

2 **1 Title**

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

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12 **Author contributions:** RG led sampling, laboratory work, data analysis, and writing. RPK
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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 in each of the areas and seasons sampled, and a strong reduction in richness
27 of current diatom assemblages. Taxa finding greater suitable habitat in near-future waters are
28 more taxonomically varied and include the ubiquitous coccolithophore *Emiliania huxleyi* and the
29 harmful *Alexandrium* sp. These results suggest foundational changes for nearshore food webs under
30 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 [31, 14, 4,
37 10], very likely altering food webs [45, 8, 21] 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 [17, 12, 34], 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 [32, 26]. 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 [32, 15]. 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

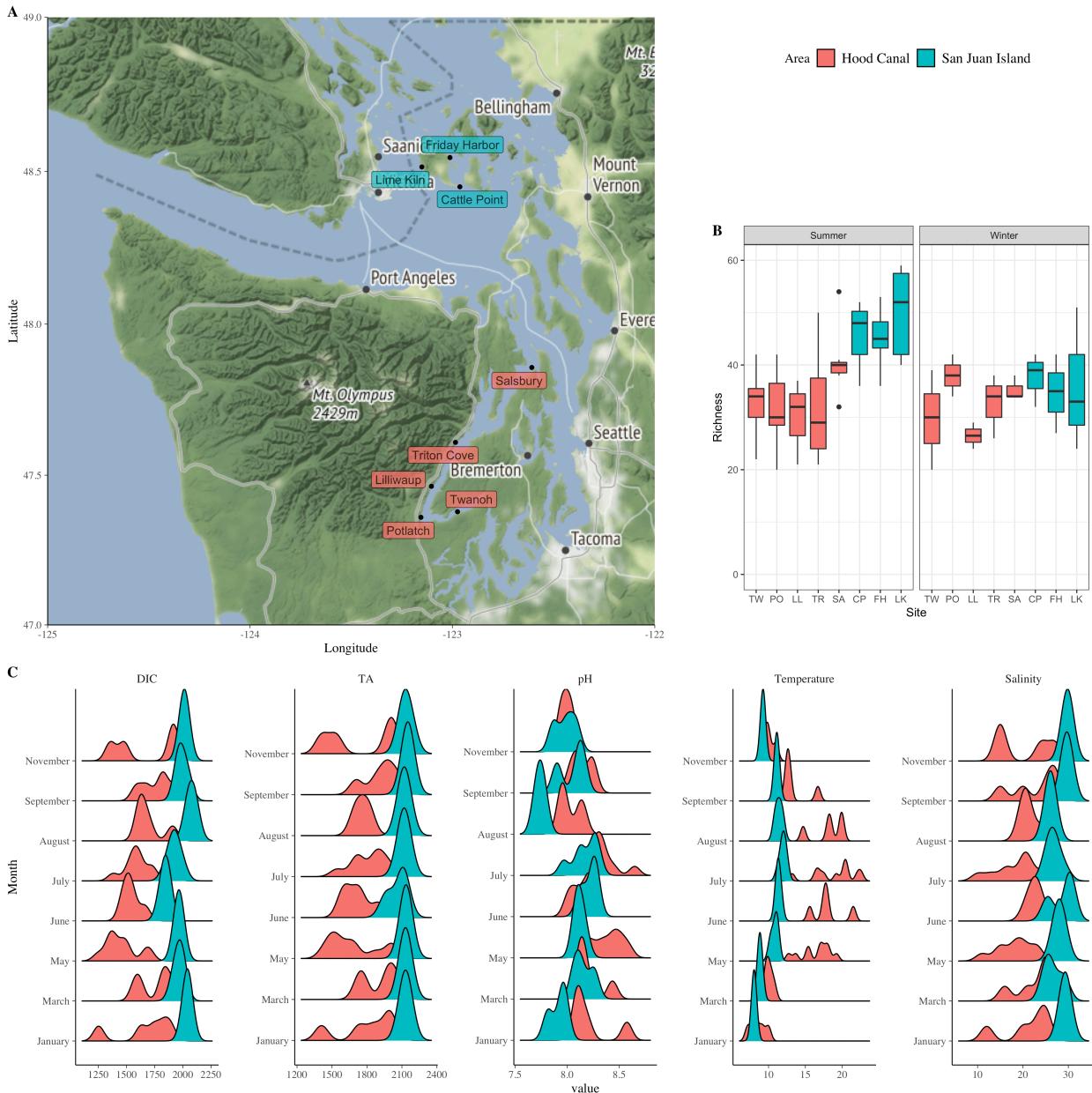


Figure 1: **A** Sampling locations in intertidal areas of the Hood Canal (Red) and San Juan Island (Blue). **B** Planktonic richness (unique taxa) per sampling locality across seasons, as reflected by the eDNA COI assay. **C** The observed environmental profiles of these two regions reflect a broad range 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.

