

2 1 Title

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

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13 conceived of the project and assisted in sampling, analysis, and writing, and received funding for
14 the work. EJP and KC assisted in sampling, laboratory work, and analysis.

15 The authors declare no conflict of interest.

16 2 Abstract

17 Studies of the ecological effects of global change often focus on one or few species at a time.
18 Consequently, we know relatively little about the changes underway at real-world scales of biological
19 communities, which typically have hundreds or thousands of interacting species. Here, we use
20 monthly samples of environmental DNA to survey over 222 planktonic taxa along a gradient of
21 temperature, salinity, dissolved oxygen, and carbonate chemistry in nearshore marine habitat. The
22 result is a high-resolution picture of changes in ecological communities using a technique replicable
23 across a wide variety of ecosystems. We estimate community-level differences associated with time,
24 space and environmental variables, and use these results to forecast near-term community changes
25 due to warming and ocean acidification. We find distinct communities in warmer and more acidified
26 conditions in both areas sampled, and a strong reduction in richness of current diatom assemblages.
27 Taxa finding greater suitable habitat in near-future waters are more taxonomically varied and
28 include the ubiquitous coccolithophore *Emiliania huxleyi* and the harmful *Alexandrium sp.* These
29 results 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 [34, 17, 4,
36 12], very likely altering food webs [49, 9, 24] 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 [20, 14, 37], 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 [35, 29]. 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 [35, 18]. 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 [33], creating an opportunity to test the effect of these
54 measures on marine communities under conditions expected worldwide in the near future [45], 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 [26, 30]
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 backs up the efficacy of eDNA metabarcoding for monitoring
64 biodiversity (see a review in [50]), and this approach has been successfully used to detect community
65 composition variation across environmental changes in aquatic [16], estuarine [11, 38], and marine
66 ecosystems [5].

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 [39] 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 $0.45 \mu\text{m}$ cellulose filter, and the filter preserved
79 in Longmire buffer until DNA extraction [47]. 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 [48]. 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’ [22].

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 [45], 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 [47]. 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 [39]. 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 [46].
105 PCR conditions and protocols for sample identification followed [31], 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 [51].

111 Code for all quality-screening and bioinformatics is available in the Supporting Information.
112 Briefly, we used a Unix script that calls secondary programs for primer-trimming and preliminary
113 quality-control [41, 10] we estimated the likely composition of each sample using DADA2. This
114 approach avoids clustering, such that we retained all of the amplicon sequence variants (ASVs, *i.e.*,
115 unique sequences); we subsequently carried out secondary quality-control and decontamination
116 following [31]. We then assigned sequences to known taxa using phylogenetic tree placement with
117 insect v1.1 [57]. We conservatively kept only taxa annotated at the level of taxonomic family,
118 genus, or species, so we could reliably infer taxon natural history. Using published literature and
119 online databases, we placed every recovered taxon into a benthic/planktonic category and focused
120 our analysis on the planktonic community (see Supporting Information).

121 By treating amplification efficiency as consistent within a given taxon, we created an index of
122 abundance for each taxon across space and time [32], using pooled data from technical replicates
123 and mean proportions across biological replicates. The multivariate tests (PERMANOVA, CAP,
124 k-means clustering) used these index values as the species abundances and Bray-Curtis as the
125 dissimilarity measurement between samples.

126 Year 2095 Environmental Scenario and Biological Responses

127 We estimated the distribution of environmental parameters for the overall Salish Sea in 2095 from
128 the results of [33], which estimated an annual mean increase in temperature of 1.51 °C and mean
129 pH decrease of 0.18 for the Salish Sea as a whole. We then subsampled this distribution to reflect
130 the characteristic environments of different areas (San Juan Island and Hood Canal) and seasons
131 (Summer and Winter, with April - September coded as "Summer"). To do this, we fit beta
132 parameters to describe the fraction of the overall observed data (2017 - 2018) reflected in each
133 subset; we then subsampled the 2095 distribution accordingly, under the assumption that the
134 Hood Canal in Summer (for example) would occupy the same fraction of the overall annual Salish
135 Sea temperature and pH distributions in 2095 as it did in 2017. See Supporting Information for
136 details of sampling parameters.

