

2 **1 Title**

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

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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. Con-
18 sequently, 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 222 planktonic taxa along a gradient of tem-
21 perature, 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 replica-
23 ble 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 in
27 dinoflagellates. Individual taxa finding greater suitable habitat in near-future waters are more tax-
28 onomically 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 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 in
75 water chemistry and other environmental conditions despite geographic proximity (ca. 300km; Fig-
76 ure 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 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; 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 Supplementary 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 Supplementary Information,
112 implemented in Unix and R [55]. Briefly, we used a Unix script that calls secondary programs for
113 primer-trimming and preliminary quality-control [46, 11] we estimated the likely composition of
114 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 Supplementary Information).

124 By treating amplification efficiency as consistent within a given taxon, we created an index
125 of abundance for each taxon across space and time ("eDNA index" [35]), using pooled data from
126 technical replicates and mean proportions across biological replicates. We used this index of abun-
127 dience in the multivariate community analysis, and used binary (presence/absence) data to capture
128 species-level responses to environmental conditions. In the Supplementary Information, we provide
129 a function in R to calculate this index.

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 strongly 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 cal-
144 culated the probability of each community, given these predictors, using a multinomial logistic
145 regression in the R package *nnet* [63].

146 Year-2095 Environmental Scenario and Biological Responses

147 We estimated the distribution of environmental parameters for the overall Salish Sea in 2095 from
148 the results of [36], which estimated an annual mean increase in temperature of 1.51 °C and mean pH
149 decrease of 0.18 for the Salish Sea as a whole. We fit a normal distribution to our 2017 environmental
150 observations to create baseline conditions, and modeled the change in mean parameter values
151 between 2017 and 2095 as a linear function of time. We then used the modeled distribution of
152 environmental parameters to generate 1000 simulations of each year scenario. The scenario labeled
153 as 2095 is the set of parameters falling within the 95% percentile in simulations for the years
154 2091-2095. See the R code in the Supplementary Information (lines 98-135).