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 [30], creating an opportunity to test the effect of these
 55 measures on marine communities under conditions expected worldwide in the near future [41], 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 [23, 27]
 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-level
 63 identification.

64 Here we use a temporal and spatial series of metabarcoding samples to track changes in nearshore
 65 ecological communities associated with differences in pH, water temperature, and other environmen-
 66 tual variables. We use broad-spectrum PCR primers [35] to target eukaryotes specifically, identifying
 67 the likely effects of future climate scenarios on suites of planktonic taxa.

68 Results

69 Variation in Carbonate Chemistry and in Ecological Communities

70 We collected intertidal environmental and genetic data from bottle samples each month between
 71 March 2017 and August 2018 (Figure 1A). Despite geographic proximity and similar overall species

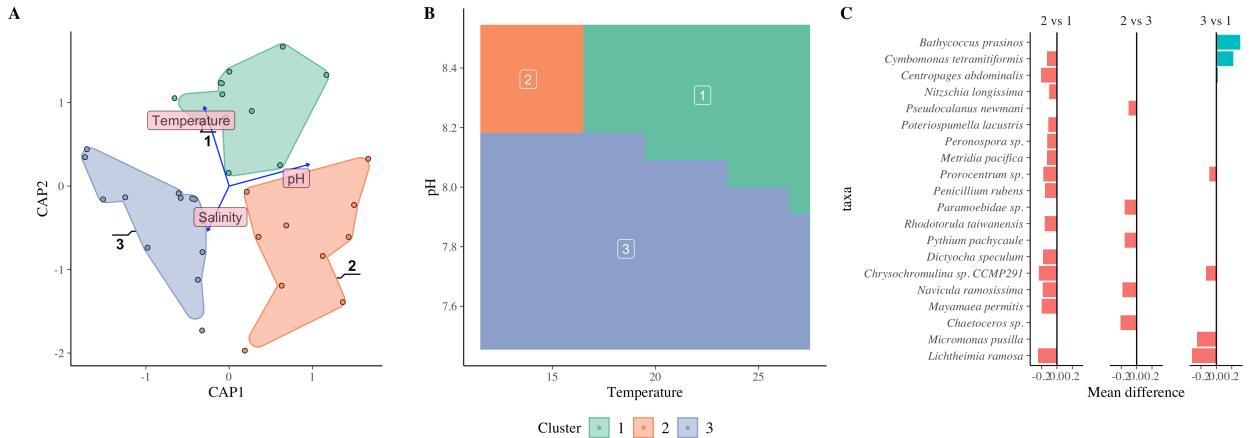


Figure 2: Biological communities and their relationship with environmental variables in the summer months of the Hood Canal. **A** Constrained Analysis of Principal Coordinates (CAP) of Bray-Curtis dissimilarity among biological communities, as constrained by pH, temperature and salinity (arrows). **B** Most-likely cluster as a function of temperature and pH given a multinomial logistic model. **C** Relative abundance (eDNA index; see [29]) of the taxa best distinguishing the three communities illustrated (SIMPER analysis). See Supporting Information for full analysis.

composition (127 of the 222 planktonic taxa were found in both regions and accounted for 98% of the sequences), the areas under study – San Juan Island and the Hood Canal – varied widely in pH, temperature, and other environmental parameters (Figure 1C), with different points along the environmental gradient simultaneously showing differences equivalent to those predicted between present-day and future oceans [7].

Metabarcoding analysis of eDNA samples generated more than 52.5M sequences from 745 samples. These samples represented biological and technical replicates from 86 unique sampling events. After bioinformatics quality-control (See Methods) the dataset included ~41M sequences, from 4782 unique amplicon sequence variants (ASVs). Of these, 1374 ASVs (22.8M reads) could be 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). Because we expect planktonic taxa to vary with water mass [28] and therefore with bottle-sampled carbonate chemistry, here we focus on only the planktonic taxa ($N = 222$). These taxa showed a seasonal richness gradient between study areas, consistent with documented biodiversity clines in the area (Fig. 1B) [13].