137 For community-level projections, we coded community-cluster identity (Figure 2) as an un-
138 ordered response variable in a multinomial logistic model, with temperature, pH, area, and season
139 as predictor variables. We calculated the probability of each community, given these predictors,
140 using the package 'nnet' [54]. Most-common combinations of T and pH were estimated from 2-
141 dimensional density plots for each year.

142 Within a geographic area and season, temperature and pH were most strongly associated with
143 community-level changes. We therefore developed a hierarchical logistical regression model relat-
144 ing the presence of each taxon to temperature and pH (as fixed effects) **-salinity is predicted to**
145 **remain largely unchanged in future scenarios** [33]. **We allowed** slope to vary by geographic area
146 and intercept to vary by season, to capture large-scale seasonal differences associated with sunlight,
147 photosynthesis, and related factors. For each taxon, we fit these models using the Bayesian gener-
148 alized linear mixed effects functions in R package rstanarm [23] for R. See Supporting Information
149 for analytical code.

150 Given the estimated sea-surface temperatures and pH values for 2095 and observed data from
151 2017, we then used taxon-specific logistic regression models to estimate the suitability of habitat
152 for each taxon in the future scenario. For each point in the pH - temperature grid, we calculated
153 species richness as the mean result of 100 independent draws of the posterior distributions of all
154 modeled taxa, grouping the individual taxa models by phylum.

155 We can only model responses of taxa present in our data set. That is, we may predict that the
156 number of (for example) diatom species present will decline relative to those present today, but our
157 data do not allow us to predict whether new species will immigrate from elsewhere or how species
158 might evolutionarily adapt to future conditions. It is beyond the scope of our work to account
159 for the latter, and furthermore, because of the extreme uncertainty of evolutionary responses, the
160 predictions of species distribution models are often interpreted without considering adaptation or
161 phenotypic plasticity [43].

162 Results

163 Variation in Carbonate Chemistry and in Ecological Communities

164 We collected intertidal environmental and genetic data from bottle samples each month between
165 March 2017 and August 2018 (Figure 1A). Despite geographic proximity and similar overall species
166 composition (127 of the 222 planktonic taxa were found in both regions and accounted for 98% of
167 the sequences), the areas under study – San Juan Island and the Hood Canal – varied widely in
168 pH, temperature, and other environmental parameters (Figure 1C), with different points along the

