

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 [58, 10, 27] and species' interactions in ways that are poorly  
38 understood. Laboratory or mesocosm-based manipulation experiments have documented a wide  
39 variety of biological responses under projected climate scenarios of  $p\text{CO}_2$ , pH, solar radiation,  
40 salinity and temperature [23, 16, 40], showing an array of species-specific responses among particular  
41 taxa of interest. However, information regarding multi-species or community-wide responses to  
42 these stressors is far more limited [38, 32]. The scarcity of such data is likely attributable to the  
43 difficulty of simultaneously tracking the responses of many species in the field, and to the difficulty  
44 of identifying natural systems that adequately reflect the environmental gradients under study.

45 Two natural  $\text{CO}_2$  seeps in nearshore marine habitats – one in Italy and one in Papua New Guinea  
46 – have demonstrated shifts in benthic communities associated with especially acute acidification in  
47 the present day, previewing those we might expect at a more global scale under future conditions  
48 [38, 21]. But beyond these exceptional sites, it is difficult to measure changes in ecological com-  
49 munities associated with the relatively subtle shifts in nearshore ocean chemistry observed to date,  
50 particularly in light of naturally large spatial and temporal variation in these communities. The

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

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

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

## 71 5 Methods

### 72 Sampling

73 We collected water samples to assess eDNA communities in two regions of the Salish Sea (Wash-  
74 ington, USA): San Juan Island and the Hood Canal. These sites experience substantial variation  
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 [56]. Concurrently, we collected one 120 ml water sample  
80 from each site and poisoned it with 0.1 ml of saturated  $\text{HgCl}_2$  for carbonate chemistry analysis,  
81 following [57]. We also collected *in situ* measurements of temperature, salinity and dissolved oxy-  
82 gen using a handheld multiprobe (Hanna Instruments, USA) and a portable refractometer. We  
83 note that many unmeasured variables influence planktonic communities (e.g., nutrients, sunlight,  
84 wave energy), but that our set of measured parameters clearly distinguished communities and was  
85 adequate for our purposes.

86 We characterized sample carbonate chemistry by measuring Total Alkalinity (TA; open-cell  
87 automated titration based on a 876 Dosimat plus (Metrohm AG) as part of a custom system  
88 assembled by Andrew Dickson (UCSD) and used in the laboratory of Alex Gagnon at UW) and  
89 Dissolved Inorganic Carbon (DIC; Apollo Instruments, USA;  $\text{CO}_2$  extraction system with 10%  
90 (v/v) phosphoric acid). Both measurements were calibrated and validated with certified reference  
91 material from the Scripps Institution of Oceanography. Using DIC and TA, we calculated pH and  
92 the remaining carbonate system parameters using the R package ‘seacarb’ [25].

93 Our sampled areas differed in the environmental variables driving changes in carbonate chem-  
94 istry. San Juan Island was less seasonally variable than the Hood Canal in every measured param-  
95 eter (Figure 1C); the island is more directly affected by summer coastal upwelling as a function  
96 of bathymetry and circulation patterns [50], and this appears to be the dominant influence on  
97 carbonate chemistry there. By contrast, photosynthesis and respiration likely drive much of the  
98 carbonate chemistry variation in the Hood Canal (See 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 [56]. After reducing inhibition via a 1/10 to 1/100 dilution, the extract was used as  
102 template for a PCR reaction targeting a 313bp fragment of cytochrome oxidase I [43]. PCR  
103 reactions were performed in triplicate and sequenced individually to quantify the stochasticity of  
104 PCR reactions on a mixed template sample, and we attached secondary indexing tags using a two-  
105 step PCR process [51]. PCR conditions and protocols for sample identification followed [34], and  
106 batches of 49 to 178 multiplexed samples were sequenced using MiSeq v2-500 or v3-600 sequencing  
107 kits using manufacturer protocols. On each sequencing run, we added triplicate samples consisting  
108 on DNA obtained from species not present in the marine environment under study (Red Kangaroo

109 (*Macropus rufus*) and Ostrich (*Struthio camelus*)) to establish quality controls of sample assignment  
110 and to quantify levels of 'tag-jumping' or sample-cross-talk [60].

111 Code for all quality-screening and bioinformatics is available in the Supporting Information,  
112 implemented in Unix and R [55]. 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 [67]; where *insect* could not place individual  
118 taxa, we supplemented assignment by classification against a custom COI database using *anacapa*  
119 [15] and *bowtie2* [42]. We conservatively kept only taxa annotated at the level of taxonomic family,  
120 genus, or species, so we could reliably infer taxon natural history under the assumption that taxa  
121 within the same family shared broad natural-history characteristics. Using published literature and  
122 online databases, we placed every recovered taxon into a benthic/planktonic category and focused  
123 our analysis on the planktonic community (see 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. **An implementation in R of this index is provided in the Supplementary  
129 information.**

### 130 Present scenario community analyses

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

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

139 For community-level projections, we coded community-cluster identity (Figure 2) as an un-  
140 ordered response variable in a multinomial logistic model, with temperature, pH, and area (Hood  
141 Canal vs. San Juan Island), as predictor variables. Salinity is predicted to remain largely unchanged  
142 in future scenarios [36], and because salinity was correlated with temperature in our dataset, it was  
143 not an important predictor variable and we subsequently dropped it from our models. We calculated  
144 the probability of each community, given these predictors, using the R package *nnet* [63].