Bray-Curtis dissimilarities among samples revealed large differences in metabarcoding communities due to season (Summer vs. Winter; PERMANOVA F-model = 14.4; $p < 0.001$) and geographic Area (Hood Canal vs. San Juan Island; F-model = 15.4; $p < 0.001$). We therefore performed a constrained analysis of principal components (CAP) for samples within each combination of Season and Area, showing the differences among communities as a function of temperature, pH, and salinity (Fig. 2 shows results for Hood Canal in summer as an example; full analysis in the Supporting Information). Independent of environmental parameters, we separately clustered samples by pairwise Bray-Curtis dissimilarities among metabarcoding communities (k-means; $N = 3$ or 4) to identify groups of samples that were similar to one another with respect to biological community. Each biological cluster (colored hulls, Fig. 2) occupied a unique area of environmental parameter space. Planktonic communities therefore varied predictably with water temperature, salinity, and pH, across a range of those parameters likely to be encountered in many near-term future-ocean scenarios [41]. Multinomial logistic regression yielded predictions of the most-likely community for any combination of environmental parameters (Fig. 2B).

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

Climate envelopes and future distributions

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

To explore the suitability of different environmental conditions for each taxon, we modeled the likelihood of taxon presence as a function of area and season (random effects) and temperature and pH (fixed effects). Salinity is predicted to remain largely unchanged in future scenarios [30]. Using predicted conditions for Puget Sound for the year 2095 [30], we then projected the probability of

presence of each taxon across these conditions over 100 draws of the posterior probabilities of all taxa. We used these draws to 1) show the change in the probability of presence of each taxon for each scenario (i.e. 2017 vs 2095, Fig.3 top panel); 2) calculate the taxon richness within taxonomic groups across the pH-temperature continuum (Fig. 3 center panel),and 3) visualized the change in suitability for each taxon in our dataset individually (selected taxa in Fig. 3 bottom panel).

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

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

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

Taxa surveyed are a function of our metabarcoding PCR primers [35] and reflect the current status of genetic databases, rather than a complete sampling of the planktonic community; we view these results as a cross-section of common taxa useful for understanding the biological effects of ocean conditions.

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

Discussion

Temperate surface oceans worldwide average approximately 14°C and pH of 8.1 [7], and will change substantially in this century [mean $\Delta T 2.5^\circ\text{C}$, $\Delta \text{pH } -0.35$ globally; RCP 8.5; 18]. Here we document communities exposed to this same range of projected conditions in the present day, along an environmental gradient only ca. 200km wide, allowing us to project future ocean communities from a robust set of underlying observations. Our results reflect patterns in a diverse selection of species from nearshore marine communities in the Salish Sea, consisting of 222 planktonic taxa obtained from the metabarcoding analysis of 227 discrete samples across 77 space-time points (eight sites, 1.5 years). We find that changes in the composition of biological communities closely mirrored the variation in pH and temperature, with clear winners (e.g., *Emiliania huxleyi*, *Alexandrium*, and others) and losers (many, but not all, diatoms) likely to shift the structure and function of future marine communities.

A vast amount of evidence suggests climate-associated effects on marine species, and broad patterns of sensitivity are discernible within major taxonomic groups [18, 47, among many others]. However, because the strength and direction of these effects are variable and species-specific [33], very little is known about community-level impacts. Our work illustrates the nearshore planktonic communities that can thrive in low pH - high temperature conditions; such communities are therefore likely to become more prevalent under future conditions.

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

Among the taxa surveyed, diatoms are of particular interest for their ubiquity in the world's oceans and their important roles in marine food webs [3, 50], as well as in ecological and evolutionary theory [36]. Our model suggests that diatoms will decrease in richness between the present and 2095, particularly in the Hood Canal summer months, when extreme temperatures are more likely. Although the most prevalent response among diatoms is a decrease in suitability, some substantial variability in responses exists within the group. For example, the centric diatom *Coscinodiscus* spp., which is a food source for *Acartia* spp. copepods [25] and many other animal species, will see

