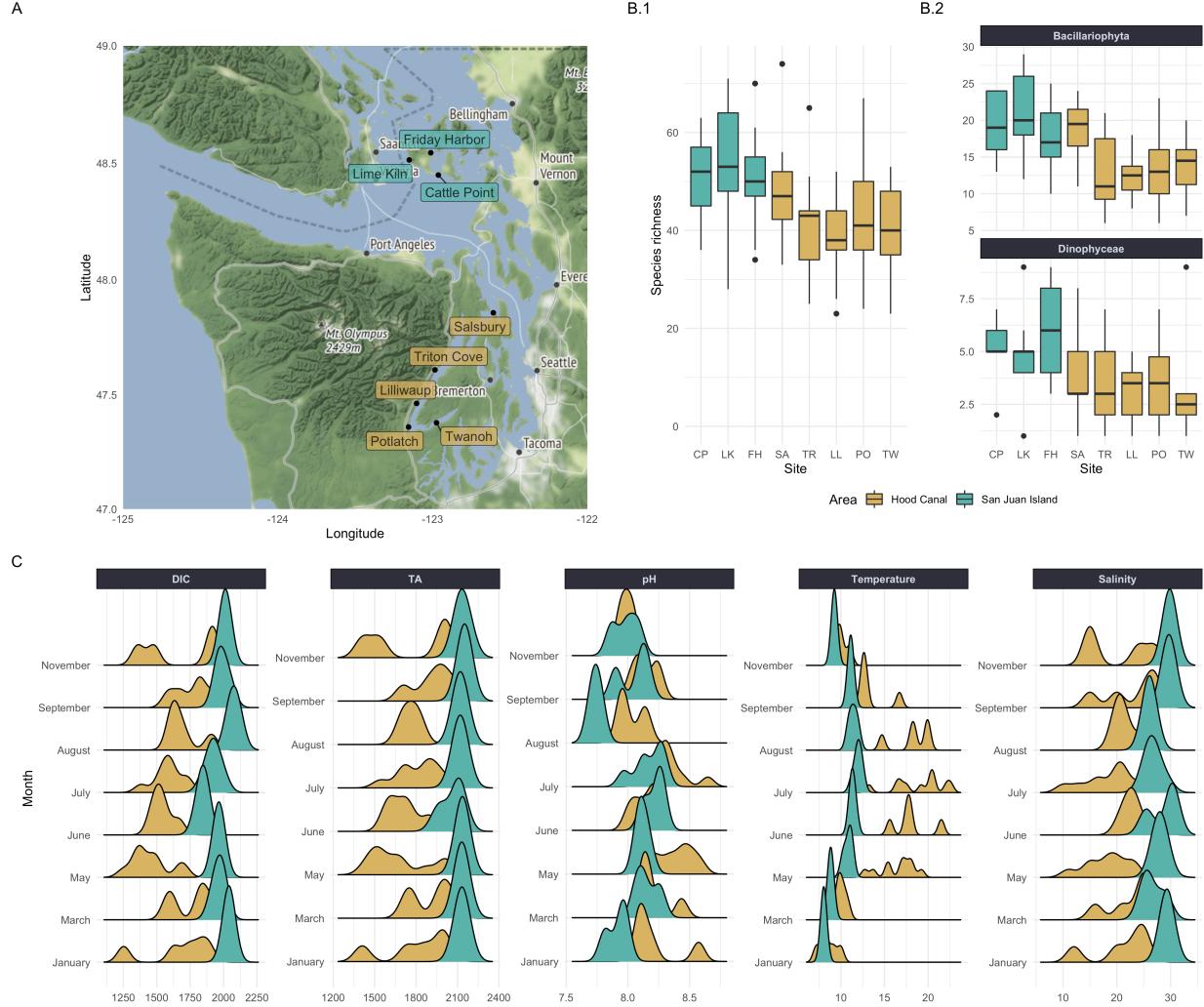


Figure 1: **A** Sampling locations in intertidal areas of the Hood Canal (dark gold) and San Juan Island (turquoise). **B** Planktonic richness (unique taxa) per sampling locality, as reflected by the eDNA COI assay. Boxplots represent the variability in richness across all time points for samples taken 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,  $\mu\text{moles}/\text{kg}$ ); Total Alkalinity (TA,  $\mu\text{moles}/\text{kg}$ ); Temperature ( $^{\circ}\text{C}$ ) and Salinity (PSU). Individual sampling sites shown as vertical facets.