### 145 Year-2095 Environmental Scenario and Biological Responses

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

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

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

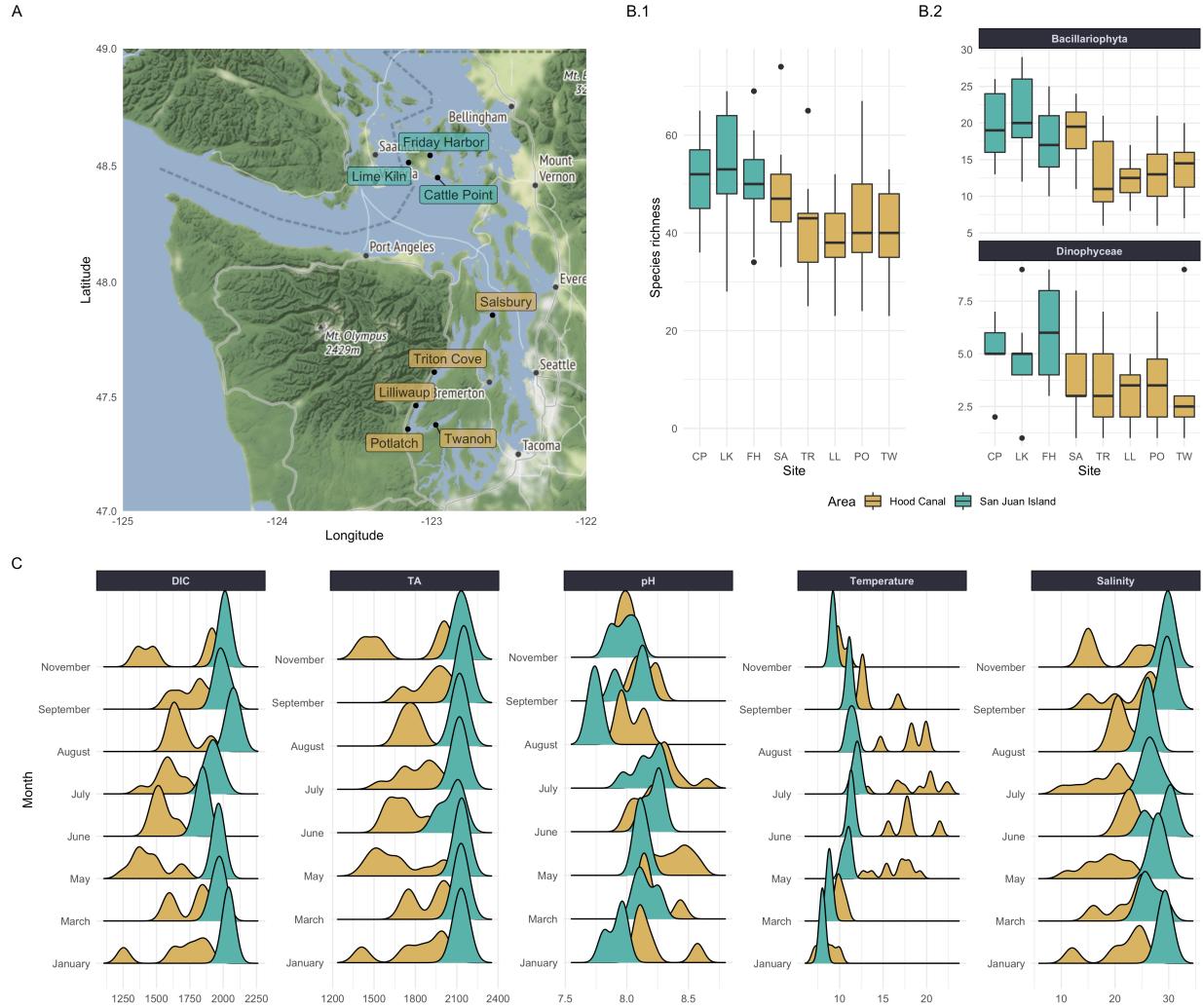


Figure 1: **A** Sampling locations in intertidal areas of the Hood Canal (dark gold) and San Juan Island (turquoise). **B** Planktonic richness (unique taxa) per sampling locality, as reflected by the eDNA COI assay. Boxplots represent the variability in richness across all time points for samples taken 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 species richness) and on each phylum independently. RYAN: Move paragraph below to discussion?  
 170 Taxa surveyed are a function of our metabarcoding PCR primers [43] and reflect the current status  
 171 of genetic databases, rather than a complete sampling of the planktonic community; we view these  
 172 results as a cross-section of common taxa useful for understanding the biological effects of ocean  
 173 conditions.

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

## 181 Results

### 182 Variation in Carbonate Chemistry and in Ecological Communities

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

190 Metabarcoding analysis of eDNA samples generated more than \*\*\*50.8M sequences from 778  
 191 samples. These samples represented biological and technical replicates from 86 unique sampling  
 192 events. After bioinformatics quality-control (See Methods) the dataset included ~\*\*\*45M se-

193 quences, from \*\*\*4849 unique amplicon sequence variants (ASVs). Of these, \*\*\*1364 ASVs (22.6M  
194 reads) could be annotated to a taxonomic level of Family or lower. These ~ 500 taxa from 43 phyla  
195 were split according to their natural history and habitat (benthic vs. planktonic; see Supporting  
196 Information). Because we expect planktonic taxa to vary with water mass [34] and therefore  
197 with bottle-sampled carbonate chemistry, here we focus on only the planktonic taxa (N = 221).  
198 These taxa showed a seasonal richness gradient between study areas, consistent with documented  
199 biodiversity clines in the area (Fig. 1B.1) [17].

