

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

31 **3 Keywords**

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

33 **4 Main Text**

34 **4.1 Background**

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

45 Two natural CO_2 seeps in nearshore marine habitats – one in Italy and one in Papua New Guinea
46 – have demonstrated shifts in benthic communities associated with especially acute acidification in
47 the present day, previewing those we might expect at a more global scale under future conditions
48 [38, 21]. But beyond these exceptional sites, it is difficult to measure changes in ecological com-
49 munities associated with the relatively subtle shifts in nearshore ocean chemistry observed to date,
50 particularly in light of naturally large spatial and temporal variation in these communities. The

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

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

67 Here we use series of metabarcoding samples taken across space and time to track changes in
68 nearshore ecological communities associated with differences in pH, water temperature, and other
69 environmental variables. We use broad-spectrum PCR primers [43] to target eukaryotes specifically,
70 identifying the likely effects of future climate scenarios on suites of planktonic taxa.

71 5 Methods

72 Sampling

73 We collected water samples to assess eDNA communities in two regions of the Salish Sea (Wash-
74 ington, USA): San Juan Island and the Hood Canal. These sites experience substantial variation
75 in water chemistry and other environmental conditions despite geographic proximity (ca. 300km;
76 Fig. 1). We sampled eight sites monthly for approximately 1.5 years (March 2017 to August 2018),
77 taking three 1L samples (biological replicates; ca. 10m apart) each month at each site (261 bottle
78 samples total). Each sample was filtered through a $0.45\ \mu\text{m}$ cellulose filter, and the filter preserved
79 in Longmire buffer until DNA extraction [55]. Concurrently, we collected one 120 ml water sample
80 from each site and poisoned it with 0.1 ml of saturated HgCl_2 for carbonate chemistry analysis,
81 following [56]. We also collected *in situ* measurements of temperature, salinity and dissolved oxy-
82 gen using a handheld multiprobe (Hanna Instruments, USA) and a portable refractometer. We
83 note that many unmeasured variables influence planktonic communities (e.g., nutrients, sunlight,
84 wave energy), but that our set of measured parameters clearly distinguished communities and was
85 adequate for our purposes.

86 We characterized sample carbonate chemistry by measuring Total Alkalinity (TA; open-cell
87 automated titration based on a 876 Dosimat plus (Metrohm AG) as part of a custom system
88 assembled by Andrew Dickson (UCSD) and used in the laboratory of Alex Gagnon at UW) and
89 Dissolved Inorganic Carbon (DIC; Apollo Instruments, USA; CO_2 extraction system with 10%
90 (v/v) phosphoric acid). Both measurements were calibrated and validated with certified reference
91 material from the Scripps Oceanographic Institute. Using DIC and TA, we calculated pH and the
92 remaining carbonate system parameters using the R package ‘seacarb’ [25].

93 Our sampled areas differed in the environmental variables driving changes in carbonate chem-
94 istry. San Juan Island was less seasonally variable than the Hood Canal in every measured param-
95 eter (Figure 1B); the island is more directly affected by summer coastal upwelling as a function
96 of bathymetry and circulation patterns [50], and this appears to be the dominant influence on
97 carbonate chemistry there. By contrast, photosynthesis and respiration likely drive much of the
98 carbonate chemistry variation in the Hood Canal (See Supporting Information).

99 eDNA sequencing and bioinformatic processes

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

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

111 Code for all quality-screening and bioinformatics is available in the Supporting Information,
112 implemented in Unix and R [54]. Briefly, we used a Unix script that calls secondary programs
113 for primer-trimming and preliminary quality-control [46, 11] we estimated the likely composition
114 of each sample using DADA2. This approach avoids clustering, such that we retained all of the
115 amplicon sequence variants (ASVs, *i.e.*, unique sequences); we subsequently carried out secondary
116 quality-control and decontamination following [34]. We then assigned sequences to known taxa
117 using phylogenetic tree placement with *insect* v1.1 [66]; where *insect* could not place individual
118 taxa, we supplemented assignment by classification against a custom COI database using *anacapa*
119 [15] and *bowtie2* [42]. We conservatively kept only taxa annotated at the level of taxonomic family,
120 genus, or species, so we could reliably infer taxon natural history under the assumption that taxa
121 within the same family shared broad natural-history characteristics. Using published literature and
122 online databases, we placed every recovered taxon into a benthic/planktonic category and focused
123 our analysis on the planktonic community (see Supporting Information).

