

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.

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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 [36, 19, 4,
36 12], very likely altering food webs [52, 9, 26] 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 [22, 15, 39], 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 [37, 31]. 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 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 [37, 20]. 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 [35], creating an opportunity to test the effect of these
54 measures on marine communities under conditions expected worldwide in the near future [47], 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 [28, 32]
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 [53]), and this approach has been successfully used to detect community
65 composition variation across environmental changes in aquatic [18], estuarine [11, 40], and marine
66 ecosystems [5, 17].

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 [41] 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 μm cellulose filter, and the filter preserved
79 in Longmire buffer until DNA extraction [50]. Concurrently, we collected one 120 ml water sample
80 from each site and poisoned it with 0.1 ml of saturated HgCl_2 for carbonate chemistry analysis,
81 following [51]. 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; 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’ [24].

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 [47], 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 [50]. 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 [41]. 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 [48].
105 PCR conditions and protocols for sample identification followed [33], 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 [54].

111 Code for all quality-screening and bioinformatics is available in the Supporting Information,
112 implemented in Unix and R [49]. Briefly, we used a Unix script that calls secondary programs
113 for primer-trimming and preliminary quality-control [43, 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 [33]. We then assigned sequences to known taxa
117 using phylogenetic tree placement with insect v1.1 [60]; where insect could not place individual
118 taxa, we supplemented assignment with a COI database [14]. We conservatively kept only taxa
119 annotated at the level of taxonomic family, genus, or species, so we could reliably infer taxon
120 natural history under the assuming that taxa within the same family shared broad natural-history
121 characteristics. Using published literature and online databases, we placed every recovered taxon
122 into a benthic/planktonic category and focused our analysis on the planktonic community (see
123 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 [34], using pooled data from technical replicates
126 and mean proportions across biological replicates.

127 Present scenario community analyses

128 We performed a series of multivariate tests to investigate the community changes across the en-
129 vironmental space we observed. We used the index of abundance as the species abundances and
130 Bray-Curtis as the dissimilarity measurement between samples. A global PERMANOVA test (Bray-
131 Curtis Area) further justified the subsequent separated analysis of the Hood Canal and San Juan
132 Island samples; a Constrained Analysis of Principal Components (CAP, [?]) investigated the
133 distinctiveness of plankton communities across the gradient of temperature, pH and salinity.

134 Independent of environmental parameters, we separately clustered samples by pairwise Bray-
135 Curtis dissimilarities among metabarcoding communities (k-means; N = 3) to identify groups of
136 samples that were similar to one another with respect to biological community. The SIMPER pro-
137 cedure from the r package *vegan* revealed the taxa 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 [35], 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 cal-
143 culated the probability of each community, given these predictors, using the r package *nnet* [57].
144

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 [35], which estimated an annual mean increase in temperature of 1.51 °C and mean
148 pH decrease of 0.18 for the Salish Sea as a whole. We fit our current environmental observations to
149 a normal distribution, and used the parameters of that distribution alongside a linear variation of
150 temperature and pH between the present and the 2095 scenario to generate 1000 simulations of each
151 year scenario. The scenario labelled as 2095 is the result of 5 simulations for the years 2091-2095.
152 We removed outliers from these simulations by keeping the 95% percentile of observations. See the
153 R code in the supplementary information to follow the process of generating these datasets (lines
154 98-135).

155 ~~For community level projections, we coded community cluster identity (Figure 2) as an un-~~
156 ~~ordered response variable in a multinomial logistic model, with temperature, pH, and area (Hood~~
157 ~~Canal vs. San Juan Island), as predictor variables. We calculated the probability of each commu-~~
158 ~~nity, given these predictors, using the package ‘nnet’ . Most common combinations of T and pH~~
159 ~~were estimated from 2 dimensional density plots for each year.~~

160 Within a geographic area, temperature and pH were most strongly associated with community-
161 level changes, although these conditions varied substantially between the Hood Canal and San Juan
162 Island sites. We therefore developed a hierarchical logistical regression model relating the presence
163 of each taxon to temperature and pH, in which the slopes of temperature and pH effects varied by
164 taxon, and each taxon had a unique intercept that was allowed to vary by geographic area. For
165 each taxon, we fit these models using the Bayesian generalized linear mixed effects functions in R
166 package rstanarm [25] for R. Model selection using WAIC supported this as the preferred model
167 over several similar ones (see Supporting Information).