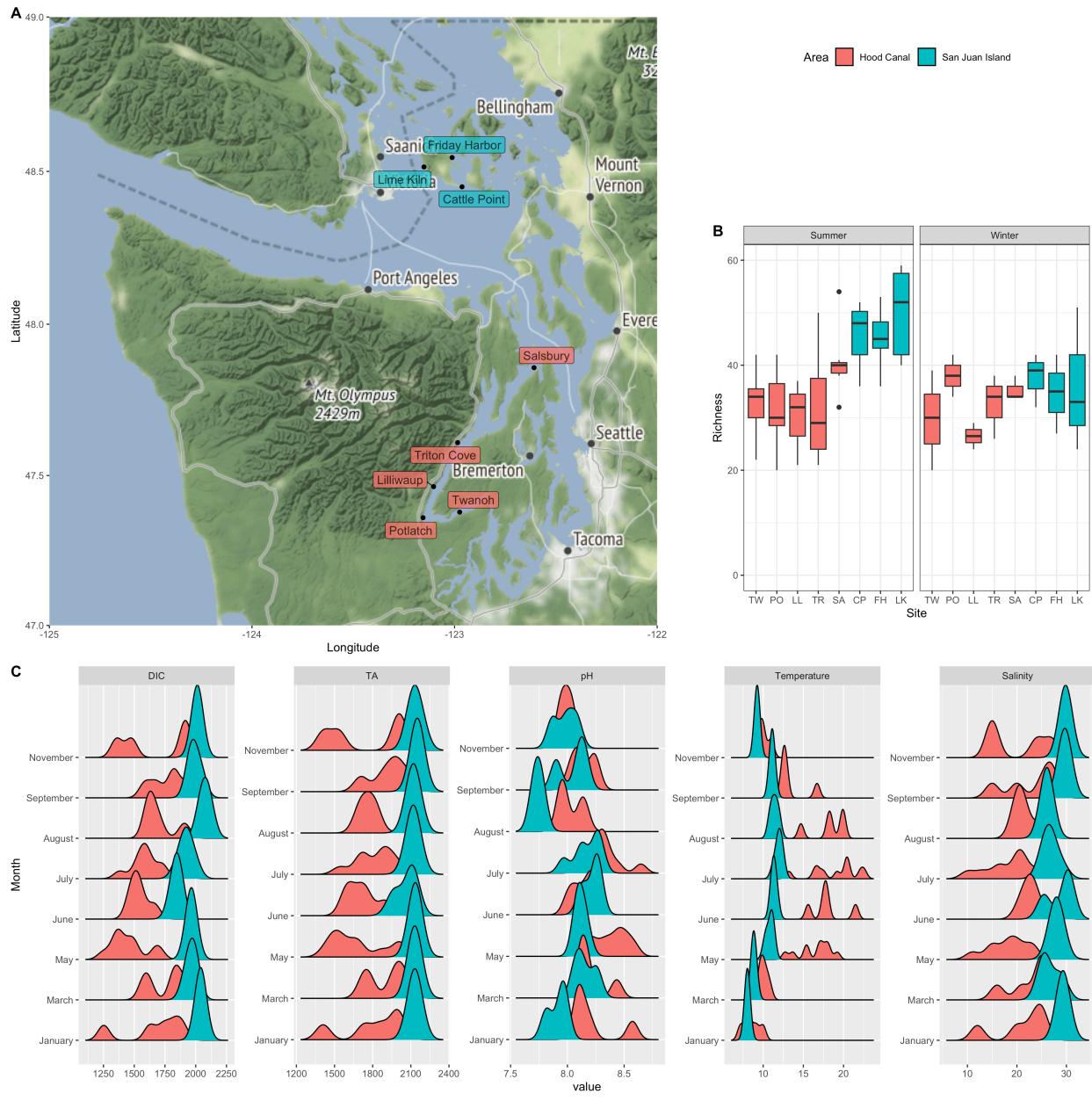


Figure 1: **A** Sampling locations in intertidal areas of the Hood Canal (Red) and San Juan Island (Blue). **B** Planktonic richness (unique taxa) per sampling locality across seasons, as reflected by the eDNA COI assay. **C** The observed environmental profiles of these two regions reflect a broad range 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); Temperature ($^{\circ}\text{C}$) and Salinity (PSU). Individual sampling sites shown as facets.