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

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

210 These communities were distinguished by changes in the relative abundances of a wide variety of  
211 taxa. In the Hood Canal for example, the community linked with colder water and higher-pH (com-  
212 munity \*\*2 in Fig. 2C) showed higher eDNA indexes of diatoms \*\*\* (*Minutocellus polymorphus*),  
213 green algae (*Bathycoccus prasinus* and *Micromonas pusilla*), and dinoflagellates like *Karlodinium*  
214 sp than that on the low pH spectrum. In that community (cluster 1) *Emiliania huxleyi*, *Ditylum*  
215 *brightwellii* and the copepod *Paracalanus* sp. C are more prevalent. The community thriving in the  
216 high temperature range (community 3 in Fig. 2C) shows high values of *Phaeocystis globosa*, *Ditylum*  
217 *brightwellii* among other species. Community 3 (Fig. 2A,B) and similar planktonic communities  
218 occupy the spectrum of environmental conditions most likely to be encountered in near-future cli-  
219 mate scenarios as temperature rises and pH falls. For example, we expect the Hood Canal in 2095  
220 to have the conditions in which community 3 is the most likely community 66% of the time, an  
221 increase of 11% compared to 2017 (see Supplemental Material). On the other hand, conditions that  
222 make cluster 2 the most likely community drop from 9% to 1% of the time between 2017 and 2095.

#### 223 Climate envelopes and future distributions

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

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

238 Likely winners under future conditions are more widely scattered among higher taxonomic  
239 groups. The haptophytes *Emiliania huxleyi* and *Chrysochromulina* sp. the dinoflagellate *Alexan-  
240 drium* sp. all find more suitable habitat in both of our study areas. Among others not shown in Fig  
241 3, *Chaetoceros* (diatom) and many hydrozoans (Cnidaria) likely increase in San Juan Island, and  
242 the potentially fish-killing heterokont flagellate *Pseudochattonella* increases in both study areas.  
243 See Supporting Information for a complete list of taxon-specific projections.

244 Given such heterogeneity in projections, gains and losses tend to balance one another out  
245 when looking at overall richness variation; we find no change in median richness for the year 2095  
246 relative to the present in the Hood Canal (overall taxon richness by year, 95% confidence interval  
247 in median species richness -0.08, +0.04; Wilcoxon p = 0.5); while higher diversity is expected in  
248 the San Juan Island in 2095 (increase in median species richness of 1.84-2.2, p < 10<sup>-16</sup>). Diatoms,  
249 in particular, show small but significant declines in richness in the Hood Canal (0.46-0.55 species,  
250 p < 10<sup>-16</sup>), while the changes on the San Juan Island are negligible (median change 0-0.08 p =  
251 0.04). Dinoflagellates see their richness increase in both regions with the future scenario (median  
252 change 0.14-0.18 Hood Canal; 0.21-0.25 San Juan Island; p < 10<sup>-16</sup> for each).

253 The bulk of our projected community changes result from now-rare conditions occurring more  
254 frequently in the future. For example, in the Hood Canal at present, we expect surface waters  
255 to have pH < 7.9 and T > 19°C only 1% of the time. In 2095, we expect these conditions 6 times