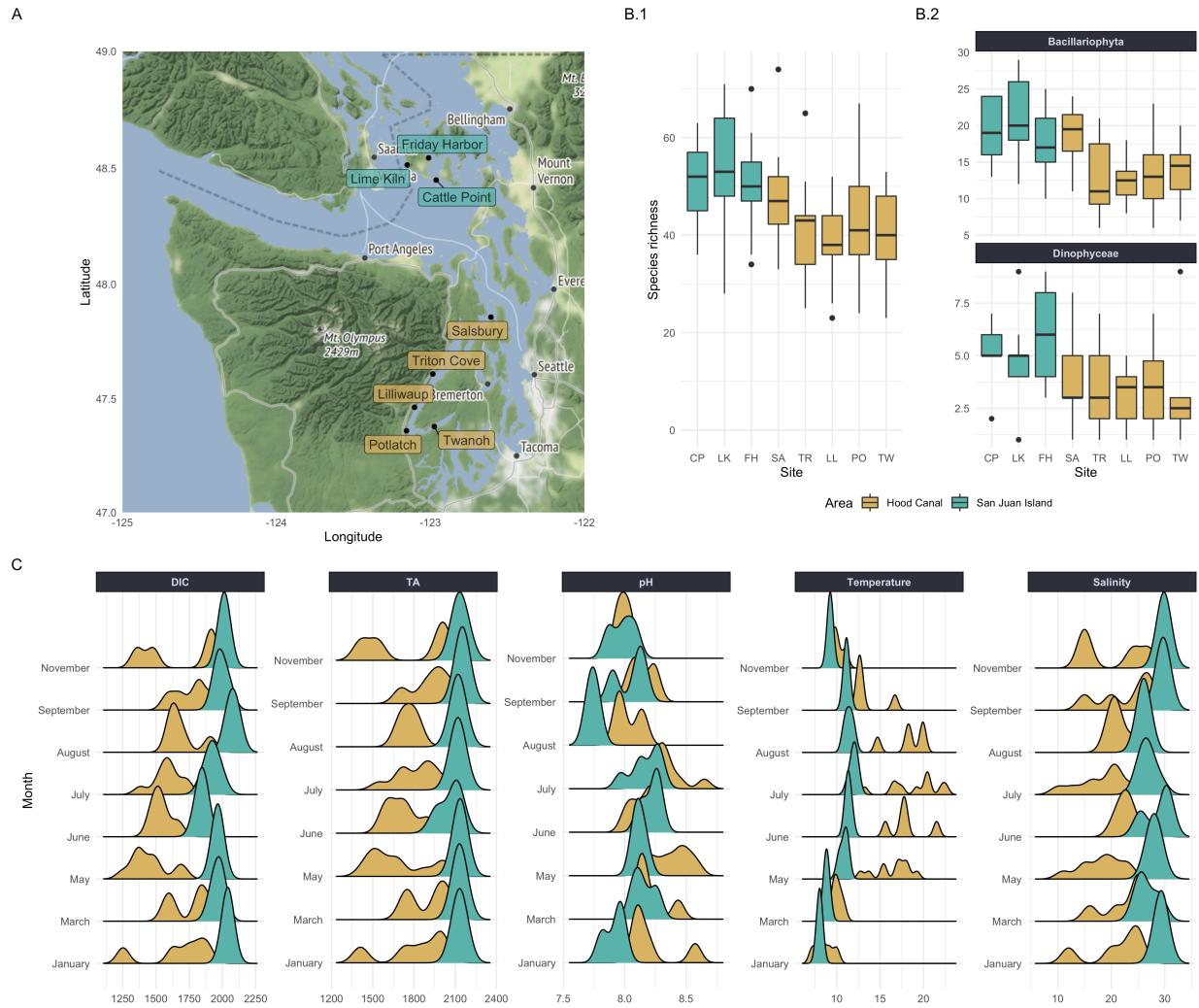


Figure 1: **A** Sampling locations in intertidal areas of the Hood Canal (dark gold) and San Juan Island (turquoise). **B** Planktonic richness (unique taxa) per sampling locality, as reflected by the eDNA COI assay. **B.1** Taxa from all phyla; **B.2** Diatoms (above) and dinoflagellates (below) shown separately. **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 facets. **CHANGE y-scale for Dinophyceae DONE**

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

We can only model responses of taxa present in our data set. That is, we may predict that the number of (for example) diatom species present will decline relative to those present today, but our data do not allow us to predict whether new species will immigrate from elsewhere or how species might evolutionarily adapt to future conditions. It is beyond the scope of our work to account for the latter, and furthermore, because of the extreme uncertainty of evolutionary responses, the predictions of species distribution models are often interpreted without considering adaptation or phenotypic plasticity [45].

