Estimating relative sensitivities of zooplankton to ocean acidification and comparing to observations *in situ*

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Abstract

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Laboratory studies show that low pH and high pCO_2 associated with ocean acidification can significantly affect the survival, growth, calcification, development, reproduction, and metabolism of planktonic organisms. To understand how sensitivity to ocean acidification varies among species, I conducted a meta-analysis of six taxonomic groups of zooplankton that are common within marine waters of the Pacific Northwest. I determined that pteropods are the taxon most sensitive to increasing levels of pCO_2, and that calcification is the process most severely affected. I then sought to determine whether observed distributions of zooplankton in Puget Sound, Washington were statistically associated with carbon system variables, and whether field observations supported findings from the meta-analysis regarding taxon-specific sensitivities. During two research cruises in June and August of 2017, I collected zooplankton and physical and chemical data at six research stations from South Hood Canal to South Puget Sound Basin. Zooplankton community composition and density varied across location and time, with copepods, a highly sensitive taxon, and larvaceans, the least sensitive taxon, accounting for the highest densities (70.2% and 14.8% respectively) over the two sampling periods. Statistical analyses revealed that a combination of temperature, fluorescence, dissolved oxygen, and salinity was the primary determinant of zooplankton abundance at these stations during the two sampling periods. I found little association between empirical measures of station pH and taxon abundance by sensitivity category (as revealed by the meta-analysis), suggesting that sensitivity to ocean acidification plays a subordinate role in determining the abundance of some zooplankton taxa.

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Introduction

Atmospheric carbon dioxide (CO_2) has increased by more than 45% since the Industrial Revolution (Dlugokencky et al. 2018), rising to levels unprecedented in the last 800,000 years (Lüthi et al. 2008). The ocean has absorbed approximately one third of anthropogenic emissions (Sabine et al. 2004), causing a suite of chemical changes in the oceans in a process known as ocean acidification (OA). OA is quantified by changes in carbon system variables including decreases in pH and can pose significant threats to organisms (Kroeker et al. 2013), ecosystems (Royal Society, 2005; Kleypas et al. 2006; Fabry et al. 2008), and human systems (Branch et al. 2013).

Average ocean surface pH has decreased by 0.1 units since preindustrial times (from 8.2 to 8.1), corresponding to a 26% increase in hydrogen concentration (Caldeira & Wickett, 2003). Under the Intergovernmental Panel on Climate Change (IPCC) business-as-usual emission scenario, pH is projected to decrease by an additional 0.30-0.32 units by the year 2100 (IPCC, 2013). This rate of decline in pH is faster than anything observed in the past 50 million years (Hönisch et al. 2012).

Oceanic uptake of CO_2 helps dampen the effects of global warming but, at the same time, altered ocean chemistry can have significant deleterious impacts on marine organisms. Laboratory studies show that low pH and high pCO_2 associated with OA can significantly affect the survival, growth, calcification, development, reproduction, and abundance of planktonic organisms (Kroeker et al. 2013; Busch & McElhany, 2016). Moreover, the saturation state (Ω), or the measure of solubility or degree to which seawater is saturated with respect to aragonite or calcite, decreases as ocean pH falls, causing calcifying marine organisms to have greater difficulty precipitating the calcium carbonate they need to build, maintain, and repair their shells and skeletons. Sensitivity to ocean acidification is highly variable among species and includes responses such as shell dissolution in pteropods (Bednaršek et al. 2012), impaired development of euphausiids (McLaskey et al. 2016), and reduced survival in crab larvae (Miller et al. 2016).

Zooplankton are well-known bioindicators of ecosystem health, food web function, and water quality (Beaugrand, 2005; Hooff & Peterson, 2006) because their short life cycles allow them to respond to seasonal variations and abrupt environmental changes. Pteropods, for example, are small pelagic molluscs with thin calcium carbonate shells, and have a documented sensitivity to OA. Bednaršek et al. (2014) estimate that shell dissolution among pteropods in nearshore environments of the California Current System has doubled since the beginning of the Industrial Revolution and is expected to triple by 2050. Because pteropods are an important component of marine food webs and serve as a food source for a variety of economically important species such as salmon, advancing OA is predicted to have not only ecosystem-level impacts, but economic and recreational impacts as well.

Patterns of seawater pH and aragonite saturation state vary spatially and temporally due to a variety of natural and human-influenced processes. On the U.S. west coast, seasonal upwelling naturally brings deep ocean water rich in CO_2 and low in pH to the coast, exacerbating the impacts of anthropogenically-caused OA. Washington state is particularly prone to OA due to coastal upwelling (Feely et al. 2008), eutrophication in enclosed bays (Strong et al. 2014) and freshwater inputs low in pH (Bianucci et al. 2018). Puget Sound, a semi-enclosed estuary in Washington with approximately 4,000 km of shoreline (Emmett et al. 2000) and a rapidly increasing population, is acidifying more quickly than the outer Washington coastline and the global average.