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

129 Present scenario community analyses

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

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

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

144 Year-2095 Environmental Scenario and Biological Responses

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

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

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

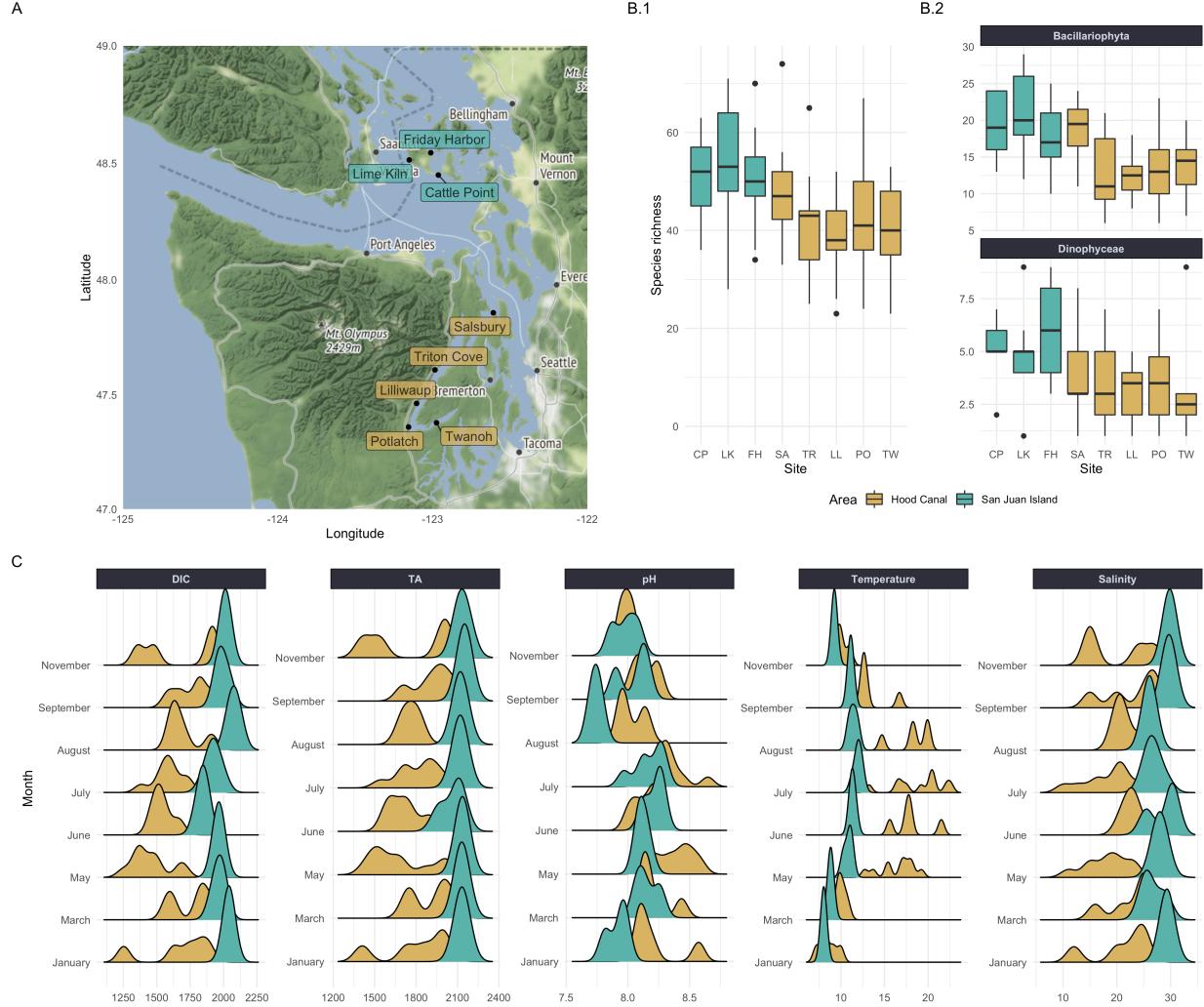


Figure 1: **A** Sampling locations in intertidal areas of the Hood Canal (dark gold) and San Juan Island (turquoise). **B** Planktonic richness (unique taxa) per sampling locality, as reflected by the eDNA COI assay. Boxplots represent the variability in richness across all time points for samples taken at the indicated site. **B.1** Taxa from all phyla; **B.2** Diatoms (above) and dinoflagellates (below) shown separately; note change in the scale of the y-axis. **C** The observed environmental profiles of these two regions reflect a broad range of environmental conditions, with the Hood Canal resembling future conditions in temperate areas worldwide. Shown: Dissolved Inorganic Carbon (DIC, $\mu\text{moles/kg}$); Total Alkalinity (TA, $\mu\text{moles/kg}$); Temperature ($^{\circ}\text{C}$) and Salinity (PSU). Individual sampling sites shown as vertical facets.