Results

Variation in Carbonate Chemistry and in Ecological Communities

We collected intertidal environmental and genetic data from bottle samples each month between March 2017 and August 2018 (Figure 1A). Despite geographic proximity and similar overall species composition (127 of the 222 planktonic taxa were found in both regions and accounted for 98% of the sequences), the areas under study – San Juan Island and the Hood Canal – varied widely in pH, temperature, and other environmental parameters (Figure 1C), with a smooth gradient in conditions along the Hood Canal, and San Juan Island more closely resembling full marine conditions. Different points along the environmental continuum simultaneously showed differences equivalent to those predicted between present-day and future oceans [8].

Metabarcoding analysis of eDNA samples generated more than 52.5M sequences from 745 samples. 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 [33] 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) [16].

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). **RPK:**
202 **still up-to-date? no adonis code in the Rmd file that I saw. RGS: that's true - I added new chunk**
203 **of code line 382 & updated results.** We therefore performed a constrained analysis of principal
204 components (CAP) for samples within each Area, showing the differences among communities as a
205 function of temperature, pH, and salinity (Fig. 2 shows results for Hood Canal; full analysis in the
206 Supporting Information). **Independent of environmental parameters, we separately clustered sam-**
207 **ples by pairwise Bray Curtis dissimilarities among metabarcoding communities (k means; N = 3)**
208 **to identify groups of samples that were similar to one another with respect to biological community.**
209 Each biological cluster (colored hulls, Fig. 2A) occupied a unique area of environmental parameter
210 space. Planktonic communities therefore varied predictably with water temperature, salinity, and
211 pH, across a range of those parameters likely to be encountered in many near-term future-ocean
212 scenarios [47]. Multinomial logistic regression yielded predictions of the most-likely community for
213 any combination of environmental parameters (Fig. 2B).

214 **RPK: I think everything below needs updating, according to some final analysis. TODO: RGS**
215 These communities were distinguished by changes in the relative abundances of many taxa: in
216 the Hood Canal for example (Fig. 2C), the colder water and higher pH communities showed higher
217 values of *Centropages* sp., *Nitzschia longissima*, *Prorocentrum* sp., *Chrysochromulina* sp., *Navicula*
218 sp. and *Chaetoceros* sp. Warmer waters were dominated by *Bathycoccus prasinus* and *Cymbomonas*
219 sp., while at lower pH values, marine communities showed *Litchtheimia ramosa*, *Micromonas pusilla*
220 at higher relative abundances.

221 Climate envelopes and future distributions

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

227 To explore the suitability of different environmental conditions for each taxon, we modeled the
228 likelihood of taxon presence as a function of area and season (random effects) and temperature and
229 pH (fixed effects). Salinity is predicted to remain largely unchanged in future scenarios [35]. Using
230 predicted conditions for Puget Sound for the year 2095 [35], we then projected the probability of
231 presence of each taxon across these conditions over 100 draws of the posterior probabilities of all
232 taxa. We used these draws to 1) show the change in the probability of presence of each taxon for
233 each scenario (i.e. 2017 vs 2095, Fig. 3 top panel); 2) calculate the taxon richness within taxonomic
234 groups across the pH-temperature continuum (Fig. 3 center panel), and 3) visualized the change in
235 suitability for each taxon in our dataset individually (selected taxa in Fig. 3 bottom panel).

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

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

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

253 Taxa surveyed are a function of our metabarcoding PCR primers [41] and reflect the current
254 status of genetic databases, rather than a complete sampling of the planktonic community; we view