Specifically, Hood Canal, the southwestern-most basin in Puget Sound, is characterized by constrained circulation due to an underwater sill at Admiralty Inlet that limits water exchange with the Strait of Juan de Fuca. Moreover, Hood Canal is subject to abundant freshwater inputs from rivers and streams that cause vertical stratification of the water column. Run-off from on-site septic systems, agriculture, and other human activities stimulates the growth of phytoplankton that die, sink, and are consumed by microbes that respire CO_2. This process can produce hypoxic conditions and decreased pH that exacerbate Hood Canal's naturally low-pH waters, leading to surface pH values as low as 7.4 and waters undersaturated with respect to aragonite (PSEMP, 2012). These conditions make Puget Sound a suitable natural laboratory for studying the role of OA in structuring zooplankton communities.

Laboratory studies are useful in demonstrating organismal response to acidified conditions, whereas field studies are important in measuring spatial and temporal variation in OA conditions and the distribution and abundance of organisms exposed to OA. A combination of both approaches is crucial for comprehensively understanding OA and its impacts in dynamic natural systems.

Using both meta-analytic techniques and the analysis of samples taken on two cruises in Puget Sound, I sought to identify the primary drivers of zooplankton density and community composition at several stations and determine whether field observations support findings from the literature regarding relative sensitivities to OA. To understand and quantify taxon-specific sensitivity to OA, I compiled relevant published data into a database and conducted a sensitivity analysis, which explored zooplankton response to elevated levels of pCO_2. Using data collected from two oceanographic cruises, I investigated associations between zooplankton community structure and environmental factors (temperature, dissolved oxygen, fluorescence and specifically chlorophyll a, salinity, aragonite saturation state, and carbonate chemistry parameters) to determine the role pCO_2 and pH played in structuring zooplankton community assemblages in Puget Sound during the summer of 2017.

Methods

Data Collection for Meta-analysis

Using Web of Science and Google Scholar, I searched the scientific literature and identified studies that analyzed an organism's direct response to manipulated carbonate chemistry. Data were collected for seasonally-abundant, holoplanktonic species known to occur in the nearshore and offshore waters of Washington and Oregon. Shellfish and crab larvae were included due to their regional economic and ecological importance.

Keywords included "ocean acidification", "pH", "carbonate chemistry", taxonomic group of interest (e.g., "krill") and scientific name (e.g., "*Euphausia pacifica*"). Studies were included if they were peer reviewed and published during or after 2009. Data pertaining to indirect effects such as predation, habitat modification, and impacts to food quality were excluded from analysis. Applicable studies were added to the database through February 10, 2019.

For each study, I recorded the reported carbon system variables (e.g., pCO_2, pH, DIC), temperature, experiment duration, sample size, mean response, variance (as standard error or deviation), lifestage, collection location, and calcification type (if applicable). If a range of values was presented (e.g., start and end values of pH), the mean was calculated and entered into the database. If a paper reported more than one experiment, each was included and analyzed separately. Because some studies did not explicitly state their results in numerical format, I used a WebPlotDigitizer Version 3.9 (Rohatgi, 2015) to quantify biological responses and experimental conditions based on information presented in figures.

When a study included several species, each species meeting the established criteria was entered into the database and analyzed separately from other species in the same study. I did not include data that had multiple experimental variables (e.g., starvation scenarios or extreme temperatures) that could obscure the interpretation of the organism's response to changes in carbonate chemistry alone.

I recorded each organism's response to varying pCO_2/pH conditions as positive, negative, or no significant response detected for six major response categories: survival, calcification, development, reproduction, metabolism, and growth (Table 1). Variables that did not clearly fit within one of response categories, such as heart rate and swimming speed, were excluded from the analysis. Positive and negative responses relative to controls were only recorded if the author stated that the change was statistically significant or provided a statistically significant p-value ($p<.05$).

Table 1. The six response categories, their definition, and three examples of the types of responses recorded in the database. Any variable that did not clearly fit within one of these six response categories was excluded from analyses.

Response Categories	**Definition**	**Examples of Response Variables**
Survival	Measure of an organism's ability to stay alive	Mortality, hatching success, survival rate
Calcification	Process by which an organism forms calcium carbonate	Amount of $CaCO_3$ precipitated, shell condition score, calcification rate
Development	Transformation of an organism and its progression through various life stages	Proportion to reach C1 stage, percent stage II zoea, abnormality
Reproduction	Ability of adult organisms to produce offspring; fecundity	Egg production rate, fertilization, buds produced per polyp
Metabolism	All processes associated with energy capture, assimilation, and expenditure, and their associated proxies	Oxygen consumption, ammonia excretion, ingestion rate
Growth	The increase in size and changes in shape of an organism	Biomass, shell area, telson length

If an author did not provide the aforementioned information or included it in a format that was incompatible with my analysis (e.g., significance at a specific pCO_2 could not be determined from an ANOVA table), then the effect was only considered statistically significant if an experiment's error bars did not overlap with the control's error bars in the published figures. Occasionally, significance could not be verified from the paper, so the study was excluded from the sensitivity analysis. If the data were not explicitly presented, but the results were mentioned in the text and noted as “not significant”, then the data were included.