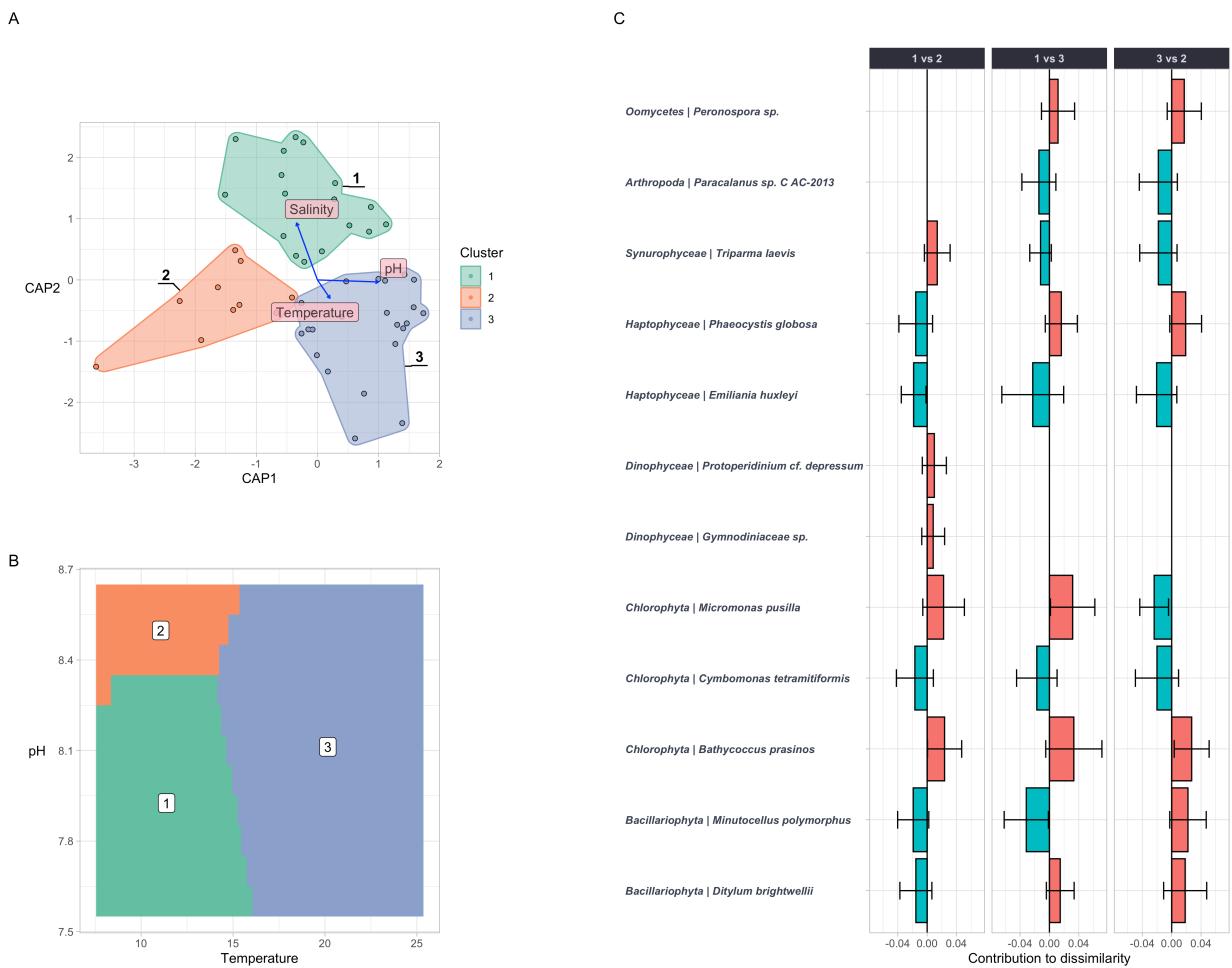
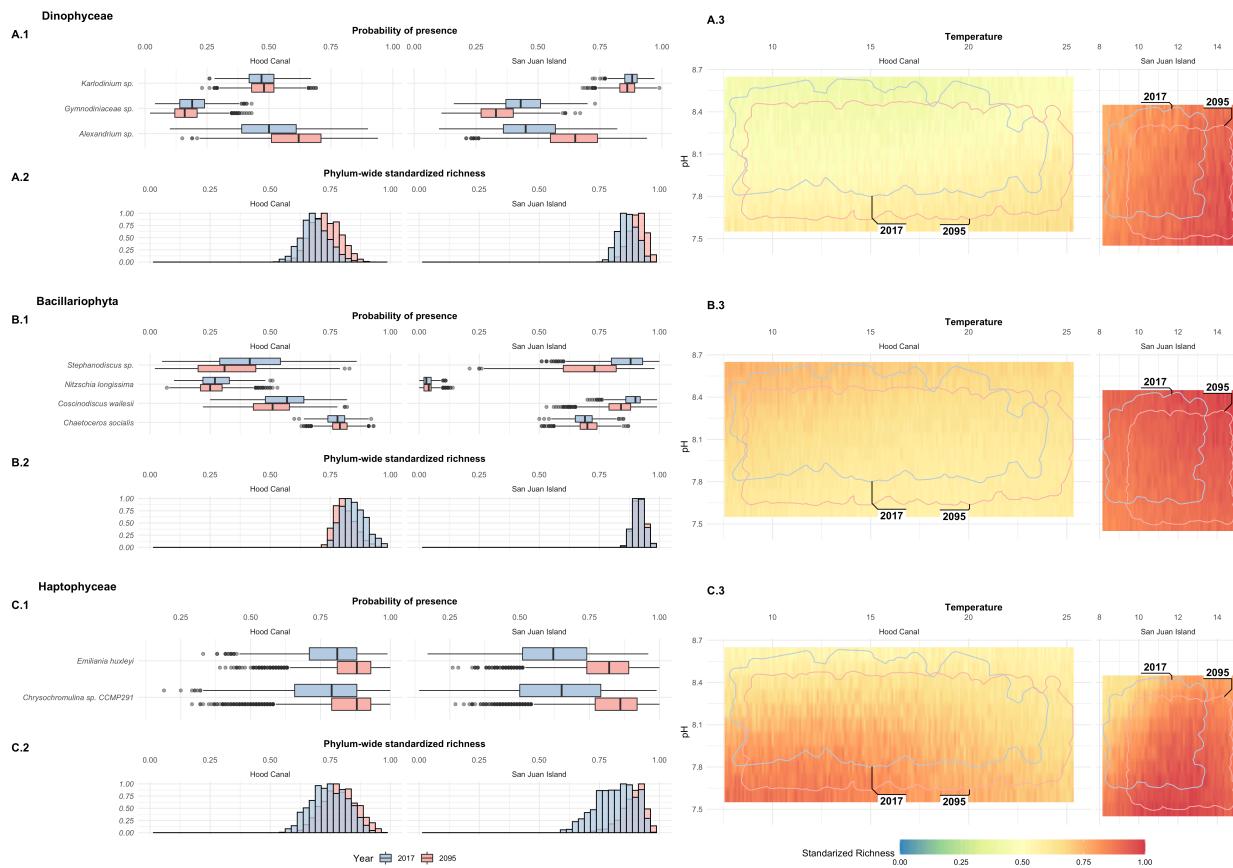


Figure 2: Biological communities and their relationship with environmental variables in the summer months of the Hood Canal. **A** Constrained Analysis of Principal Coordinates (CAP) of Bray-Curtis dissimilarities among biological communities, as constrained by pH, temperature and salinity (arrows).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



**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.

more frequently (i.e., 6% 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 = 0.83). By contrast, the large centric diatom *Coscinodiscus* – a potentially key source of carbon for zooplankton and small fishes [53, 69] with effects on dissolved oxygen and other water-column characteristics [44] – occurs only one-third of the time under these same conditions (mean frequency = 0.35).