169 species richness) and on each phylum independently. RYAN: Move paragraph below to discussion?  
 170 Taxa surveyed are a function of our metabarcoding PCR primers [43] and reflect the current status  
 171 of genetic databases, rather than a complete sampling of the planktonic community; we view these  
 172 results as a cross-section of common taxa useful for understanding the biological effects of ocean  
 173 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

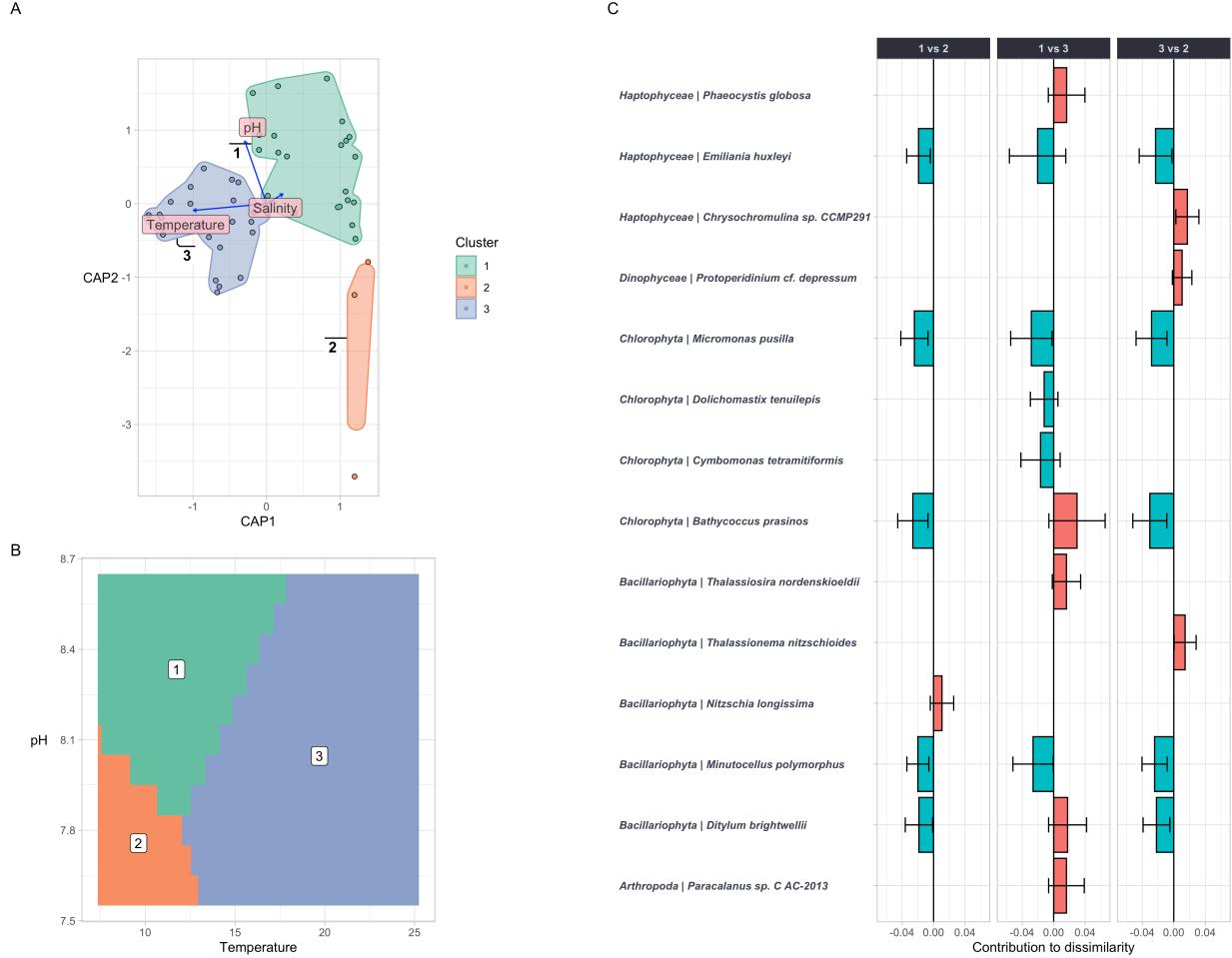


Figure 2: Biological communities and their relationship with environmental variables in the summer months of the Hood Canal. **A** Constrained Analysis of Principal Coordinates (CAP) of Bray-Curtis dissimilarities among biological communities, as constrained by pH, temperature and salinity (arrows). **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.