In order to be compatible with the sensitivity analysis, I converted pH and ppm CO_2 to pCO_2. If a paper provided data for pH, total alkalinity, temperature, and salinity, I used the spreadsheet version of CO2SYS to calculate pCO_2 (Pierrot & Wallace, 2006). When the authors did not provide the environmental data necessary for a conversion, I equated ppm with pCO_2 because the measures are similar and the differences between them are unlikely to substantially influence the results.

Sensitivity Analysis

All analyses were performed in R version 1.1.442 using the Vegan, MASS, and corrplot packages.

I modified the approach of Wittmann and Pörtner (2013) to determine the effect of increasing pCO_2 on taxonomic groups, and to compare sensitivity across groups. I created bins of pCO_2 adapted from carbon chemistry conditions currently observed or projected for Puget Sound, WA as described by Busch et al. (2014): the current summer surface (200-550 µatm), current deep water or surface conditions during upwelling (550-950 µatm; 950-1300 µatm; 1300-1800 µatm) and future deep water and future surface conditions (1800-3200 µatm). I included a final bin for occasionally observed conditions and extreme conditions (3200-10000 µatm) for a total of six bins.

Before analyzing the data, I cleaned my dataset to eliminate duplicate response categories for each species. By only including one experiment per response category, I ensured that some categories were not overrepresented. To select the representative experiment, I chose the longest running study. If two experiments had the same duration, I prioritized the experiment with the sharpest response (e.g., negative response at low pCO_2 chosen over negative or no significant response at higher pCO_2). If there was no significant response or the direction of response was the same for several parameters, then I chose the experiment that reported the more comprehensive parameter (e.g., shell area over shell length for growth). When several life stages were reported for a specific taxon, I included data for all lifestages so that, for example, both hatching success and mortality of larvae were included for a single species, even though they are both variables under the survival response category.

Per the methods of Wittmann and Pörtner (2013), data were extrapolated to help compensate for missing values. I extrapolated the data so that when a species exhibited a significant negative/positive response at low pCO_2, I recorded that it would also exhibit a negative/positive response at higher pCO_2. Moreover, if a response was exhibited at a low and high pCO_2, the same response was recorded at pCO_2 levels between the two.

I then quantified the percent of significant negative effect for each bin and summed across the six bins for each taxon. If a taxon was positively affected at high pCO_2, the sum of significant positive effect across bins was subtracted from the sum of negative effects. The highest total percent denotes the most sensitive taxonomic group and the lowest total percent denotes the least sensitive.

To compare these sensitivities to observations made *in situ*, I created four categories of susceptibility to OA: most susceptible, susceptible, least susceptible, and insufficient data. I grouped taxa into each category based on a visual determination of the similarity of their total percent of significant negative effect. I then applied this result to observational data collected on two cruises to determine whether relative abundance was consistent with sensitivities as revealed by my analysis.

Field Data Collection and Processing

In June and August 2017, I collected zooplankton and associated seawater samples on two research cruises in Puget Sound, Washington aboard the R/V Clifford A. Barnes. I sampled water chemistry by deploying a CTD and collected zooplankton using vertical net tows. I collected data at six stations established by the Puget Sound Regional Synthesis Model (PRISM) program (P8, P12, P15, P28, P38, and P402; Figure 1) from June 23-30 (hereafter referred to as "June") and again from August 25-September 1 (hereafter referred to as "August"). P8, P12, P15, and P402 are located in the Hood Canal Basin, whereas P28 is in the Puget Sound Main Basin and P38 is in the South Puget Sound Basin (Appendix A). These sampling sites and dates were selected to cover a range of physical and chemical properties (Feely et al. 2010).