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

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

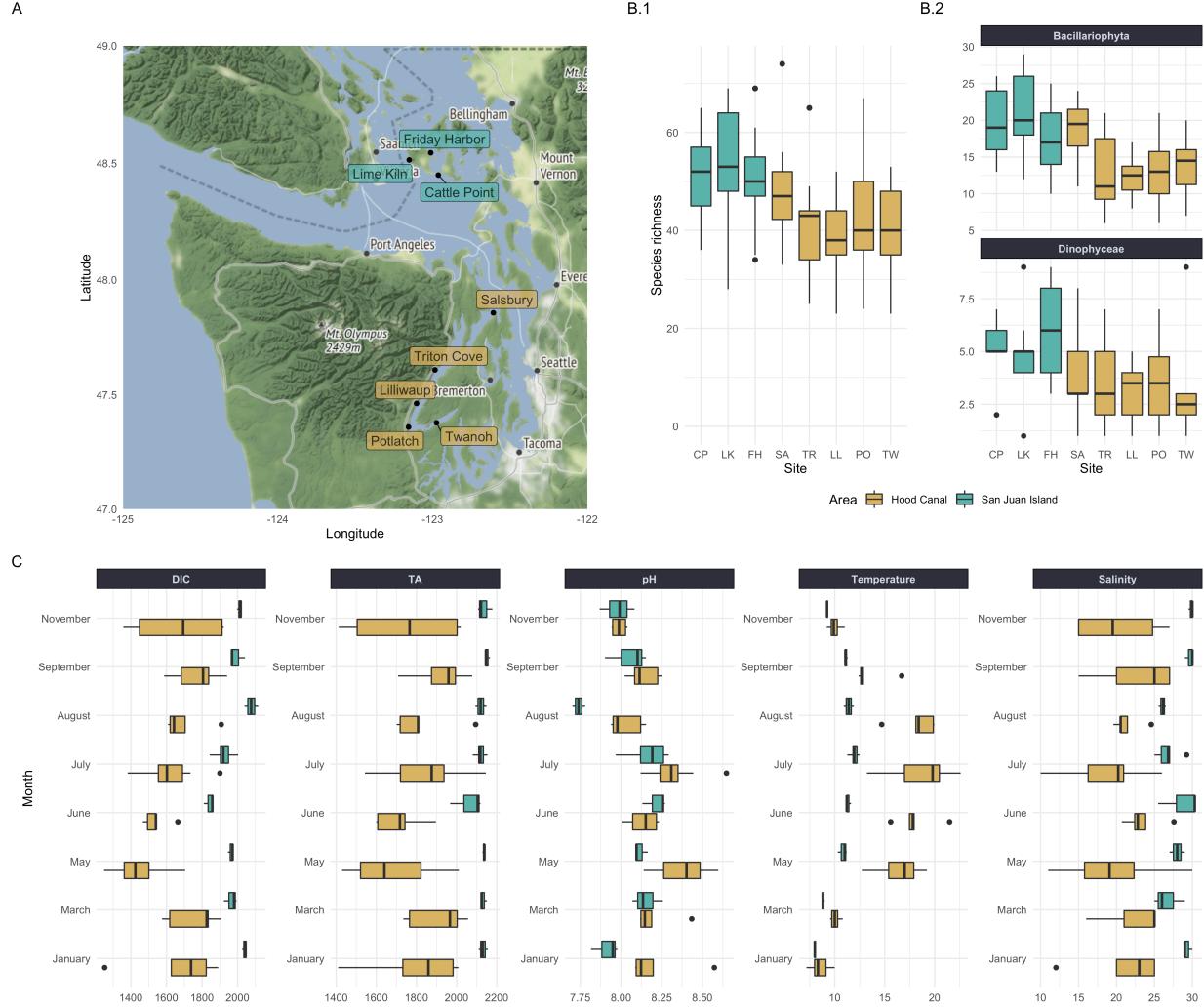


Figure 1: **A** Sampling locations in intertidal areas of the Hood Canal (dark gold) and San Juan Island (turquoise). **B** Planktonic richness (unique taxa) per sampling locality, as reflected by the eDNA COI assay. Boxplots represent the variability in richness across all time points for samples taken 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).

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

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

178 Results

179 Variation in Carbonate Chemistry and in Ecological Communities

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

187 Metabarcoding analysis of eDNA samples generated more than 50.8M sequences from 778 sam-
 188 ples. These samples represented biological and technical replicates from 86 unique sampling events.
 189 After bioinformatics quality-control the dataset included ~45M sequences, from 4849 unique am-
 190 plicon sequence variants (ASVs). Of these, 1364 ASVs (22.6M reads) could be annotated to a
 191 taxonomic level of Family or lower. These ~ 500 taxa from 43 phyla were split according to their
 192 natural history and habitat (benthic vs. planktonic; see Supplementary Information). Because we