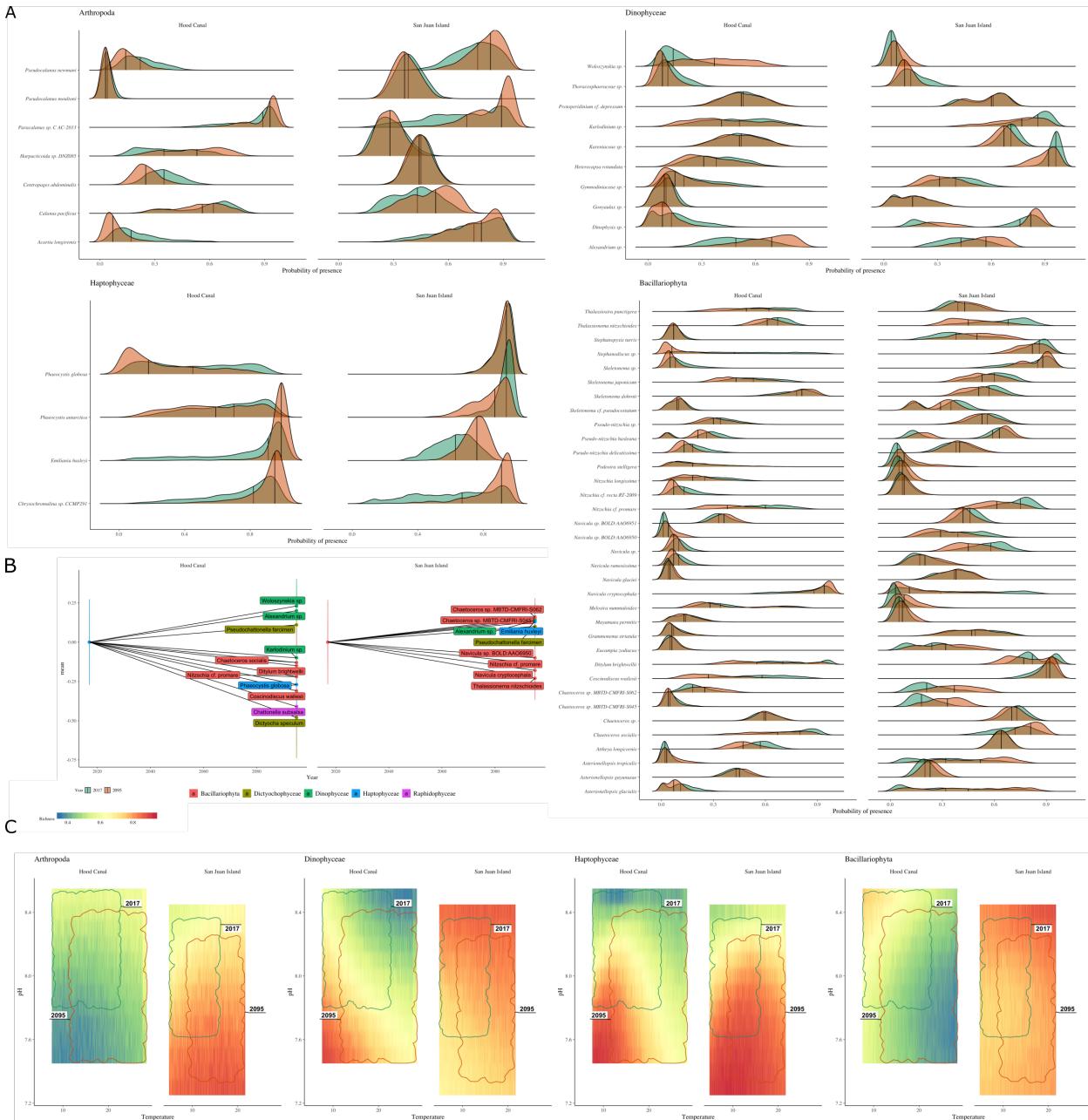


Figure 3: Forecasted changes in plankton in the Salish Sea. **A** Probability densities for the occurrence of individual taxa (species- and genus-level) for 2017 (blue) and 2095 (red); data are mean probabilities over 100 model draws, and variance in probability is due to differences in underlying environmental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

178 future suitable habitat only in colder waters such as those in San Juan Island, while *Skeletonema*
179 spp. and the HAB-forming species *Pseudo-nitzschia* spp. will see their habitat suitability remain
180 constant or slightly increased, especially at low pH levels.

181 More strikingly, we see a dramatic increase in suitable environment for the HAB-forming di-
182 noflagellate *Alexandrium* sp., which can substantially harm local ecosystems [11] and economies [1].
183 This increase is particularly high in the summer months of the Hood Canal, when pH is low and
184 temperatures are high. Both archaeological and experimental evidence suggest *Alexandrium* sp.
185 blooms with warmer temperatures [40], and models [39] also predict an increase in bloom-favorable
186 conditions for *Alexandrium* sp. in future oceans.