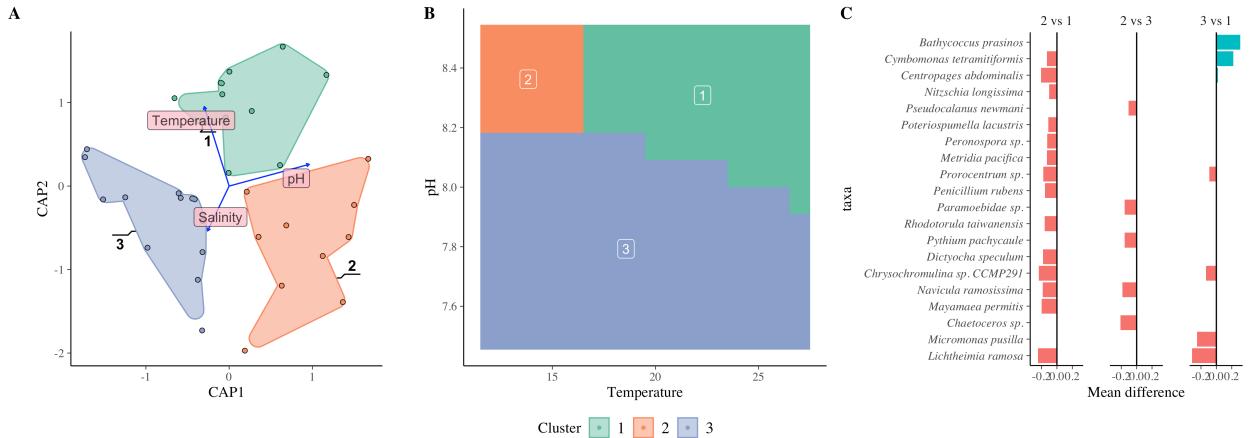


Figure 2: Biological communities and their relationship with environmental variables in the summer months of the Hood Canal. **A** Constrained Analysis of Principal Coordinates (CAP) of Bray-Curtis dissimilarities among biological communities, as constrained by pH, temperature and salinity (arrows). **B** Most-likely cluster as a function of temperature and pH given a multinomial logistic model. **C** Relative abundance (eDNA index; see [32]) of the taxa best distinguishing the three communities illustrated (SIMPER analysis). See Supporting Information for full analysis.

169 environmental gradient simultaneously showing differences equivalent to those predicted between
170 present-day and future oceans [8].

171 Metabarcoding analysis of eDNA samples generated more than 52.5M sequences from 745 sam-
172 ples. These samples represented biological and technical replicates from 86 unique sampling events.
173 After bioinformatics quality-control (See Methods) the dataset included ~41M sequences, from
174 4782 unique amplicon sequence variants (ASVs). Of these, 1374 ASVs (22.8M reads) could be
175 annotated to a taxonomic level of Family or lower. These ~ 500 taxa from 43 phyla were split ac-
176 cording to their natural history and habitat (benthic vs. planktonic; see Supporting Information).
177 Because we expect planktonic taxa to vary with water mass [31] and therefore with bottle-sampled
178 carbonate chemistry, here we focus on only the planktonic taxa ($N = 222$). These taxa showed a
179 seasonal richness gradient between study areas, consistent with documented biodiversity clines in
180 the area (Fig. 1B) [15].

181 Bray-Curtis dissimilarities among samples revealed large differences in metabarcoding com-
182 munities due to season (Summer vs. Winter; PERMANOVA F-model = 14.4; $p < 0.001$) and
183 geographic Area (Hood Canal vs. San Juan Island; F-model = 15.4; $p < 0.001$). We therefore
184 performed a constrained analysis of principal components (CAP) for samples within each combina-
185 tion of Season and Area, showing the differences among communities as a function of temperature,
186 pH, and salinity (Fig. 2 shows results for Hood Canal in summer as an example; full analysis in
187 the Supporting Information). Independent of environmental parameters, we separately clustered
188 samples by pairwise Bray-Curtis dissimilarities among metabarcoding communities (k-means; $N =$
189 3 or 4) to identify groups of samples that were similar to one another with respect to biological
190 community. Each biological cluster (colored hulls, Fig. 2) occupied a unique area of environmental
191 parameter space. Planktonic communities therefore varied predictably with water temperature,
192 salinity, and pH, across a range of those parameters likely to be encountered in many near-term
193 future-ocean scenarios [45]. Multinomial logistic regression yielded predictions of the most-likely
194 community for any combination of environmental parameters (Fig. 2B).

195 These communities were distinguished by changes in the relative abundances of many taxa: in
196 the Hood Canal in summer for example (Fig. 2C), the colder water communities showed higher
197 values of *Centropages* sp., *Nitzschia longissima*, *Prorocentrum* sp., *Chrysochromulina* sp., *Navicula*
198 sp. and *Chaetoceros* sp. Warmer waters were dominated by *Bathycoccus prasinus* and *Cymbomonas*
199 sp., while at lower pH values, marine communities showed *Litchtheimia ramosa*, *Micromonas pusilla*
200 at higher relative abundances.