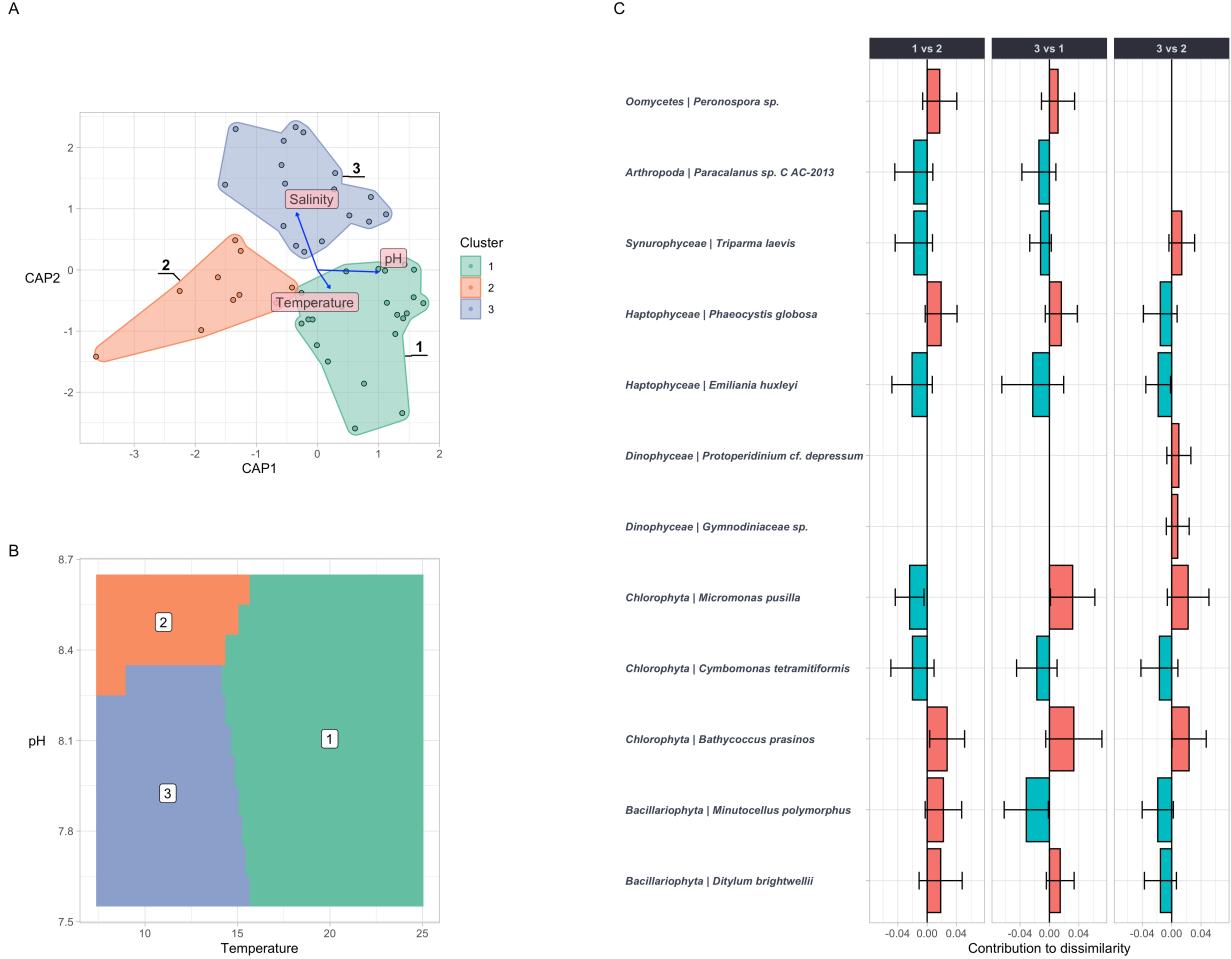


Figure 2: Biological communities and their relationship with environmental variables in the summer months of the Hood Canal. **A** Constrained Analysis of Principal Coordinates (CAP) of Bray-Curtis dissimilarities among biological communities, as constrained by pH, temperature and salinity (arrows).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

193 expect planktonic taxa to vary with water mass [34] and therefore with bottle-sampled carbonate
194 chemistry, here we focus on only the planktonic taxa ($N = 221$). These taxa showed a seasonal
195 richness gradient between study areas, consistent with documented biodiversity clines in the area
196 (Figure 1B.1) [17].

197 Bray-Curtis dissimilarities among samples revealed large differences in metabarcoding communi-
198 ties due to geographic Area (Hood Canal vs. San Juan Island; $F = 1.6184$; $p < 0.01$). We therefore
199 performed a constrained analysis of principal components (CAP) for samples within each Area,
200 showing the differences among communities as a function of temperature, pH, and salinity (Figure
201 2; for clarity, results for Hood Canal shown; full analysis in the Supplementary Information).