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

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

180 Results

181 Variation in Carbonate Chemistry and in Ecological Communities

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

189 Metabarcoding analysis of eDNA samples generated more than 52.5M sequences from 745 sam-
 190 ples. These samples represented biological and technical replicates from 86 unique sampling events.
 191 After bioinformatics quality-control (See Methods) the dataset included ~41M sequences, from
 192 4782 unique amplicon sequence variants (ASVs). Of these, 1374 ASVs (22.8M reads) could be

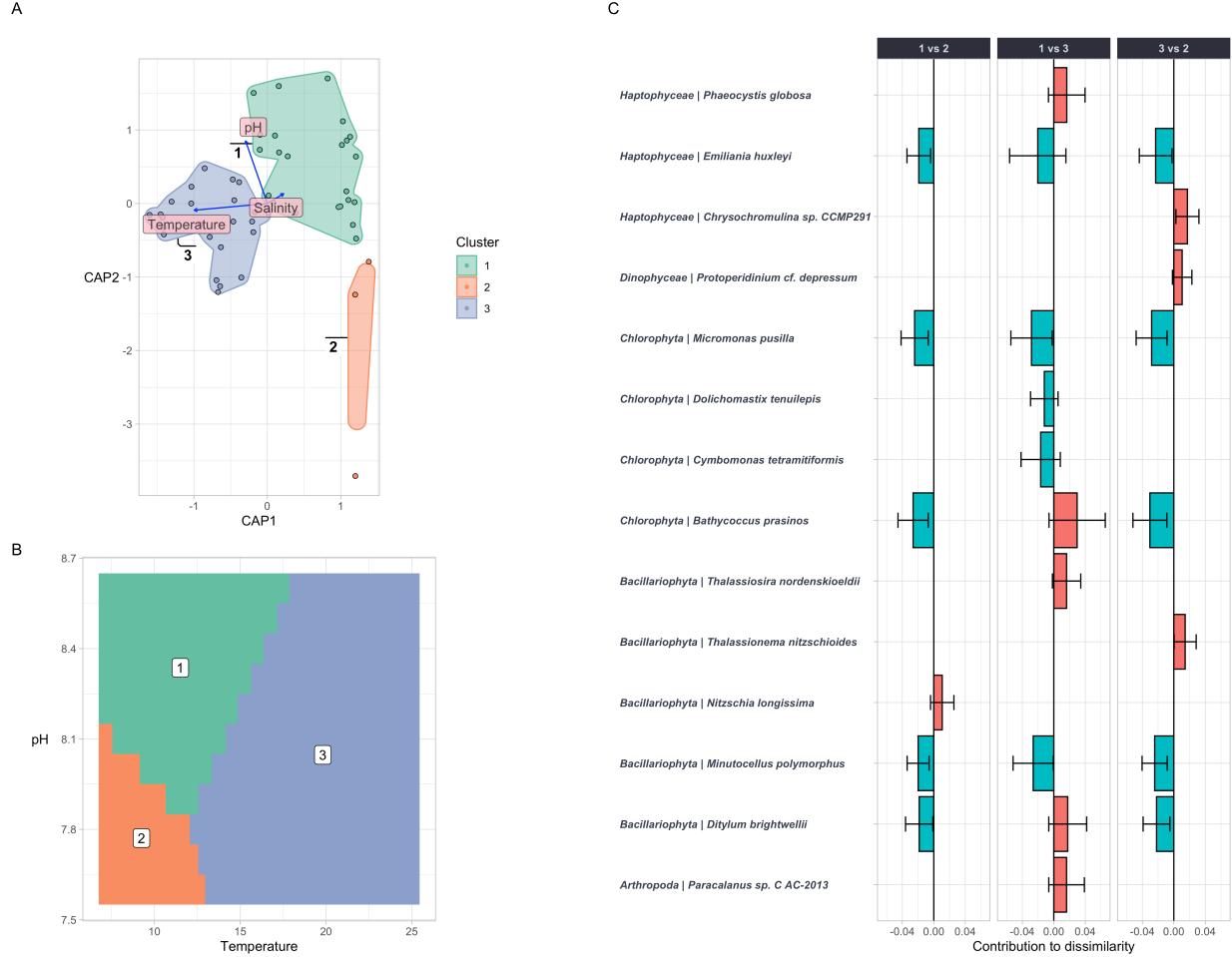


Figure 2: Biological communities and their relationship with environmental variables in the summer months of the Hood Canal. **A** Constrained Analysis of Principal Coordinates (CAP) of Bray-Curtis dissimilarities among biological communities, as constrained by pH, temperature and salinity (arrows). **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 annotated to a taxonomic level of Family or lower. These ~ 500 taxa from 43 phyla were split according to their natural history and habitat (benthic vs. planktonic; see Supporting Information).
 194 Because we expect planktonic taxa to vary with water mass [34] and therefore with bottle-sampled
 195 carbonate chemistry, here we focus on only the planktonic taxa ($N = 222$). These taxa showed a
 196 seasonal richness gradient between study areas, consistent with documented biodiversity clines in
 197 the area (Fig. 1B.1) [17].

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

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

202 These communities were distinguished by changes in the relative abundances of a wide variety of taxa. In the Hood Canal for example, the colder water and higher-pH community (community 1 in Fig. 2C) showed higher eDNA indexes of diatoms *Thalassiosira nordenskioeldii*, *Nitzschia cf. promare*, and *Ditylum brightwellii* – among other species – relative to a higher-temperature community (community 3 in Fig. 2C). Communities 2 and 3 had relatively higher values for taxa from a mix of groups including *Emiliania huxleyi*, *Minutocellus polymorphus*, *Micromonas pusilla*, and *Bathycoccus prasinus*. Community 3 (Fig. 2A,B) and similar planktonic communities occupy the spectrum of environmental conditions most likely to be encountered in near-future climate scenarios as temperature rises and pH falls. For example, we expect the Hood Canal in 2095 to have the conditions in which community 3 is the most likely community 75% of the time, an increase of 22% compared to 2017 (see Supplemental Material).