## Discussion

Temperate surface oceans worldwide average approximately 14°C and pH of 8.1 [9], and will change substantially in this century [mean  $\Delta$  T 2.5°C,  $\Delta$  pH -0.35 globally; RCP 8.5; 24]. 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 [24, 61, among many others]. However, because the strength and direction of these effects are variable and species-specific [39], 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 [4, 64], as well as in ecological and evolutionary theory [45]. Our model suggests that diatoms will decrease in richness between the present and

285 2095, particularly in the Hood Canal, where extreme temperatures are more common. Although  
286 the most prevalent response among diatoms is a decrease in suitability, some substantial variability  
287 in responses exists within the group. For example, the centric diatom *Coscinodiscus* spp., which is  
288 a food source for *Acartia* spp. copepods [31] and many other animal species, will see future suitable  
289 habitat only in colder waters such as those in San Juan Island, while *Skeletonema* spp. and the  
290 harmful algal bloom (HAB)-forming species *Pseudo-nitzschia* spp. will see their habitat suitability  
291 remain constant or slightly increased, especially at low pH levels (see Supplemental Material).

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

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

309 Our model also suggests increased environmental suitability for the coccolithophore *Emiliania*  
310 *huxleyi*. There is evidence supporting increased calcification and respiration rates with higher pCO<sub>2</sub>  
311 levels [30] for this ubiquitous species, although the many strains of this species and its adaptive  
312 capacity make it difficult to predict longer-term effects with confidence [8].

313 Changes in environmental conditions and associated shifts in planktonic communities will likely  
314 reshape ecosystems and food webs, although some environmental processes may be conserved even  
315 as the particular taxa change. A switch from a diatom-dominated ecosystem to one in which  
316 dinoflagellate blooms extend in space and time could provoke cascade effects [68] including fish  
317 mortality, anoxia [2], and ~~carbon sinking dynamics alter current benthopelagic coupling processes~~  
318 [7]. Beyond the phylum-specific patterns, the increase in suitable habitat for harmful algae species  
319 will alone be an engine for ecosystem change [62, 66].

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

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

## 329 6 Acknowledgments

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335 carbonate-chemistry expertise and equipment.

## 336 References

- 337 [1] D. M. Anderson, P. Hoagland, Y. Kaoru, and A. W. White. Estimated annual economic  
338 impacts from harmful algal blooms (HABs) in the United States. Technical report, National  
339 Oceanic and Atmospheric Administration, 2000.
- 340 [2] D. M. Anderson, P. M. Glibert, and J. M. Burkholder. Harmful algal blooms and eutrophica-  
341 tion: nutrient sources, composition, and consequences. *Estuaries*, 25(4):704–726, 2002.

- 342 [3] M. J. Anderson and T. J. Willis. Canonical analysis of principal coordinates: a useful method  
343 of constrained ordination for ecology. *Ecology*, 84(2):511–525, 2003.
- 344 [4] E. V. Armbrust. The life of diatoms in the world’s oceans. *Nature*, 459(7244):185, 2009.
- 345 [5] N. Bednarsek, R. A. Feely, E. L. Howes, B. Hunt, F. Kessouri, P. León, S. Lischka, A. E. Maas,  
346 K. McLaughlin, N. Nezlin, et al. Systematic review and meta-analysis towards synthesis  
347 of thresholds of ocean acidification impacts on calcifying pteropods and interactions with  
348 warming. *Frontiers in Marine Science*, 6:227, 2019.
- 349 [6] T. E. Berry, B. J. Saunders, M. L. Coghlan, M. Stat, S. Jarman, A. J. Richardson, C. H. Davies,  
350 O. Berry, E. S. Harvey, and M. Bunce. Marine environmental dna biomonitoring reveals  
351 seasonal patterns in biodiversity and identifies ecosystem responses to anomalous climatic  
352 events. *PLoS genetics*, 15(2), 2019.
- 353 [7] P. Bienfang and P. Harrison. Sinking-rate response of natural assemblages of temperate and  
354 subtropical phytoplankton to nutrient depletion. *Marine Biology*, 83(3):293–300, 1984.
- 355 [8] C. T. Bolton, M. T. Hernández-Sánchez, M.-A. Fuertes, S. González-Lemos, L. Abrevaya,  
356 A. Mendez-Vicente, J.-A. Flores, I. Probert, L. Giosan, J. Johnson, et al. Decrease in cocol-  
357 lithophore calcification and co 2 since the middle miocene. *Nature Communications*, 7:10284,  
358 2016.
- 359 [9] L. Bopp, L. Resplandy, J. C. Orr, S. C. Doney, J. P. Dunne, M. Gehlen, P. Halloran, C. Heinze,  
360 T. Ilyina, R. Seferian, et al. Multiple stressors of ocean ecosystems in the 21st century:  
361 projections with cmip5 models. *Biogeosciences*, 10:6225–6245, 2013.
- 362 [10] D. S. Busch, C. J. Harvey, and P. McElhany. Potential impacts of ocean acidification on  
363 the Puget Sound food web. *ICES Journal of Marine Science*, 70(4):823–833, 07 2013. ISSN  
364 1054-3139. doi: 10.1093/icesjms/fst061. URL <https://doi.org/10.1093/icesjms/fst061>.
- 365 [11] B. J. Callahan, P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes.  
366 DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13  
367 (7):581, 2016.
- 368 [12] A. A. Chariton, S. Stephenson, M. J. Morgan, A. D. Steven, M. J. Colloff, L. N. Court,  
369 and C. M. Hardy. Metabarcoding of benthic eukaryote communities predicts the ecological  
370 condition of estuaries. *Environmental pollution*, 203:165–174, 2015.
- 371 [13] J. C. Clements and E. S. Darrow. Eating in an acidifying ocean: a quantitative review of  
372 elevated CO<sub>2</sub> effects on the feeding rates of calcifying marine invertebrates. *Hydrobiologia*, 820  
373 (1):1–21, 2018.
- 374 [14] S. P. Colin and H. G. Dam. Latitudinal differentiation in the effects of the toxic dinoflagellate  
375 *Alexandrium spp.* on the feeding and reproduction of populations of the copepod *Acartia*  
376 *hudsonica*. *Harmful Algae*, 1(1):113–125, 2002.
- 377 [15] E. E. Curd, Z. Gold, G. S. Kandlikar, J. Gomer, M. Ogden, T. O’Connell, L. Pipes, T. M.  
378 Schweizer, L. Rabichow, M. Lin, et al. Anacapa toolkit: an environmental dna toolkit for  
379 processing multilocus metabarcode datasets. *Methods in Ecology and Evolution*, 10(9):1469–  
380 1475, 2019.
- 381 [16] N. J. Delorme and M. A. Sewell. Temperature and salinity: two climate change stressors  
382 affecting early development of the new zealand sea urchin *Evechinus chloroticus*. *Marine*  
383 *Biology*, 161(9):1999–2009, 2014.
- 384 [17] M. N. Dethier and H. Berry. Decadal changes in shoreline biota in Westcott and Garrison  
385 Bays, San Juan County. *Report to the Washington State Dept. of Natural Resources, Nearshore*  
386 *Habitat Program*, 2008.
- 387 [18] A. Djurhuus, C. Closek, R. P. Ryan, K. Pitz, R. Michisaki, H. Starks, K. Walz, E. Andruszkiewicz,  
388 E. Olesin, K. Hubbard, E. Montes, D. Otis, F. Muller-Karger, F. Chavez,  
389 A. Boehm, and M. Breitbart. Microbes to mammals: Detecting ecosystem shifts through  
390 environmental dna. *Nature Ecology and Evolution*, Submitted.
- 391 [19] C. E. Emilson, D. G. Thompson, L. A. Venier, T. M. Porter, T. Swystun, D. Chartrand,  
392 S. Capell, and M. Hajibabaei. Dna metabarcoding and morphological macroinvertebrate met-  
393 rics reveal the same changes in boreal watersheds across an environmental gradient. *Scientific*  
394 *reports*, 7(1):1–11, 2017.