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

207 These clusters were distinguished by changes in the relative abundances of a wide variety of
208 taxa. In the Hood Canal for example, the cluster linked with colder water and higher-pH (cluster
209 2 in Figure 2B) showed higher eDNA indexes of diatoms (*Minutocellus polymorphus*), green algae
210 (*Bathycoccus prasinus* and *Micromonas pusilla*), and dinoflagellates like *Karlodinium* sp. relative
211 to the lower-pH cluster at the same temperature range. In that community (cluster 3) *Emiliania*
212 *huxleyi*, *Ditylum brightwellii* and the copepod *Paracalanus* sp. C are more prevalent. The cluster
213 common in the high temperature range (cluster 1 in Figure 2B) shows high values of *Phaeocystis*
214 *globosa*, *Emiliania huxleyi*, *Ditylum brightwellii* among other species. Cluster 1 (Figure 2A,B) and
215 similar planktonic communities occupy the spectrum of environmental conditions most likely to be
216 encountered in near-future climate scenarios as temperature rises and pH falls. For example, we
217 expect the Hood Canal in 2095 to have the conditions in which community 1 is the most likely
218 community 67% of the time, an increase of 11% compared to 2017 (see Supplementary Information).
219 On the other hand, conditions that make cluster 2 the most likely community drop from 12% to
220 2% of the time between 2017 and 2095.