187 Our results therefore suggest a possible change in relative dominance between diatoms and
188 other phytoplankton species such as dinoflagellates, consistent with those seen at ecological regime
189 shifts found elsewhere [50, 22]. Such a shift could affect ecosystems in many ways; even under the
190 assumption that the surviving taxa would maintain the primary production levels, for example,
191 the smaller cell-size of dinoflagellates and the differential sinking rates of the two groups would
192 likely alter regional patterns of nutrient cycling[53, 2, 5]. Although the north Atlantic has shown
193 an increase in diatom abundance[22], the increase in wind stress and associated mixing in the
194 water column in the open ocean is unlikely to occur in the Hood Canal, where stratification is
195 the strongest in the Salish Sea [38]. Furthermore, locally focused models support an increase in
196 dinoflagellate dominance with climate change, particularly during summer months [30].

197 Our model also suggests increased environmental suitability for the coccolithophore *Emiliania*
198 *huxleyi*. There is evidence supporting increased calcification and respiration rates with higher pCO₂
199 levels [24] for this ubiquitous species, although the many strains of this species and its adaptive
200 capacity make it difficult to predict longer-term effects with confidence [6].

201 Changes in environmental conditions and associated shifts in planktonic communities will likely
202 reshape ecosystems and food webs, although some environmental processes may be conserved even
203 as the particular taxa change. A switch from a diatom-dominated ecosystem to one in which
204 dinoflagellate blooms extend in space and time could provoke cascade effects [53] including fish
205 mortality, eutrophication [2], and benthopelagic coupling [5]. Beyond the phylum-specific patterns,
206 the increase in suitable habitat for HAB-forming species will alone be an engine for ecosystem change
207 [48, 51].

208 One general challenge for model-based work is a tendency to extrapolate from observed condi-
209 tions in ways that are often untestable – by necessity, projections frequently operate outside the
210 range of parameters on which the model was trained [16]. Our study system lets us avoid this
211 pitfall, in that our observed conditions encompass much of the environmental range predicted for
212 future temperate oceans. That is, the changes we predict for the year 2095 do not primarily come
213 from extreme values of pH and temperature, but rather from presently-rare conditions becoming
214 more common.

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

217 5 Methods

218 Sampling

219 We collected water samples to assess eDNA communities in two regions of the Salish Sea (Wash-
220 ington, USA): San Juan Island and the Hood Canal. These sites experience substantial variation
221 in water chemistry and other environmental conditions despite geographic proximity (ca. 300km;
222 Fig. 1). We sampled eight sites monthly for approximately 1.5 years (March 2017 to August 2018),
223 taking three 1L samples (biological replicates; ca. 10m apart) each month at each site (261 bottle
224 samples total). Each sample was filtered through a 0.45 μm cellulose filter, and the filter preserved
225 in Longmire buffer until DNA extraction [43]. Concurrently, we collected one 120 ml water sample
226 from each site and poisoned it with 0.1 ml of saturated HgCl₂ for carbonate chemistry analysis,
227 following [44]. We also collected *in situ* measurements of temperature, salinity and dissolved oxy-
228 gen using a handheld multiprobe (Hanna Instruments, USA) and a portable refractometer. We
229 note that many unmeasured variables influence planktonic communities (e.g., nutrients, sunlight,
230 wave energy), but that our set of measured parameters clearly distinguished communities and was
231 adequate for our purposes.

232 We characterized sample carbonate chemistry by measuring Total Alkalinity (TA; open-cell
233 automated titration based on a 876 Dosimat plus (Metrohm AG) as part of a custom system
234 assembled by Andrew Dickson (UCSD) and used in the laboratory of Alex Gagnon at UW) and
235 Dissolved Inorganic Carbon (DIC; Apollo Instruments, USA; CO₂ extraction system with 10%
236 (v/v) phosphoric acid). Both measurements were calibrated and validated with certified reference
237 material from the Scripps Oceanographic Institute. Using DIC and TA, we calculated pH and the
238 remaining carbonate system parameters using the R package ‘seacarb’ [19].