Climate envelopes and future distributions

201 The low-pH planktonic communities (cluster 3, Fig. 2) occupy the spectrum of environmental
202 conditions most likely to be encountered in near-future climate scenarios as temperature rises and
203 pH falls. For example, by 2095 we expect summer in the Hood Canal to be $T > 12$ and $\text{pH} < 8.2$
204 – where cluster 3 is the most likely community – to occur more than two-thirds of the time (68%).

205 To explore the suitability of different environmental conditions for each taxon, we modeled the
206 likelihood of taxon presence as a function of area and season (random effects) and temperature and
207 pH (fixed effects). Salinity is predicted to remain largely unchanged in future scenarios [33]. Using
208 predicted conditions for Puget Sound for the year 2095 [33], we then projected the probability of
209 presence of each taxon across these conditions over 100 draws of the posterior probabilities of all
210 taxa. We used these draws to 1) show the change in the probability of presence of each taxon for
211 each scenario (i.e. 2017 vs 2095, Fig.3 top panel); 2) calculate the taxon richness within taxonomic

213 groups across the pH-temperature continuum (Fig. 3 center panel), and 3) visualized the change in
214 suitability for each taxon in our dataset individually (selected taxa in Fig. 3 bottom panel).

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

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

228 Given such heterogeneity in projections, gains and losses tend to balance one another out when
229 looking at richness variation within phyla; we find no change in median richness for the year 2095
230 relative to the present (ANOVA; taxon richness within phyla by year, $p = 0.3$). Diatoms, however,
231 show consistent and significant declines in richness (Wilcoxon $p < 10^{-15}$).

232 Taxa surveyed are a function of our metabarcoding PCR primers [39] and reflect the current
233 status of genetic databases, rather than a complete sampling of the planktonic community; we view
234 these results as a cross-section of common taxa useful for understanding the biological effects of
235 ocean conditions.

236 The bulk of our projected community changes result from now-rare conditions occurring more
237 frequently in the future. For example, in summer in the Hood Canal at present, we expect surface
238 waters to have $\text{pH} < 7.9$ and $T > 19^\circ\text{C}$ only 1% of the time. In 2095, we expect these conditions
239 18 times more frequently (i.e., 18% of the time). At these values of T and pH, our model predicts
240 the harmful *Alexandrium* sp. to occur more often than not (mean frequency of occurrence = 0.7).
241 By contrast, the large centric diatom *Coscinodiscus* – a key source of carbon for zooplankton and
242 small fishes – occurs only one-third of the time under these same conditions (mean frequency =
243 0.34).

244 Discussion

245 Temperate surface oceans worldwide average approximately 14°C and pH of 8.1 [8], and will change
246 substantially in this century [mean $\Delta T 2.5^\circ\text{C}$, $\Delta \text{pH } -0.35$ globally; RCP 8.5; 21]. Here we
247 document communities exposed to this same range of projected conditions in the present day, along
248 an environmental gradient only ca. 200km wide, allowing us to project future ocean communities
249 from a robust set of underlying observations. Our results reflect patterns in a diverse selection
250 of species from nearshore marine communities in the Salish Sea, consisting of 222 planktonic taxa
251 obtained from the metabarcoding analysis of 227 discrete samples across 77 space-time points (eight
252 sites, 1.5 years). We find that changes in the composition of biological communities closely mirrored
253 the variation in pH and temperature, with clear winners (e.g., *Emiliania huxleyi*, *Alexandrium*, and
254 others) and losers (many, but not all, diatoms) likely to shift the structure and function of future
255 marine communities.

256 A vast amount of evidence suggests climate-associated effects on marine species, and broad
257 patterns of sensitivity are discernible within major taxonomic groups [21, 52, among many others].
258 However, because the strength and direction of these effects are variable and species-specific [36],
259 very little is known about community-level impacts. Our work illustrates the nearshore plank-
260 tonic communities that can thrive in low pH - high temperature conditions; such communities are
261 therefore likely to become more prevalent under future conditions.