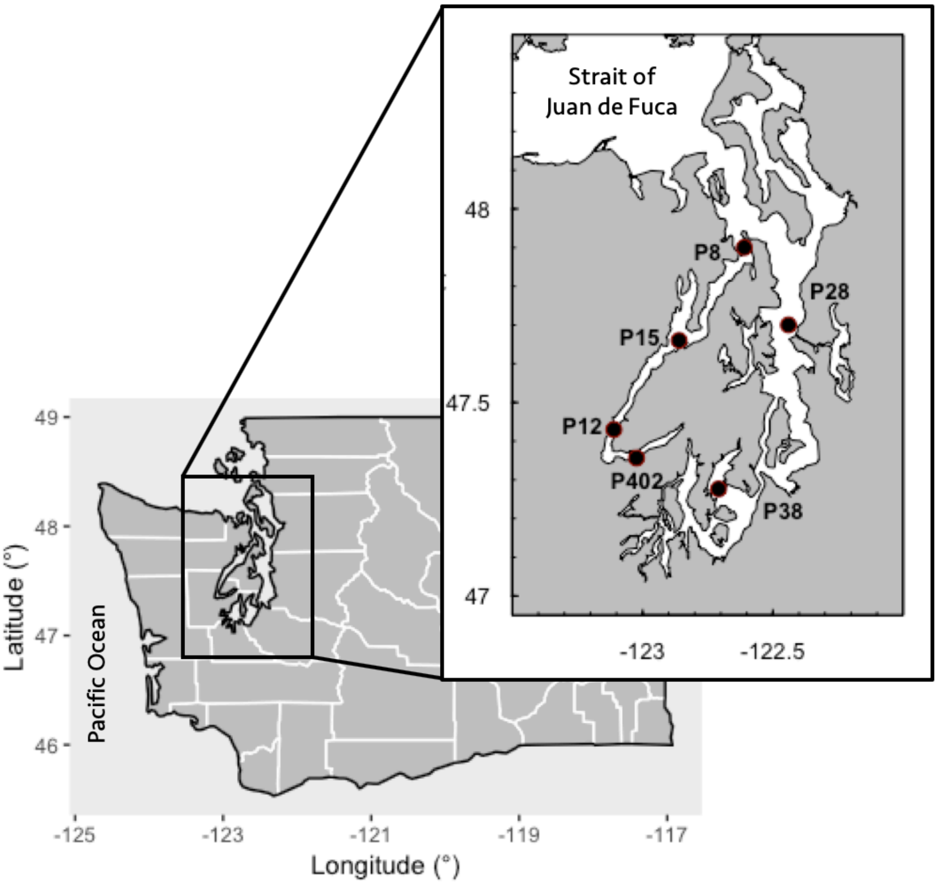


Figure 1. Approximate locations of the six PRISM sampling stations in Puget Sound, Washington, USA.

At each station, I deployed a CTD profiler (Sea-Bird SBE 9) equipped with a pH sensor (Sea-Bird SBE 18), dissolved oxygen sensor (Sea-Bird SBE 43), and Niskin bottles to quantify the physical and chemical characteristics of the site. The CTD sensors were used to determine the shape of the station's chemistry profile and provide guidance for triggering the Niskin bottles, which collected seawater at discrete depths for analysis of dissolved oxygen (DO), chlorophyll a (chl a), dissolved inorganic carbon (DIC), and total alkalinity (TA). All water samples collected for carbonate chemistry analysis were collected and analyzed according to Dickson et al. (2007). DO was analyzed through the Winkler titration method outlined in Carpenter (1965).

I estimated phytoplankton biomass by measuring chl a from Niskin bottle water collected at the surface and fluorescence maximum layer as determined by the CTD. I quantitatively filtered this water with a GF/F filter, extracted and wrapped the filter in foil, and froze it until it could be analyzed on shore. In the laboratory, I extracted the chl a in 90% acetone with sonification and measured its fluorescence with a fluorometer (Turner Designs TD-700).

For each CTD cast, I calculated the offset between the CTD pH profile and the discrete bottle samples, created a shifted pH profile assuming a constant offset with depth, and used the new profile to calculate mean pH for each station. When comparing the CTD's DO sensor with the Winkler titration samples, I determined that the sensor was well calibrated, so no correction to the profile was made. I used the seacarb package in R to calculate pH, Ω_{arg}, and pCO_2 from DIC and TA measurements for each station.

Zooplankton were collected at each station using a 60-cm diameter ring net with 200-µm mesh towed vertically from approximately 10 m above the seafloor to the surface at 30 m/min. Zooplankton collections were made between 20:48 and 21:49 in the evening. A TSK flow meter was used to measure the volume of water filtered during each net tow. Zooplankton samples were preserved in 5% buffered formalin in seawater and taken back to the laboratory for taxonomic identification.

In the laboratory, each of the 12 preserved samples was diluted to 4-10 times its settled volume with the goal of achieving approximately 200 organisms per 1 mL aliquot. Large organisms (>1 cm) were removed from the sample and identified to broad taxonomic groups, such as larvacean, krill, and chaetognath, and the remaining solution was subsampled with 2-3 1 mL Stempel pipettes. Additionally, one 10 mL Stempel pipette subsample was searched for unrecorded taxa to reduce the likelihood that less abundant zooplankton present in the sample were excluded from analysis. Every organism in the sample (aside from eggs and phytoplankton, which were excluded from this analysis) was counted and identified to broad taxonomic groups (see Appendix B for full list). Because pteropods are easily identified and are a documented bioindicator of OA, they were identified to species. Copepods, euphausiids, barnacles, crabs, polychaetes, and shrimp were identified to life history stage.