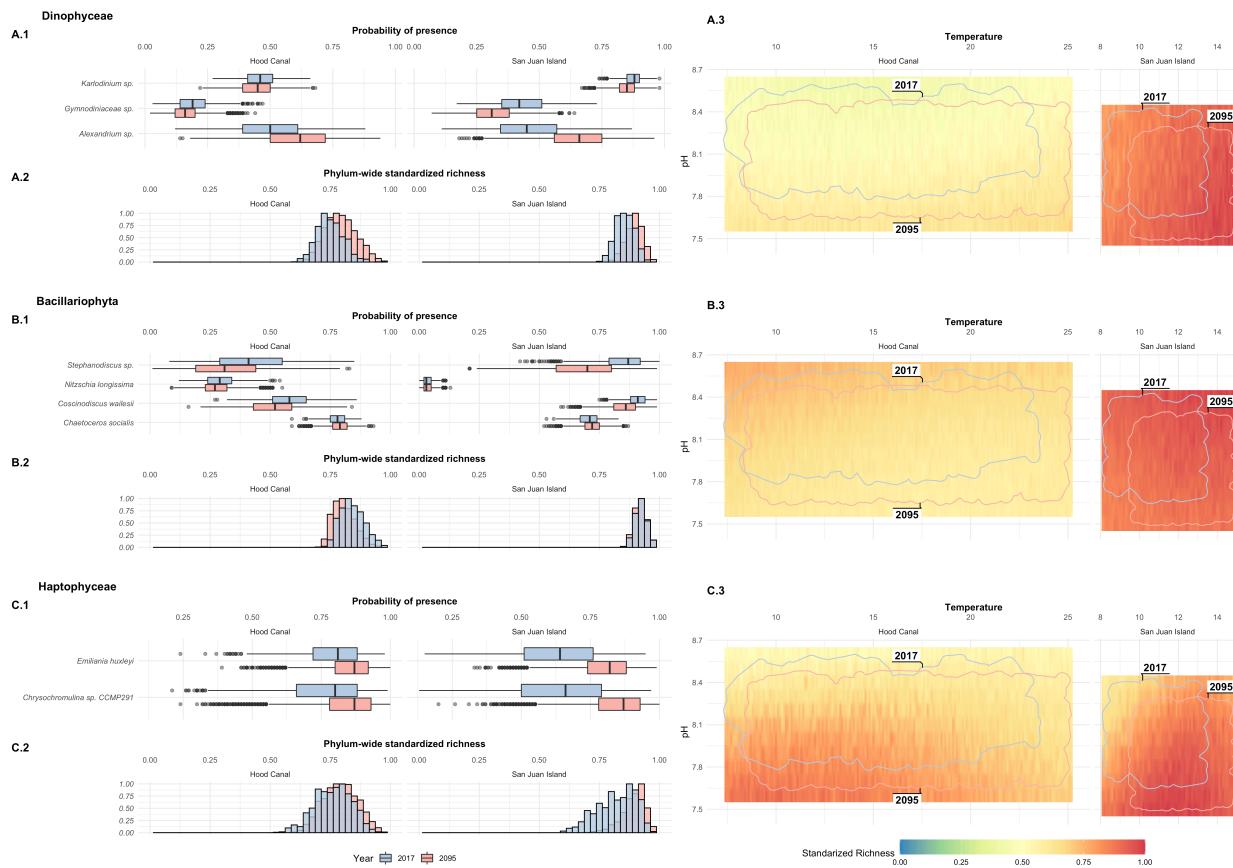
193 4782 unique amplicon sequence variants (ASVs). Of these, 1374 ASVs (22.8M reads) could be  
 194 annotated to a taxonomic level of Family or lower. These ~ 500 taxa from 43 phyla were split ac-  
 195 cording to their natural history and habitat (benthic vs. planktonic; see Supporting Information).  
 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 have the conditions in which community 3 is the most likely community 75% of the time, an increase  
 220 of 22% compared to 2017 (see Supplemental Material).

221 Climate envelopes and future distributions



**Figure 3:** Forecasted changes in plankton in the Salish Sea for Dinoflagellates (panel **A**), Diatoms (**B**) and Haptophytes (**C**). Each panel 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.