- 395 [20] N. Espinel-Velasco, L. Hoffmann, A. Agüera, M. Byrne, S. Dupont, S. Uthicke, N. S. Webster,  
396 and M. Lamare. Effects of ocean acidification on the settlement and metamorphosis of marine  
397 invertebrate and fish larvae: a review. *Marine Ecology Progress Series*, 606:237–257, 2018.
- 398 [21] K. Fabricius, A. Kluibenschedl, L. Harrington, S. Noonan, and G. De’Ath. *In situ* changes  
399 of tropical crustose coralline algae along carbon dioxide gradients. *Scientific Reports*, 5:9537,  
400 2015.
- 401 [22] M. C. Fitzpatrick and W. W. Hargrove. The projection of species distribution models and the  
402 problem of non-analog climate. *Biodiversity and Conservation*, 18(8):2255, 2009.
- 403 [23] K. Gao, E. Helbling, D. Häder, and D. Hutchins. Responses of marine primary  
404 producers to interactions between ocean acidification, solar radiation,  
405 and warming. *Marine Ecology Progress Series*, 470:167–189, 2012. URL  
406 <https://www.int-res.com/abstracts/meps/v470/p167-189/>.
- 407 [24] J.-P. Gattuso, A. Magnan, R. Billé, W. Cheung, E. Howes, F. Joos, D. Allemand, L. Bopp,  
408 S. Cooley, C. Eakin, et al. Contrasting futures for ocean and society from different anthro-  
409 pogenic CO<sub>2</sub> emissions scenarios. *Science*, 349(6243):aac4722, 2015.
- 410 [25] J.-P. Gattuso, J.-M. Epitalon, H. Lavigne, and J. Orr. *seacarb: Seawater Carbonate Chemistry*,  
411 2019. URL <https://CRAN.R-project.org/package=seacarb>. R package version 3.2.12.
- 412 [26] B. Goodrich, J. Gabry, I. Ali, and S. Brilleman. *rstanarm: Bayesian applied regression mod-  
413 eling via Stan.*, 2018. URL <http://mc-stan.org/>. R package version 2.17.4.
- 414 [27] J. P. Heiden, C. Völkner, E. M. Jones, W. H. van de Poll, A. G. Buma, M. P. Meredith, H. J.  
415 de Baar, K. Bischof, D. Wolf-Gladrow, and S. Trimborn. Impact of ocean acidification and  
416 high solar radiation on productivity and species composition of a late summer phytoplankton  
417 community of the coastal western antarctic peninsula. *Limnology and Oceanography*, 2019.
- 418 [28] S. L. Hinder, G. C. Hays, M. Edwards, E. C. Roberts, A. W. Walne, and M. B. Gravenor.  
419 Changes in marine dinoflagellate and diatom abundance under climate change. *Nature Climate  
420 Change*, 2(4):271, 2012.
- 421 [29] L. E. Holman, M. de Bruyn, S. Creer, G. Carvalho, J. Robidart, and M. Rius. Detection of  
422 introduced and resident marine species using environmental dna metabarcoding of sediment  
423 and water. *Scientific Reports*, 9(1):1–10, 2019.
- 424 [30] M. D. Iglesias-Rodriguez, P. R. Halloran, R. E. Rickaby, I. R. Hall, E. Colmenero-Hidalgo,  
425 J. R. Gittins, D. R. Green, T. Tyrrell, S. J. Gibbs, P. von Dassow, et al. Phytoplankton  
426 calcification in a high-CO<sub>2</sub> world. *science*, 320(5874):336–340, 2008.
- 427 [31] S. Jansen. Copepods grazing on *Coscinodiscus wailesii*: a question of size? *Helgoland Marine  
428 Research*, 62(3):251, 2008.
- 429 [32] P. Jokiel, K. Rodgers, I. Kuffner, A. Andersson, E. Cox, and F. Mackenzie. Ocean acidification  
430 and calcifying reef organisms: a mesocosm investigation. *Coral Reefs*, 27(3):473–483, 2008.
- 431 [33] R. P. Kelly, J. L. O’Donnell, N. C. Lowell, A. O. Shelton, J. F. Samhouri, S. M. Hennessey,  
432 B. E. Feist, and G. D. Williams. Genetic signatures of ecological diversity along an urbanization  
433 gradient. *PeerJ*, 4:e2444, 2016.
- 434 [34] R. P. Kelly, R. Gallego, and E. Jacobs-Palmer. The effect of tides on nearshore environmental  
435 DNA. *PeerJ*, 6:e4521, 2018.
- 436 [35] R. P. Kelly, A. O. Shelton, and R. Gallego. Understanding PCR processes to draw meaningful  
437 conclusions from environmental DNA studies. *Scientific Reports*, 9(1):1–14, 2019.
- 438 [36] T. Khangaonkar, A. Nugraha, W. Xu, and K. Balaguru. Salish sea response to global climate  
439 change, sea level rise, and future nutrient loads. *Journal of Geophysical Research: Oceans*,  
440 2019.
- 441 [37] K. J. Kroeker, R. L. Kordas, R. N. Crim, and G. G. Singh. Meta-analysis reveals negative yet  
442 variable effects of ocean acidification on marine organisms. *Ecology Letters*, 13(11):1419–1434,  
443 2010.
- 444 [38] K. J. Kroeker, F. Micheli, M. C. Gambi, and T. R. Martz. Divergent ecosystem responses  
445 within a benthic marine community to ocean acidification. *Proceedings of the National  
446 Academy of Sciences*, 108(35):14515–14520, 2011.