Analysis of Environmental Data

To explore patterns among samples, environmental data were normalized using the standardize function in the Vegan package in R, and a principal components analysis (PCA) was conducted. Due to analytical requirements associated with small biological sample sizes, the number of environmental factors included in the analysis were reduced by eliminating covarying parameters. The variables that were inferred to be the most ecologically relevant parameters were selected. Following this procedure, six environmental parameters were included in the PCA:

minimum pH, maximum temperature, mean salinity, mean DO, maximum chl a, and maximum Ω_{arg}.

The average density of zooplankton at each station was calculated from the zooplankton abundance in each sample divided by the volume of water filtered. Zooplankton that were observed three or fewer times in the laboratory (i.e., isopods, arachnids, ctenophores, and fish) were excluded from analyses.

The Shannon-Wiener Index was used to quantify species diversity at each site and determine differences between months. The Bray-Curtis dissimilarity ordination was used to determine spatial and temporal differences in zooplankton community structure and quantify dissimilarity between sites.

I ran a linear model for each taxon and environmental parameter to determine significant correlations, lending insight into the conditions that influence specific taxa. To ensure that one or two data points were not driving results of significance, for each significant result I removed statistical outliers, reran the model, and compared the results. Only models for which the results of regression analysis were similar between the original linear model (with all data) and the model with outliers removed were considered ecologically meaningful.

The BIOENV function (Clarke & Ainsworth, 1993) was used to estimate the combination of environmental variables that had the highest correlation with the biological matrix. For each combination, a Spearman's correlation indicated the association between the Bray-Curtis ordination and Euclidean distances. Pairwise comparisons, the PRcomp function, and the rcorr function in the Hmisc package were used to test for multicollinearity and correlation between environmental parameters in order to eliminate covariates. The most ecologically important variables were chosen, with minimum and maximum values typically taking precedence over mean values. Minimum and maximum temperature, minimum and maximum pH, maximum omega, mean and maximum DO, minimum fluorescence, maximum ch a, and mean and maximum salinity were all included in the BIOENV procedure. No cap was set on the number of environmental variables that could be combined.

Results

Meta-analysis

Of the 43 studies that met the criteria for inclusion in the meta-analysis, 37 studies were included in the sensitivity analysis (Appendix C). The experiments reported in these studies ranged from 1 hour (Miller et al. 2014; Havenhand & Schlegel, 2009) to 122 days (Winans et al. 2010), with 7 days as the most frequent duration. Not every study reported data pertaining to lifestage, but among those that did, larval stages were the most frequently reported (37%), followed by eggs (24%). Only six studies used specimens collected from Oregon or Washington waters; the majority of specimens used in these studies were collected outside of this region.

Sensitivity Analysis and Index

Of the 17 taxa included in the sensitivity analysis, pteropods were the most frequent (30%) and larvaceans were the least frequent (3%). When summarizing effects across all taxonomic groups, significant negative impacts increased with increasing levels of pCO_2 in a linear fashion (Figure 2A). The largest percent increase in negative response (22%) occurred between 550-950 μatm and 950-1300 μatm.

Pteropods and larval bivalves were negatively affected at the lowest pCO_2 levels of 200-550 μatm, whereas shrimp, krill, and jellyfish showed no negative effects up to 950-1300 μatm (Figure 2B).