203 Climate envelopes and future distributions

204 To explore the suitability of different environmental conditions for each taxon, we modeled the

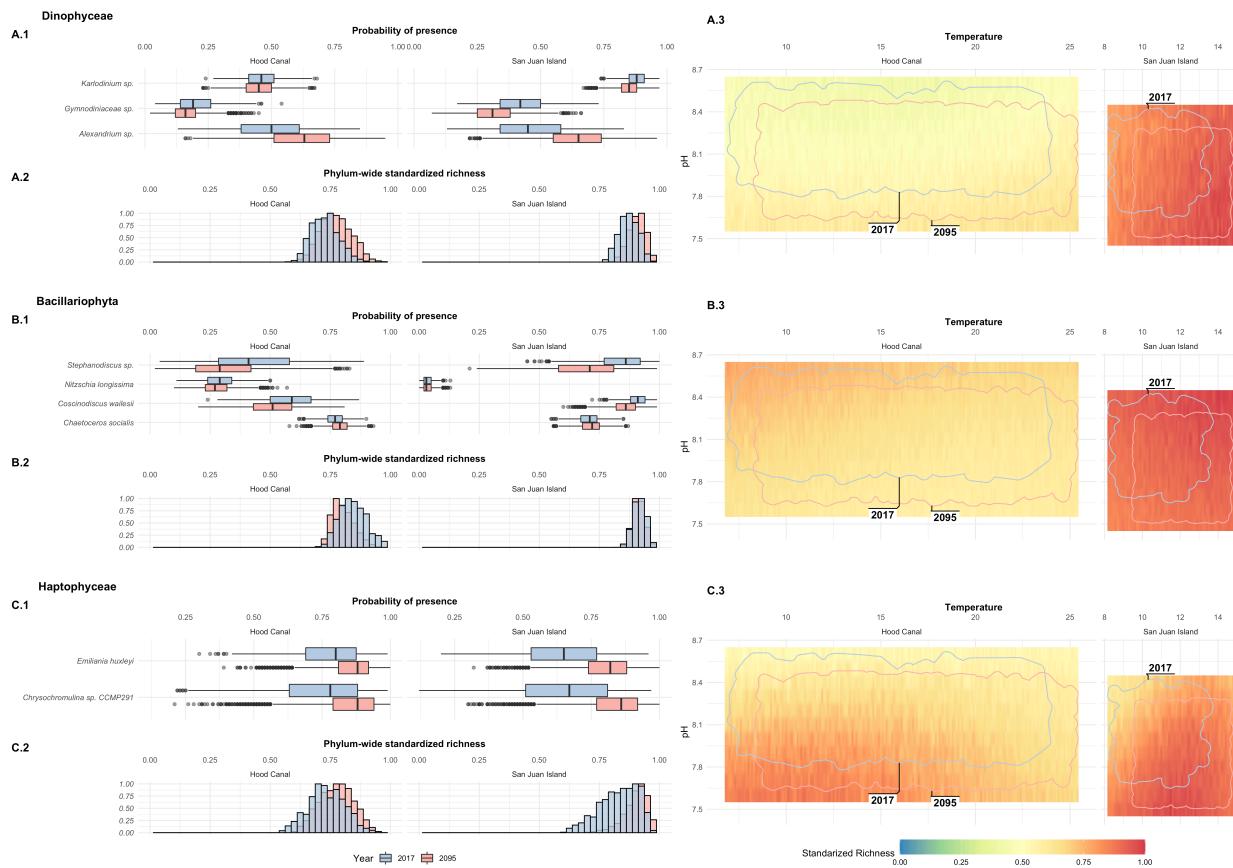


Figure 3: Forecasted changes in plankton in the Salish Sea for Dinoflagellates (panel **A**), Diatoms (B) and Haptophytes (C). Each panel shows: (1) probability densities for the occurrence of selected taxa (species- and genus-level) for 2017 (blue) and 2095 (red); data are mean probabilities over 100 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.

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

229 Diatoms (Bacillariophyta) show the steepest richness decline under future conditions (Figure
 230 3 B.2); the probability of occurrence decreases markedly for diverse diatom taxa including both
 231 pennate (e.g., *Nitzschia*) and centric (e.g., *Coscinodiscus*, *Stephanodiscus*) body forms. These
 232 declines in diatom richness were more accentuated at lower pH values and higher temperatures.
 233 Other taxa likely to find less-suitable habitat in the future include dinoflagellates *Karlodinium* and
 234 *Gymnodiniaceae* sp.

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

241 Given such heterogeneity in projections, gains and losses tend to balance one another out
 242 when looking at overall richness variation; we find no change in median richness for the year 2095
 243 relative to the present in the Hood Canal (overall taxon richness by year, 95% confidence interval
 244 in median species richness -0.08, +0.04; Wilcoxon p = 0.5); while higher diversity is expected in
 245 the San Juan Island in 2095 (increase in median species richness of 1.84-2.2, p < 10⁻¹⁶). Diatoms,
 246 in particular, show small but significant declines in richness in the Hood Canal (0.47-0.57 species,
 247 p < 10⁻¹⁶), while the changes on the San Juan Island are negligible (median change 0-0.08 p =
 248 0.04). Dinoflagellates see their richness increase in both regions with the future scenario (median
 249 change 0.14-0.18 Hood Canal; 0.21-0.25 San Juan Island; p < 10⁻¹⁶ for each).

250 The bulk of our projected community changes result from now-rare conditions occurring more
 251 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, 68] 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 $\Delta T 2.5^\circ\text{C}$, $\Delta \text{pH } -0.35$ globally; RCP 8.5; 24]. Here we