- 447 [39] K. J. Kroeker, R. L. Kordas, R. Crim, I. E. Hendriks, L. Ramajo, G. S. Singh, C. M. Duarte,  
448 and J.-P. Gattuso. Impacts of ocean acidification on marine organisms: quantifying sensitivities  
449 and interaction with warming. *Global Change Biology*, 19(6):1884–1896, 2013.
- 450 [40] H. Kurihara. Effects of CO<sub>2</sub>-driven ocean acidification on the early developmental stages of  
451 invertebrates. *Marine Ecology Progress Series*, 373:275–284, 2008.
- 452 [41] D. Lallias, J. G. Hiddink, V. G. Fonseca, J. M. Gaspar, W. Sung, S. P. Neill, N. Barnes,  
453 T. Ferrero, N. Hall, P. J. D. Lambshead, et al. Environmental metabarcoding reveals het-  
454 erogeneous drivers of microbial eukaryote diversity in contrasting estuarine ecosystems. *The  
455 ISME journal*, 9(5):1208–1221, 2015.
- 456 [42] B. Langmead and S. L. Salzberg. Fast gapped-read alignment with bowtie 2. *Nature methods*,  
457 9(4):357, 2012.
- 458 [43] M. Leray, J. Y. Yang, C. P. Meyer, S. C. Mills, N. Agudelo, V. Ranwez, J. T. Boehm,  
459 and R. J. Machida. A new versatile primer set targeting a short fragment of the  
460 mitochondrial COI region for metabarcoding metazoan diversity: application for char-  
461 acterizing coral reef fish gut contents. *Frontiers in Zoology*, 10(1):34, 2013. URL  
462 <http://www.biomedcentral.com/content/pdf/1742-9994-10-34.pdf>.
- 463 [44] T. Manabe and S. Ishio. Bloom of *coccinodiscus wailesii* and do deficit of bottom water in seto  
464 inland sea. *Marine Pollution Bulletin*, 23:181–184, 1991.
- 465 [45] R. Margalef. Life-forms of phytoplankton as survival alternatives in an unstable environment.  
466 *Oceanol. Acta*, 1(4), 1978.
- 467 [46] M. Martin. Cutadapt removes adapter sequences from high-throughput sequencing reads.  
468 *EMBnet.journal*, 17(1):10pp, 2011.
- 469 [47] S. K. Moore, N. J. Mantua, J. A. Newton, M. Kawase, M. J. Warner, and J. P. Kellogg. A de-  
470 scriptive analysis of temporal and spatial patterns of variability in Puget Sound oceanographic  
471 properties. *Estuarine, Coastal and Shelf Science*, 80(4):545–554, 2008.
- 472 [48] S. K. Moore, J. A. Johnstone, N. S. Banas, and E. P. Salathe Jr. Present-day and future  
473 climate pathways affecting *Alexandrium* blooms in Puget Sound, WA, USA. *Harmful Algae*,  
474 48:1–11, 2015.
- 475 [49] P. J. Mudie, A. Rochon, and E. Levac. Palynological records of red tide-producing species  
476 in Canada: past trends and implications for the future. *Palaeogeography, Palaeoclimatology,*  
477 *Palaeoecology*, 180(1-3):159–186, 2002.
- 478 [50] J. W. Murray, E. Roberts, E. Howard, M. O'Donnell, C. Bantam, E. Carrington, M. Foy,  
479 B. Paul, and A. Fay. An inland sea high nitrate-low chlorophyll (hnlc) region with naturally  
480 high pCO<sub>2</sub>. *Limnology and Oceanography*, 60(3):957–966, 2015.
- 481 [51] J. L. O'Donnell, R. P. Kelly, N. C. Lowell, and J. A. Port. Indexed PCR primers induce  
482 template-specific bias in large-scale DNA sequencing studies. *PloS one*, 11(3):e0148698, 2016.
- 483 [52] J. Oksanen, F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L.  
484 Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. *vegan: Community Ecology Package*,  
485 2015. URL <http://CRAN.R-project.org/package=vegan>. R package version 2.3-1.
- 486 [53] S. Pasquaud, M. Pillet, V. David, B. Sautour, and P. Elie. Determination of fish trophic levels  
487 in an estuarine system. *Estuarine, Coastal and Shelf Science*, 86(2):237–246, 2010.
- 488 [54] L. Polimene, S. Sailley, D. Clark, A. Mitra, and J. I. Allen. Biological or microbial carbon  
489 pump? the role of phytoplankton stoichiometry in ocean carbon sequestration. *Journal of  
490 Plankton Research*, 39(2):180–186, 2017.
- 491 [55] R Core Development Team. R: A language and environment for statistical computing, 2013.  
492 URL <http://www.R-project.org/>.
- 493 [56] M. A. Renshaw, B. P. Olds, C. L. Jerde, M. M. McVeigh, and D. M. Lodge. The room tem-  
494 perature preservation of filtered environmental DNA samples and assimilation into a phenol–  
495 chloroform–isoamyl alcohol DNA extraction. *Molecular Ecology Resources*, 15(1):168–176,  
496 2015.
- 497 [57] U. Riebesell, V. J. Fabry, L. Hansson, and J.-P. Gattuso. *Guide to best practices for ocean  
498 acidification research and data reporting*. Office for Official Publications of the European  
499 Communities, 2011.