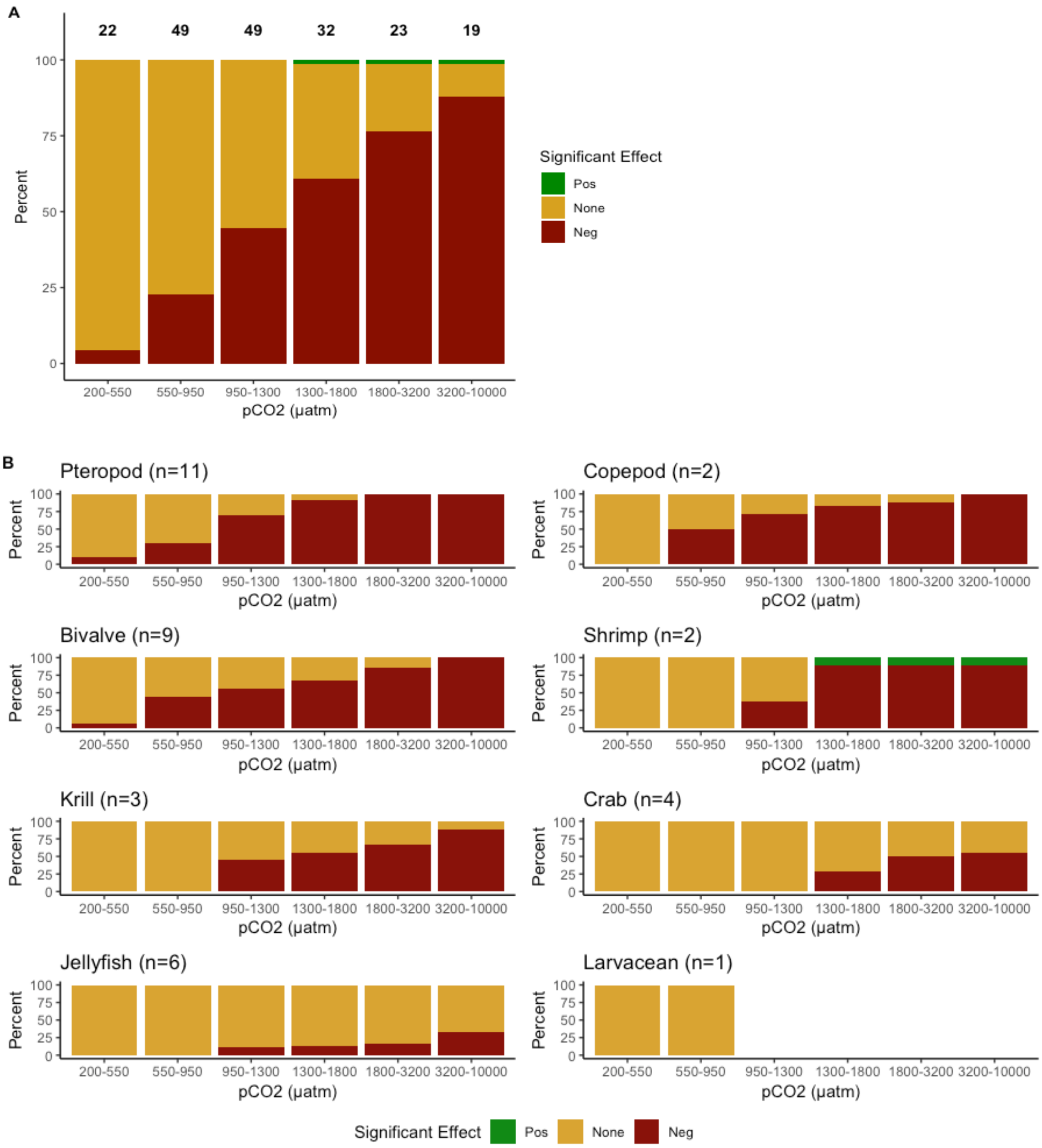
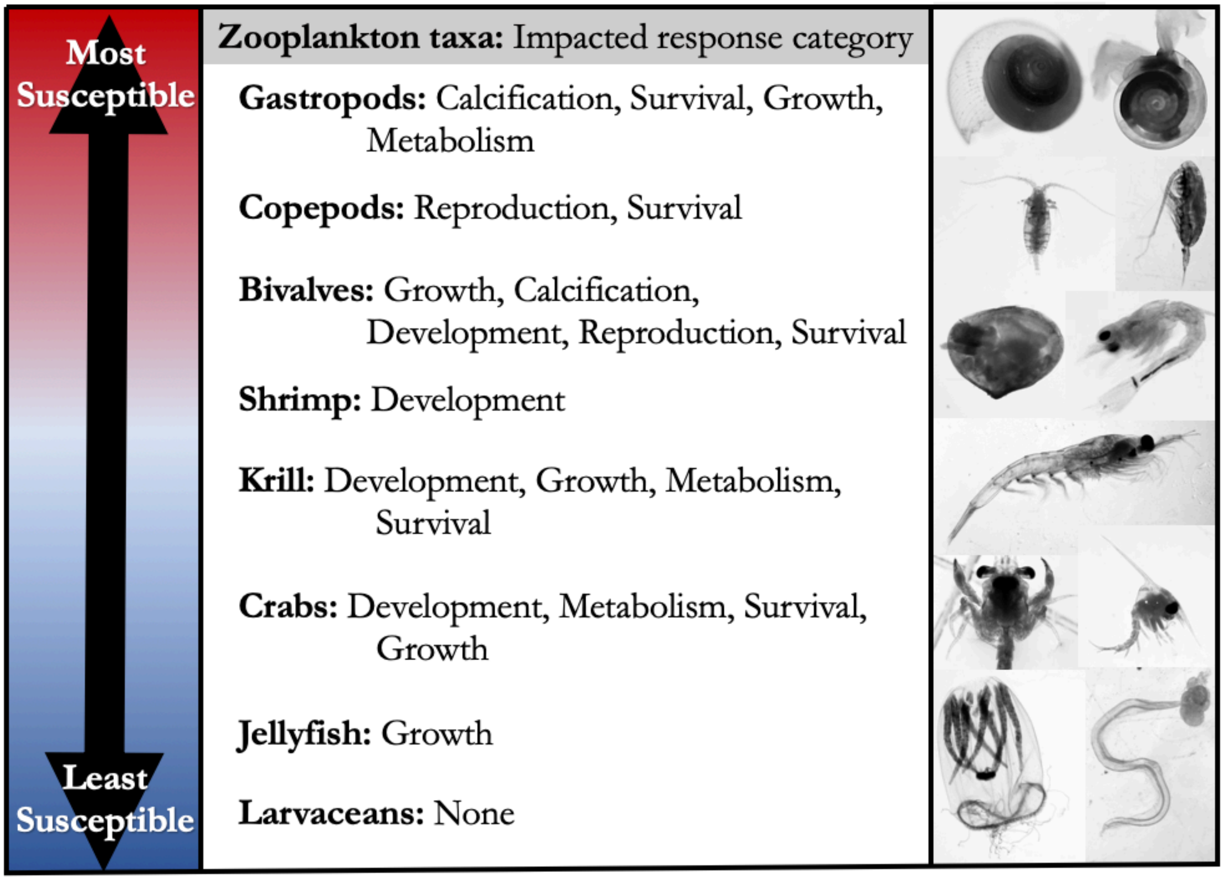