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

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

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

Given such heterogeneity in projections, gains and losses tend to balance one another out when looking at overall richness variation; we find no change in median richness for the year 2095 relative to the present in the Hood Canal (overall taxon richness by year, 95% confidence interval in median species richness -0.08, +0.04; Wilcoxon  $p = 0.5$ ); while higher diversity is expected in the San Juan Island in 2095 (increase in median species richness of 1.84-2.2,  $p < 10^{-16}$ ). Diatoms, in particular, show small but significant declines in richness in the Hood Canal (0.47-0.57 species,  $p < 10^{-16}$ ), while the changes on the San Juan Island are negligible (median change 0-0.08  $p = 0.04$ ). Dinoflagellates see their richness increase in both regions with the future scenario (median change 0.14-0.18 Hood Canal; 0.21-0.25 San Juan Island;  $p < 10^{-16}$  for each).

The bulk of our projected community changes result from now-rare conditions occurring more

252 frequently in the future. For example, in in the Hood Canal at present, we expect surface waters  
253 to have pH < 7.9 and T > 19°C only 1% of the time. In 2095, we expect these conditions 6 times  
254 more frequently (i.e., 6% of the time). At these values of T and pH, our model predicts the harmful  
255 *Alexandrium* sp. to occur more often than not (mean frequency of occurrence = 0.83). By contrast,  
256 the large centric diatom *Coscinodiscus* – a potentially key source of carbon for zooplankton and  
257 small fishes [53, 69] with effects on dissolved oxygen and other water-column characteristics [44] –  
258 occurs only one-third of the time under these same conditions (mean frequency = 0.35).

## 259 Discussion

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

271 A vast amount of evidence suggests climate-associated effects on marine species, and broad  
272 patterns of sensitivity are discernible within major taxonomic groups [24, 61, among many others].  
273 However, because the strength and direction of these effects are variable and species-specific [39],  
274 very little is known about community-level impacts. Our work illustrates the nearshore plank-  
275 tonic communities that can thrive in low pH - high temperature conditions; such communities are  
276 therefore likely to become more prevalent under future conditions.

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

280 Among the taxa surveyed, diatoms are of particular interest for their ubiquity in the world's  
281 oceans and their important roles in marine food webs [4, 64], as well as in ecological and evolutionary  
282 theory [45]. Our model suggests that diatoms will decrease in richness between the present and  
283 2095, particularly in the Hood Canal, where extreme temperatures are more common. Although  
284 the most prevalent response among diatoms is a decrease in suitability, some substantial variability  
285 in responses exists within the group. For example, the centric diatom *Coscinodiscus* spp., which is  
286 a food source for *Acartia* spp. copepods [31] and many other animal species, will see future suitable  
287 habitat only in colder waters such as those in San Juan Island, while *Skeletonema* spp. and the  
288 harmful algal bloom (HAB)-forming species *Pseudo-nitzschia* spp. will see their habitat suitability  
289 remain constant or slightly increased, especially at low pH levels (see Supplemental Material).

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

296 Our results therefore suggest a possible change in relative dominance between diatoms and  
297 other phytoplankton species such as dinoflagellates, consistent with those seen at ecological regime  
298 shifts found elsewhere [64, 28]. Such a shift could affect ecosystems in many ways; even under the  
299 assumption that the surviving taxa would maintain the primary production levels, for example, the  
300 smaller cell-size of dinoflagellates and the differential sinking rates of the two groups would likely  
301 alter regional patterns of nutrient cycling and carbon sequestration [68, 2, 7, 54]. Although the  
302 north Atlantic has shown an increase in diatom abundance [28], the increase in wind stress and  
303 associated mixing in the water column in the open ocean is unlikely to occur in the Hood Canal,  
304 where stratification is the strongest in the Salish Sea [47]. Furthermore, locally focused models  
305 support an increase in dinoflagellate dominance with climate change, particularly during summer  
306 months [36].

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

311 Changes in environmental conditions and associated shifts in planktonic communities will likely  
312 reshape ecosystems and food webs, although some environmental processes may be conserved even  
313 as the particular taxa change. A switch from a diatom-dominated ecosystem to one in which

314 dinoflagellate blooms extend in space and time could provoke cascade effects [68] including fish  
315 mortality, anoxia [2], and ~~carbon sinking dynamics alter current benthopelagic coupling processes~~  
316 [7]. Beyond the phylum-specific patterns, the increase in suitable habitat for harmful algae species  
317 will alone be an engine for ecosystem change [62, 66].

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

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

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333 carbonate-chemistry expertise and equipment.

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