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

265 Among the taxa surveyed, diatoms are of particular interest for their ubiquity in the world's
266 oceans and their important roles in marine food webs [3, 55], as well as in ecological and evolutionary
267 theory [40]. Our model suggests that diatoms will decrease in richness between the present and
268 2095, particularly in the Hood Canal summer months, when extreme temperatures are more likely.
269 Although the most prevalent response among diatoms is a decrease in suitability, some substantial
270 variability in responses exists within the group. For example, the centric diatom *Coscinodiscus*
271 spp., which is a food source for *Acartia* spp. copepods [28] and many other animal species, will see
272 future suitable habitat only in colder waters such as those in San Juan Island, while *Skeletonema*
273 spp. and the HAB-forming species *Pseudo-nitzschia* spp. will see their habitat suitability remain
274 constant or slightly increased, especially at low pH levels.

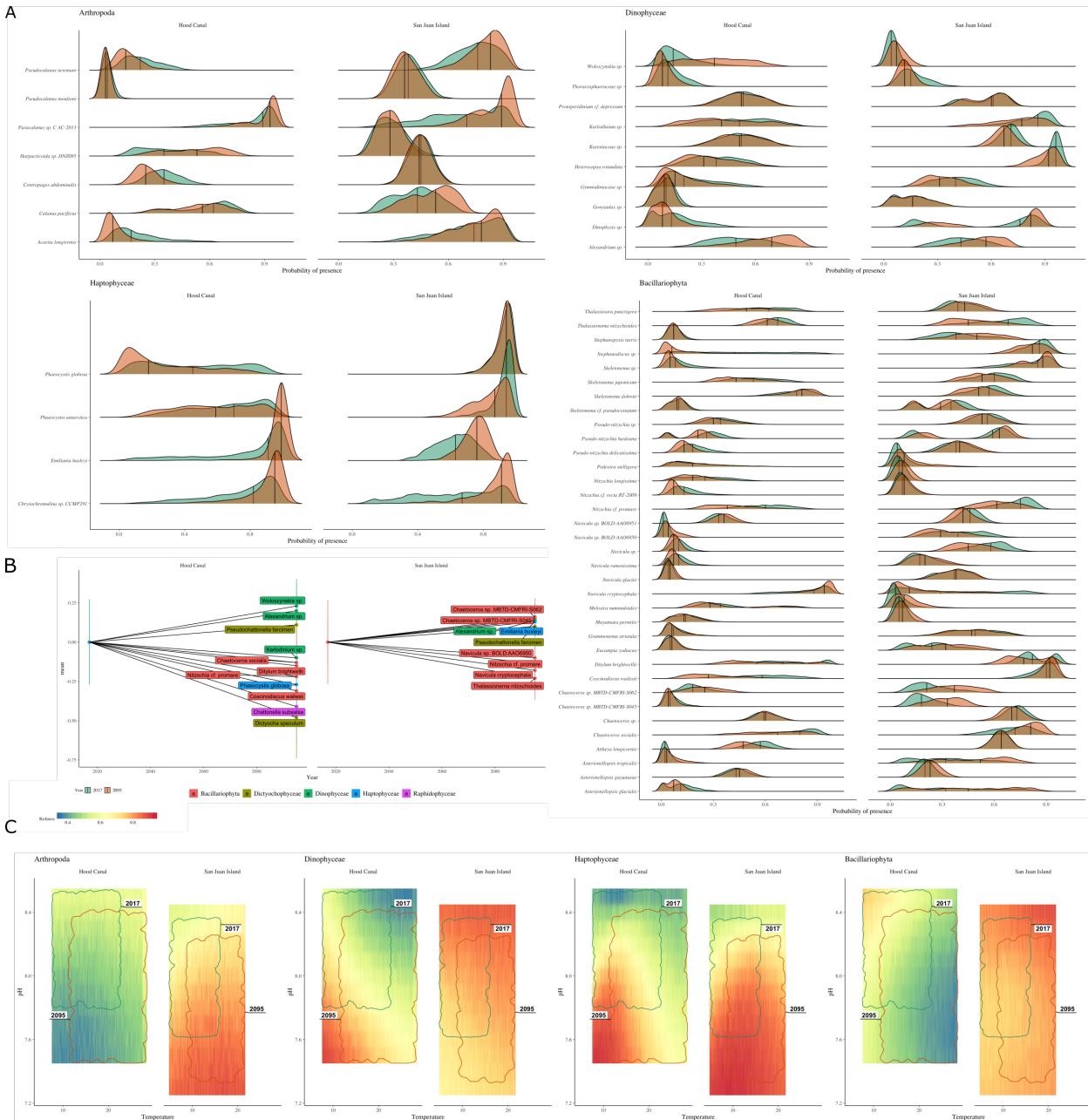


Figure 3: Forecasted changes in plankton in the Salish Sea. **A** Probability densities for the occurrence of individual taxa (species- and genus-level) for 2017 (blue) and 2095 (red); data are mean probabilities over 100 model draws, and variance in probability is due to differences in underlying environmental 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.

275 More strikingly, we see a dramatic increase in suitable environment for the HAB-forming di-
276 noflagellate *Alexandrium* sp., which can substantially harm local ecosystems [13] and economies [1].
277 This increase is particularly high in the summer months of the Hood Canal, when pH is low and
278 temperatures are high. Both archaeological and experimental evidence suggest *Alexandrium* sp.
279 blooms with warmer temperatures [44], and models [43] also predict an increase in bloom-favorable
280 conditions for *Alexandrium* sp. in future oceans.

281 Our results therefore suggest a possible change in relative dominance between diatoms and
282 other phytoplankton species such as dinoflagellates, consistent with those seen at ecological regime
283 shifts found elsewhere [55, 25]. Such a shift could affect ecosystems in many ways; even under the
284 assumption that the surviving taxa would maintain the primary production levels, for example,
285 the smaller cell-size of dinoflagellates and the differential sinking rates of the two groups would
286 likely alter regional patterns of nutrient cycling[58, 2, 6]. Although the north Atlantic has shown