Figure 2. A. Summary of all taxonomic groups (pteropod, copepod, bivalve, shrimp, krill, crab, jellyfish, and larvacean) exhibiting positive (green), negative (red), or no significant response (yellow) to elevated levels of pCO_2 (μatm). The numbers above each bar indicate the number of experiments analyzed for each level of pCO_2, not including extrapolated data. **B.** Responses to varying levels of pCO_2 by taxonomic group. N represents the number of studies included in the analysis.

Studies indicate that pteropods are the zooplankton group most sensitive to OA and are affected at pCO_2 levels as low as 530 µatm. Pteropod calcification, survival, growth, and metabolism are all negatively affected by increasing levels of pCO_2 (Appendix D-1). Copepods emerged as the second most sensitive group, with reproduction and survival negatively affected at pCO_2 levels as low as 824 µatm (Appendix D-2). Larval bivalve calcification, growth, development, reproduction, and survival were affected, with calcification affected at pCO_2 levels as low as 545 µatm (Appendix D-3). Shrimp were the only taxon to show positive response to OA conditions, with one study finding survival increasing at 1332 µatm. Shrimp development, however, was negatively affected at pCO_2 levels as low as 1237 µatm (Appendix D-4). Krill development, growth, metabolism, and survival were all negatively affected at pCO_2 levels as low as 956 µatm (Appendix D-5), while crab larvae showed tolerance up to pCO_2 levels of 1361 µatm (Appendix D-6). Jellyfish growth was affected at pCO_2 levels above 1000 µatm, but other response categories showed no effect (Appendix D-7). Larvaceans showed the least sensitivity, with no significant response at any of the tested levels of pCO_2 (Appendix D-8). Relative sensitivities based on these results are shown in Figure 3.



Zooplankton taxa: Impacted response category

Gastropods: Calcification, Survival, Growth, Metabolism

Copepods: Reproduction, Survival

Bivalves: Growth, Calcification, Development, Reproduction, Survival

Shrimp: Development

Krill: Development, Growth, Metabolism, Survival

Crabs: Development, Metabolism, Survival, Growth

Jellyfish: Growth

Larvaceans: None

Figure 3. Relative ranking of sensitivity to ocean acidification among selected zooplankton taxa. Following each taxon are the response categories that are affected by increasing pCO_2, listed in the order of highest to lowest sensitivity. All photos are by the Keister Laboratory at the University of Washington.

Pooling data across all taxa revealed that calcification was the response category most sensitive to increasing pCO_2. Approximately 25% of experiments tested for calcification at pCO_2 levels of 550 µatm or lower reported a significantly negative response (Figure 4). Metabolism was least sensitive, and the only response category unaffected at pCO_2 levels less than 1000 µatm.