221 Climate envelopes and future distributions

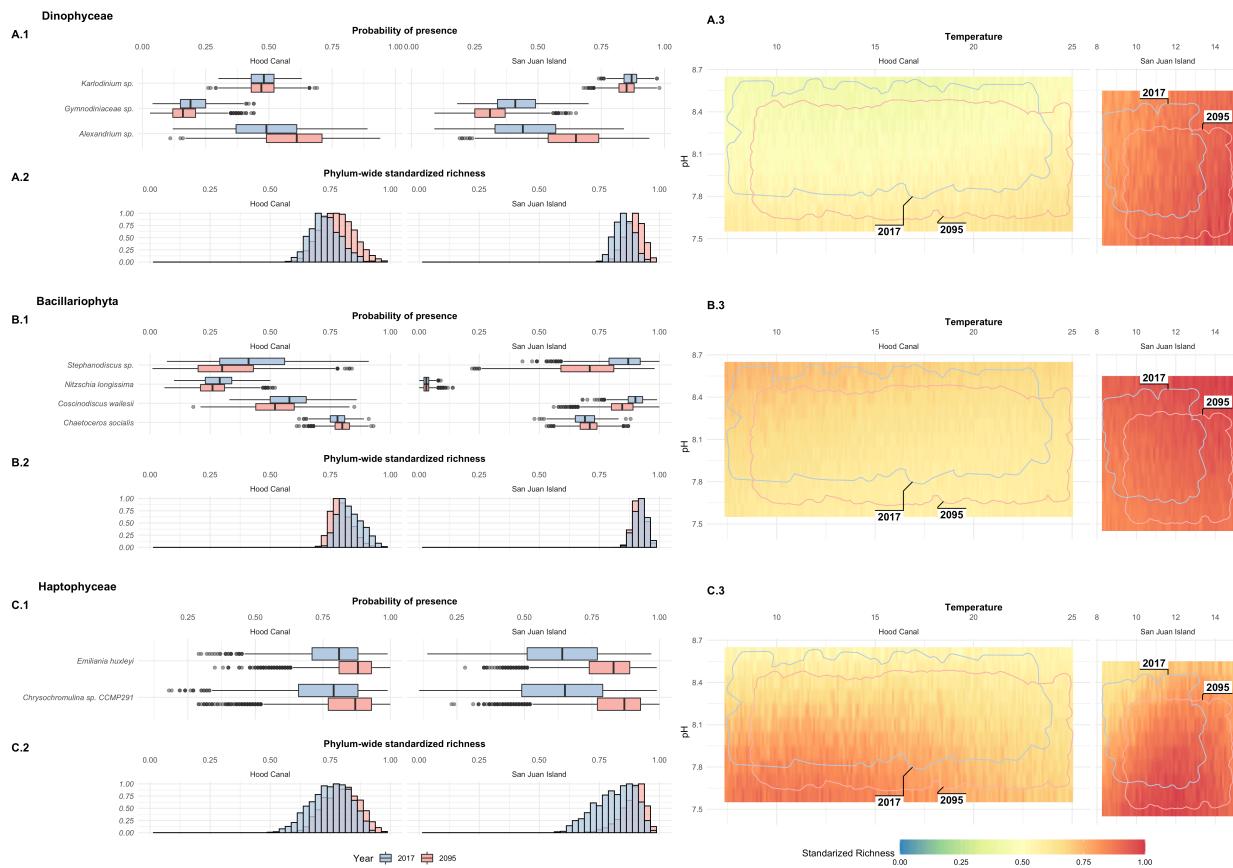


Figure 3: Forecasted changes in plankton in the Salish Sea for Dinoflagellates (panel **A**), Diatoms (**B**) and Haptophytes (**C**). Each panel shows: **(1)** probability densities for the occurrence of selected taxa (species- and genus-level) for 2017 (blue) and 2095 (red); data are mean probabilities 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 (Figure 3 A.1, B.1, C.1), estimate richness for larger taxonomic grounds as a whole for these two climates (Figure 3 A.2, B.2, C.2), and estimate richness within taxonomic groups across the pH-temperature continuum (Figure 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 diatom taxa including *Coscinodiscus* and *Stephanodiscus*, both centric 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 the dinoflagellate *Gymnodiniaceae* sp.

Likely winners under future conditions are more widely scattered among higher taxonomic groups. The haptophytes *Emiliania huxleyi* and *Chrysochromulina* sp. the dinoflagellate *Alexandrium* sp. all find more suitable habitat in both of our study areas. Among others not shown in Figure 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 Supplementary 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 (0.46-0.55 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 frequently in the future. For example, in the Hood Canal at present, we expect surface waters

252 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
253 more frequently (i.e., 6% of the time). At these values of T and pH, our model predicts the harmful
254 *Alexandrium* sp. to occur more often than not (mean frequency of occurrence = 0.83). By contrast,
255 the large centric diatom *Coscinodiscus* – a potentially key source of carbon for zooplankton and
256 small fishes [53, 69] with effects on dissolved oxygen and other water-column characteristics [44] –
257 occurs only one-third of the time under these same conditions (mean frequency = 0.35).

258 Discussion

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

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

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

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

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

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

306 Our model also suggests increased environmental suitability for the coccolithophore *Emiliania*
307 *huxleyi*. There is evidence supporting increased calcification and respiration rates with higher pCO_2
308 levels [30] for this ubiquitous species, although the many strains of this species and its adaptive
309 capacity make it difficult to predict longer-term effects with confidence [8].

310 Changes in environmental conditions and associated shifts in planktonic communities will likely
311 reshape ecosystems and food webs, although some environmental processes may be conserved even
312 as the particular taxa change. A switch from a diatom-dominated ecosystem to one in which
313 dinoflagellate blooms extend in space and time could provoke cascade effects [68] including fish

314 mortality, anoxia [2], and carbon sinking dynamics [7]. Beyond the phylum-specific patterns, the
315 increase in suitable habitat for harmful algae species will alone be an engine for ecosystem change
316 [62, 66].

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

324 The taxa surveyed here are a function of our metabarcoding PCR primers [43] and reflect the
325 current status of genetic databases, rather than a complete sampling of the planktonic community;
326 we view these results as a cross-section of common taxa useful for understanding the biological
327 effects of ocean conditions. Our observations are strong evidence of the kinds of changes likely in
328 future marine communities, and they offer testable predictions about the magnitude and direction
329 of effects on focal species.

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