287 an increase in diatom abundance[25], the increase in wind stress and associated mixing in the
288 water column in the open ocean is unlikely to occur in the Hood Canal, where stratification is
289 the strongest in the Salish Sea [42]. Furthermore, locally focused models support an increase in
290 dinoflagellate dominance with climate change, particularly during summer months [33].

291 Our model also suggests increased environmental suitability for the coccolithophore *Emiliania*
292 *huxleyi*. There is evidence supporting increased calcification and respiration rates with higher pCO₂
293 levels [27] for this ubiquitous species, although the many strains of this species and its adaptive
294 capacity make it difficult to predict longer-term effects with confidence [7].

295 Changes in environmental conditions and associated shifts in planktonic communities will likely
296 reshape ecosystems and food webs, although some environmental processes may be conserved even
297 as the particular taxa change. A switch from a diatom-dominated ecosystem to one in which
298 dinoflagellate blooms extend in space and time could provoke cascade effects [58] including fish
299 mortality, eutrophication [2], and benthopelagic coupling [6]. Beyond the phylum-specific patterns,
300 the increase in suitable habitat for HAB-forming species will alone be an engine for ecosystem change
301 [53, 56].

302 One general challenge for model-based work is a tendency to extrapolate from observed condi-
303 tions in ways that are often untestable – by necessity, projections frequently operate outside the
304 range of parameters on which the model was trained [19]. Our study system lets us avoid this
305 pitfall, in that our observed conditions encompass much of the environmental range predicted for
306 future temperate oceans. That is, the changes we predict for the year 2095 do not primarily come
307 from extreme values of pH and temperature, but rather from presently-rare conditions becoming
308 more common.

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

311 6 Acknowledgments

312 We are grateful to the UW Center for Environmental Genomics and Linda Park’s lab at NOAA
313 Fisheries for access to lab equipment and associated expertise and support. Thanks to Micah
314 Horwith and the Washington State Department of Natural Resources for in-field support, to Terrie
315 Klinger for feedback on an earlier draft, to Stephanie Moore for advice on phytoplankton generally,
316 to Ana Ramón-Laca for lab help, to Dan Drinan for field help, and to Alex Gagnon for access to
317 carbonate-chemistry expertise and equipment.

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