- 500 [58] U. Riebesell, N. Aberle-Malzahn, E. P. Achterberg, M. Algueró-Muñiz, S. Alvarez-Fernandez,  
501 J. Arístegui, L. T. Bach, M. Boersma, T. Boxhammer, W. Guan, M. Haunost, H. G.  
502 Horn, C. R. Löscher, A. Ludwig, C. Spisla, M. Sswat, P. Stange, and J. Taucher.  
503 Toxic algal bloom induced by ocean acidification disrupts the pelagic food web. *Nature Climate Change*, 8(12):1082–1086, 2018. doi: 10.1038/s41558-018-0344-1. URL  
504 <https://doi.org/10.1038/s41558-018-0344-1>.
- 506 [59] K. M. Ruppert, R. J. Kline, and M. S. Rahman. Past, present, and future perspectives of  
507 environmental dna (edna) metabarcoding: A systematic review in methods, monitoring, and  
508 applications of global edna. *Global Ecology and Conservation*, page e00547, 2019.
- 509 [60] I. B. Schnell, K. Bohmann, and M. T. P. Gilbert. Tag jumps illuminated–reducing sequence-  
510 to-sample misidentifications in metabarcoding studies. *Molecular Ecology Resources*, 15(6):  
511 1289–1303, 2015.
- 512 [61] C. A. Stock, J. G. John, R. R. Rykaczewski, R. G. Asch, W. W. Cheung, J. P. Dunne, K. D.  
513 Friedland, V. W. Lam, J. L. Sarmiento, and R. A. Watson. Reconciling fisheries catch and  
514 ocean productivity. *Proceedings of the National Academy of Sciences*, 114(8):E1441–E1449,  
515 2017.
- 516 [62] V. L. Trainer, S. K. Moore, G. Hallegraeff, R. M. Kudela, A. Clement, J. I. Mardones, and  
517 W. P. Cochlan. Pelagic harmful algal blooms and climate change: Lessons from nature’s  
518 experiments with extremes. *Harmful Algae*, In Press, 2019.
- 519 [63] W. N. Venables and B. D. Ripley. *Modern Applied Statistics with S*. Springer, New York,  
520 fourth edition, 2002. URL <http://www.stats.ox.ac.uk/pub/MASS4>. ISBN 0-387-95457-0.
- 521 [64] N. Wasmund, J. Kownacka, J. Göbel, A. Jaanus, M. Johansen, I. Jurgensone, S. Lehtinen,  
522 and M. Powilleit. The diatom/dinoflagellate index as an indicator of ecosystem changes in the  
523 Baltic Sea 1. principle and handling instruction. *Frontiers in Marine Science*, 4:22, 2017.
- 524 [65] S. Watanabe and M. Opper. Asymptotic equivalence of bayes cross validation and widely ap-  
525 plicable information criterion in singular learning theory. *Journal of machine learning research*,  
526 11(12), 2010.
- 527 [66] M. L. Wells and B. Karlson. Harmful algal blooms in a changing ocean. In *Global Ecology and*  
528 *Oceanography of Harmful Algal Blooms*, pages 77–90. Springer, 2018.
- 529 [67] S. P. Wilkinson, M. Stat, M. Bunce, and S. K. Davy. Taxonomic identification of environmental  
530 dna with informatic sequence classification trees. *PeerJ Preprints*, 6:e26812v1, 2018. doi:  
531 10.7287/peerj.preprints.26812v1. URL <https://peerj.com/preprints/26812>.
- 532 [68] W. Xiao, X. Liu, A. J. Irwin, E. A. Laws, L. Wang, B. Chen, Y. Zeng, and B. Huang. Warming  
533 and eutrophication combine to restructure diatoms and dinoflagellates. *Water Research*, 128:  
534 206–216, 2018.
- 535 [69] J. E. Zamon. Tidal changes in copepod abundance and maintenance of a summer coscinodiscus  
536 bloom in the southern san juan channel, san juan islands, usa. *Marine Ecology Progress Series*,  
537 226:193–210, 2002.