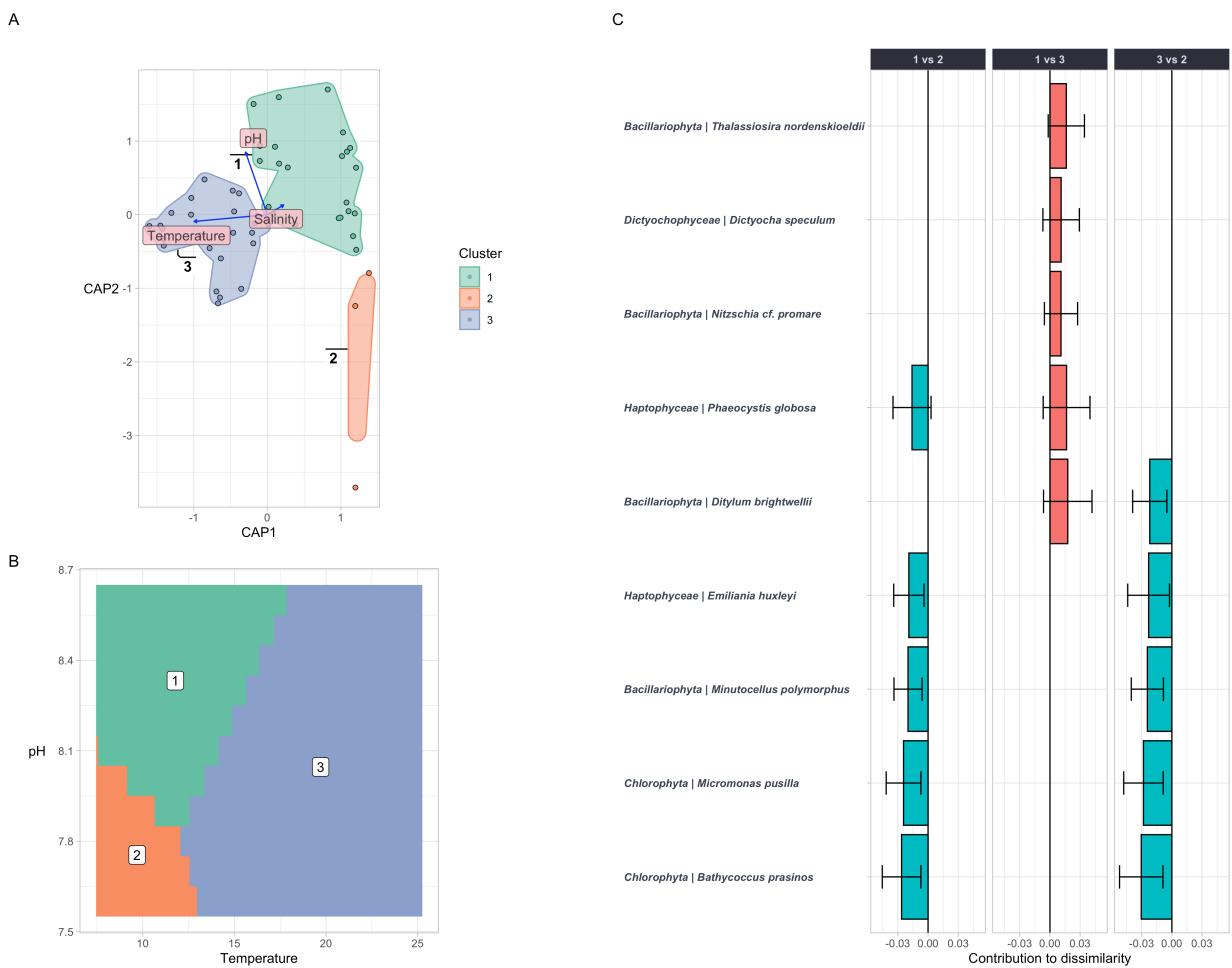


Figure 2: Biological communities and their relationship with environmental variables in the summer months of the Hood Canal. **A** Constrained Analysis of Principal Coordinates (CAP) of Bray-Curtis dissimilarities among biological communities, as constrained by pH, temperature and salinity (arrows). **B** Most-likely cluster as a function of temperature and pH given a multinomial logistic model. **C** Relative abundance (eDNA index; see [34]) 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.

255 these results as a cross-section of common taxa useful for understanding the biological effects of
256 ocean conditions.

257 The bulk of our projected community changes result from now-rare conditions occurring more
258 frequently in the future. For example, in summer in the Hood Canal at present, we expect surface
259 waters to have $\text{pH} < 7.9$ and $T > 19^\circ\text{C}$ only 1% of the time. In 2095, we expect these conditions
260 18 times more frequently (i.e., 18% of the time). At these values of T and pH, our model predicts
261 the harmful *Alexandrium* sp. to occur more often than not (mean frequency of occurrence = 0.7).
262 By contrast, the large centric diatom *Coscinodiscus* – a key source of carbon for zooplankton and
263 small fishes – occurs only one-third of the time under these same conditions (mean frequency =
264 0.34).

265 **Discussion**

266 Temperate surface oceans worldwide average approximately 14°C and pH of 8.1 [8], and will change
267 substantially in this century [mean $\Delta T 2.5^\circ\text{C}$, $\Delta \text{pH } -0.35$ globally; RCP 8.5; 23]. Here we
268 document communities exposed to this same range of projected conditions in the present day, along
269 an environmental gradient only ca. 200km wide, allowing us to project future ocean communities
270 from a robust set of underlying observations. Our results reflect patterns in a diverse selection
271 of species from nearshore marine communities in the Salish Sea, consisting of 222 planktonic taxa

272 obtained from the metabarcoding analysis of 227 discrete samples across 77 space-time points (eight
273 sites, 1.5 years). We find that changes in the composition of biological communities closely mirrored
274 the variation in pH and temperature, with clear winners (e.g., *Emiliania huxleyi*, *Alexandrium*, and
275 others) and losers (many, but not all, diatoms) likely to shift the structure and function of future
276 marine communities.