261 document communities exposed to this same range of projected conditions in the present day, along
262 an environmental gradient only ca. 200km wide, allowing us to project future ocean communities
263 from a robust set of underlying observations. Our results reflect patterns in a diverse selection
264 of species from nearshore marine communities in the Salish Sea, consisting of 222 planktonic taxa
265 obtained from the metabarcoding analysis of 227 discrete samples across 77 space-time points (eight
266 sites, 1.5 years). We find that changes in the composition of biological communities closely mirrored
267 the 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, 60, 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, 63], 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 Supplemental Material).

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 [63, 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,
299 the smaller cell-size of dinoflagellates and the differential sinking rates of the two groups would
300 likely alter regional patterns of nutrient cycling [67, 2, 7]. Although the north Atlantic has shown
301 an increase in diatom abundance [28], the increase in wind stress and associated mixing in the
302 water column in the open ocean is unlikely to occur in the Hood Canal, where stratification is
303 the strongest in the Salish Sea [47]. Furthermore, locally focused models support an increase in
304 dinoflagellate dominance with climate change, particularly during summer months [36].

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

309 Changes in environmental conditions and associated shifts in planktonic communities will likely
310 reshape ecosystems and food webs, although some environmental processes may be conserved even
311 as the particular taxa change. A switch from a diatom-dominated ecosystem to one in which
312 dinoflagellate blooms extend in space and time could provoke cascade effects [67] including fish
313 mortality, eutrophication [2], and benthic-pelagic coupling [7]. Beyond the phylum-specific patterns,

314 the increase in suitable habitat for harmful algae species will alone be an engine for ecosystem change
315 [61, 65].

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

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

325 6 Acknowledgments

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