Our sampled areas differed in the environmental variables driving changes in carbonate chemistry. San Juan Island was less seasonally variable than the Hood Canal in every measured parameter (Figure 1B); the island is more directly affected by summer coastal upwelling as a function of bathymetry and circulation patterns [41], and this appears to be the dominant influence on carbonate chemistry there. By contrast, photosynthesis and respiration likely drive much of the carbonate chemistry variation in the Hood Canal (See Supporting Information).

eDNA sequencing and bioinformatic processes

We purified DNA from each filtered sample using a Phenol Chloroform Isoamyl Alcohol protocol, following [43]. A 1/10 to 1/100 dilution of the extract was used as template for a PCR reaction targeting a 313bp fragment of cytochrome oxidase I [35]. PCR reactions were performed in triplicate and sequenced individually to quantify the stochasticity of PCR reactions on a mixed template sample, and we attached secondary indexing tags using a two-step PCR process [42]. PCR conditions and protocols for sample identification followed [28], and batches of 49 to 178 multiplexed samples were sequenced using MiSeq v2-500 or v3-600 sequencing kits using manufacturer protocols. On each sequencing run, we added triplicate samples consisting on DNA obtained from species not present in the marine environment under study (Red Kangaroo (*Macropus rufus*) and Ostrich (*Struthio camelus*)) to establish quality controls of sample assignment and quantify levels of 'tag-jumping' or sample-cross-talk [46].

Code for all quality-screening and bioinformatics is available in the Supporting Information. Briefly, we used a Unix script that calls secondary programs for primer-trimming and preliminary quality-control [37, 9] we estimated the likely composition of each sample using DADA2. This approach avoids clustering, such that we retained all of the amplicon sequence variants (ASVs, *i.e.*, unique sequences); we subsequently carried out secondary quality-control and decontamination following [28]. We then assigned sequences to known taxa using phylogenetic tree placement with insect v1.1 [52]. We conservatively kept only taxa annotated at the level of taxonomic family, genus, or species, so we could reliably infer taxon natural history. Using published literature and online databases, we placed every recovered taxon into a benthic/planktonic category and focused our analysis on the planktonic community (see Supporting Information).

By treating amplification efficiency as consistent within a given taxon, we created an index of abundance for each taxon across space and time [29], using pooled data from technical replicates and mean proportions across biological replicates. The multivariate tests (PERMANOVA, CAP, k-means clustering) used these index values as the species abundances and Bray-Curtis as the dissimilarity measurement between samples.

Year 2095 Environmental Scenario and Biological Responses

We estimated the distribution of environmental parameters for the overall Salish Sea in 2095 from the results of [30], which estimated an annual mean increase in temperature of 1.51 °C and mean pH decrease of 0.18 for the Salish Sea as a whole. We then subsampled this distribution to reflect the characteristic environments of different areas (San Juan Island and Hood Canal) and seasons (Summer and Winter, with April - September coded as "Summer"). To do this, we fit beta parameters to describe the fraction of the overall observed data (2017 - 2018) reflected in each subset; we then subsampled the 2095 distribution accordingly, under the assumption that the Hood Canal in Summer (for example) would occupy the same fraction of the overall annual Salish Sea temperature and pH distributions in 2095 as it did in 2017. See Supporting Information for details of sampling parameters.

For community-level projections, we coded community-cluster identity (Figure 2) as an unordered response variable in a multinomial logistic model, with temperature, pH, area, and season as predictor variables. We calculated the probability of each community, given these predictors, using the package 'nnet' [49]. Most-common combinations of T and pH were estimated from 2-dimensional density plots for each year.

Within a geographic area and season, temperature and pH were most strongly associated with community-level changes. We therefore developed a hierarchical logistical regression model relating the presence of each taxon to temperature and pH (as fixed effects), allowing slope to vary by geographic area and intercept to vary by season, to capture large-scale seasonal differences associated with sunlight, photosynthesis, and related factors. For each taxon, we fit these models using the Bayesian generalized linear mixed effects functions in R package rstanarm [20] for R. See Supporting Information for analytical code.

Given the estimated sea-surface temperatures and pH values for 2095 and observed data from 2017, we then used taxon-specific logistic regression models to estimate the suitability of habitat for each taxon in the future scenario. For each point in the pH - temperature grid, we calculated

298 species richness as the mean result of 100 independent draws of the posterior distributions of all
299 modeled taxa, grouping the individual taxa models by phylum.

300 We can only model responses of taxa present in our data set. That is, we may predict that the
301 number of (for example) diatom species present will decline relative to those present today, but our
302 data do not allow us to predict whether new species will immigrate from elsewhere or how species
303 might evolutionarily adapt to future conditions. It is beyond the scope of our work to account
304 for the latter, and furthermore, because of the extreme uncertainty of evolutionary responses, the
305 predictions of species distribution models are often interpreted without considering adaptation or
306 phenotypic plasticity [39].

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