277 A vast amount of evidence suggests climate-associated effects on marine species, and broad
278 patterns of sensitivity are discernible within major taxonomic groups [23, 55, among many others].
279 However, because the strength and direction of these effects are variable and species-specific [38],
280 very little is known about community-level impacts. Our work illustrates the nearshore plank-
281 tonic communities that can thrive in low pH - high temperature conditions; such communities are
282 therefore likely to become more prevalent under future conditions.

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

286 Among the taxa surveyed, diatoms are of particular interest for their ubiquity in the world's
287 oceans and their important roles in marine food webs [3, 58], as well as in ecological and evolutionary
288 theory [42]. Our model suggests that diatoms will decrease in richness between the present and
289 2095, particularly in the Hood Canal summer months, when extreme temperatures are more likely.
290 Although the most prevalent response among diatoms is a decrease in suitability, some substantial
291 variability in responses exists within the group. For example, the centric diatom *Coscinodiscus*
292 spp., which is a food source for *Acartia* spp. copepods [30] and many other animal species, will see
293 future suitable habitat only in colder waters such as those in San Juan Island, while *Skeletonema*
294 spp. and the HAB-forming species *Pseudo-nitzschia* spp. will see their habitat suitability remain
295 constant or slightly increased, especially at low pH levels.

296 More strikingly, we see a dramatic increase in suitable environment for the HAB-forming di-
297 noflagellate *Alexandrium* sp., which can substantially harm local ecosystems [13] and economies [1].
298 This increase is particularly high in the summer months of the Hood Canal, when pH is low and
299 temperatures are are high. Both archaeological and experimental evidence suggest *Alexandrium* sp.
300 blooms with warmer temperatures [46], and models [45] also predict an increase in bloom-favorable
301 conditions for *Alexandrium* sp. in future oceans.

302 Our results therefore suggest a possible change in relative dominance between diatoms and
303 other phytoplankton species such as dinoflagellates, consistent with those seen at ecological regime
304 shifts found elsewhere [58, 27]. Such a shift could affect ecosystems in many ways; even under the
305 assumption that the surviving taxa would maintain the primary production levels, for example,
306 the smaller cell-size of dinoflagellates and the differential sinking rates of the two groups would
307 likely alter regional patterns of nutrient cycling[61, 2, 6]. Although the north Atlantic has shown
308 an increase in diatom abundance[27], the increase in wind stress and associated mixing in the
309 water column in the open ocean is unlikely to occur in the Hood Canal, where stratification is
310 the strongest in the Salish Sea [44]. Furthermore, locally focused models support an increase in
311 dinoflagellate dominance with climate change, particularly during summer months [35].

312 Our model also suggests increased environmental suitability for the coccolithophore *Emiliania*
313 *huxleyi*. There is evidence supporting increased calcification and respiration rates with higher pCO₂
314 levels [29] for this ubiquitous species, although the many strains of this species and its adaptive
315 capacity make it difficult to predict longer-term effects with confidence [7].

316 Changes in environmental conditions and associated shifts in planktonic communities will likely
317 reshape ecosystems and food webs, although some environmental processes may be conserved even
318 as the particular taxa change. A switch from a diatom-dominated ecosystem to one in which
319 dinoflagellate blooms extend in space and time could provoke cascade effects [61] including fish
320 mortality, eutrophication [2], and benthic-pelagic coupling [6]. Beyond the phylum-specific patterns,
321 the increase in suitable habitat for HAB-forming species will alone be an engine for ecosystem change
322 [56, 59].

323 One general challenge for model-based work is a tendency to extrapolate from observed condi-
324 tions in ways that are often untestable – by necessity, projections frequently operate outside the
325 range of parameters on which the model was trained [21]. Our study system lets us avoid this
326 pitfall, in that our observed conditions encompass much of the environmental range predicted for
327 future temperate oceans. That is, the changes we predict for the year 2095 do not primarily come
328 from extreme values of pH and temperature, but rather from presently-rare conditions becoming
329 more common.

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

332 **6 Acknowledgments**

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338 carbonate-chemistry expertise and equipment.

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