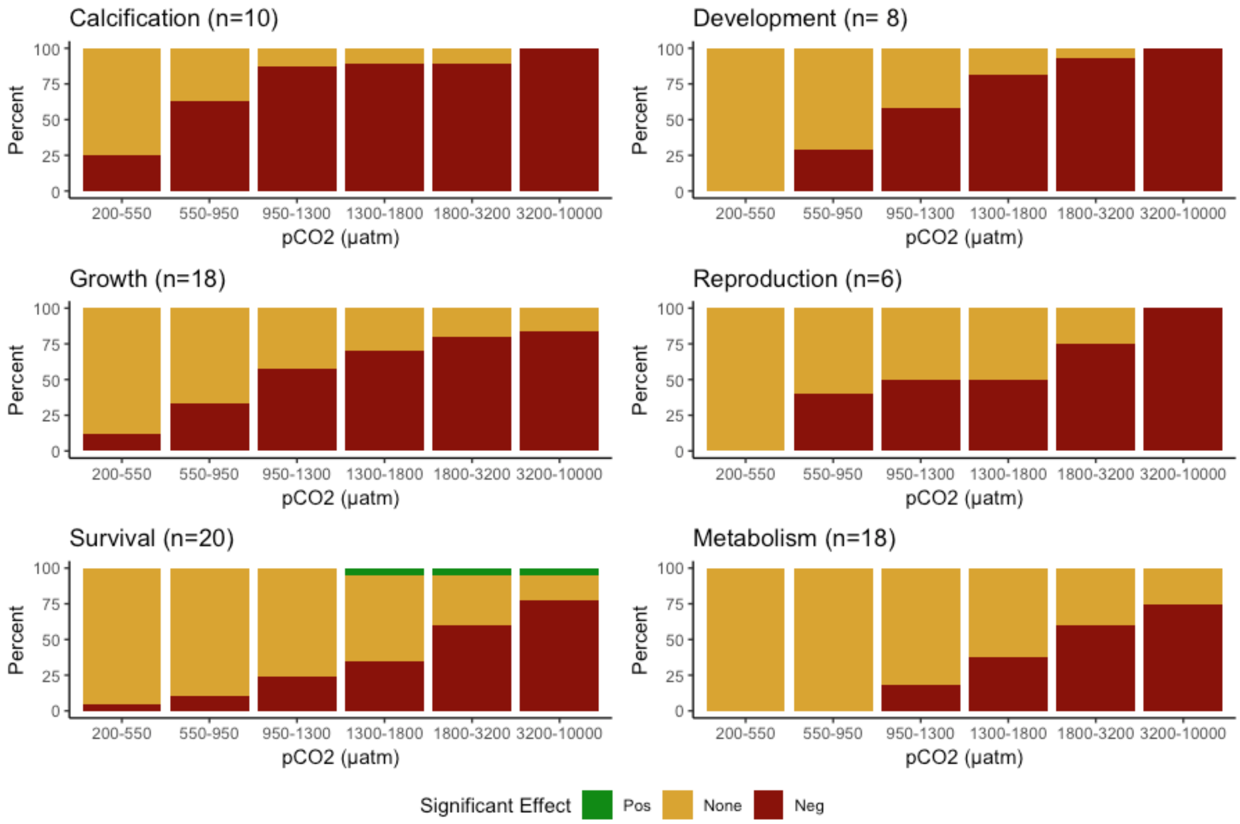


Figure 4. Summary of responses to varying levels of pCO₂ (µatm) pooled across taxonomic groups. N represents the number of studies included in the analysis.

Cruise Data

Observed Physical Conditions

Average water temperature over all stations and depths increased by about one degree Celsius between June and August (10.8°C and 11.7°C on average, respectively). Salinity ranged from 19.3 to 31.0 across all six stations over the two sampling periods. Low dissolved oxygen (DO) was recorded in South Hood Canal with an average of 4.7 mg/L over the two sampling periods. The lowest recorded DO value was 1.9 mg/L at P12 (South Hood Canal) in August. Fluorescence ranged from 0.8-33.2 µg/L and 2.5-20.8 µg/L in June and August, respectively.

Bottle-corrected CTD profiles showed lower mean pH values for all sites in August than in June (7.63 and 7.74 respectively), but pH was lower at depth in June than in August. The lowest pH in both June and August occurred at station P12 in South Hood Canal (7.32 and 7.30 respectively). Minimum Ω_{arg} ranged from severely undersaturated waters (0.32) at P12 to saturated waters (2.77) at P38. All South Hood Canal sites had lower pH and Ω_{arg} than sites in the other basins.

The results of the principal components analysis (Figure 5) indicate that environmental variables varied across sites and sampling points. Spatial variation among sites tended to exceed temporal variation between the two time-points sampled. PC1 and PC2 describe 49.6% and 25.5% of the variability in the data, respectively. Minimum pH and maximum temperature explained most of the variation in PC1, whereas maximum Omega and mean salinity were the primary explanatory variables of PC2. The majority of the variation between months is captured by PC2. The two South Hood Canal sites (P12 and P402) are tightly clustered and somewhat distinct from the other sites, which is consistent with basin-specific observations drawn from field-collected data (Feely et al. 2010).

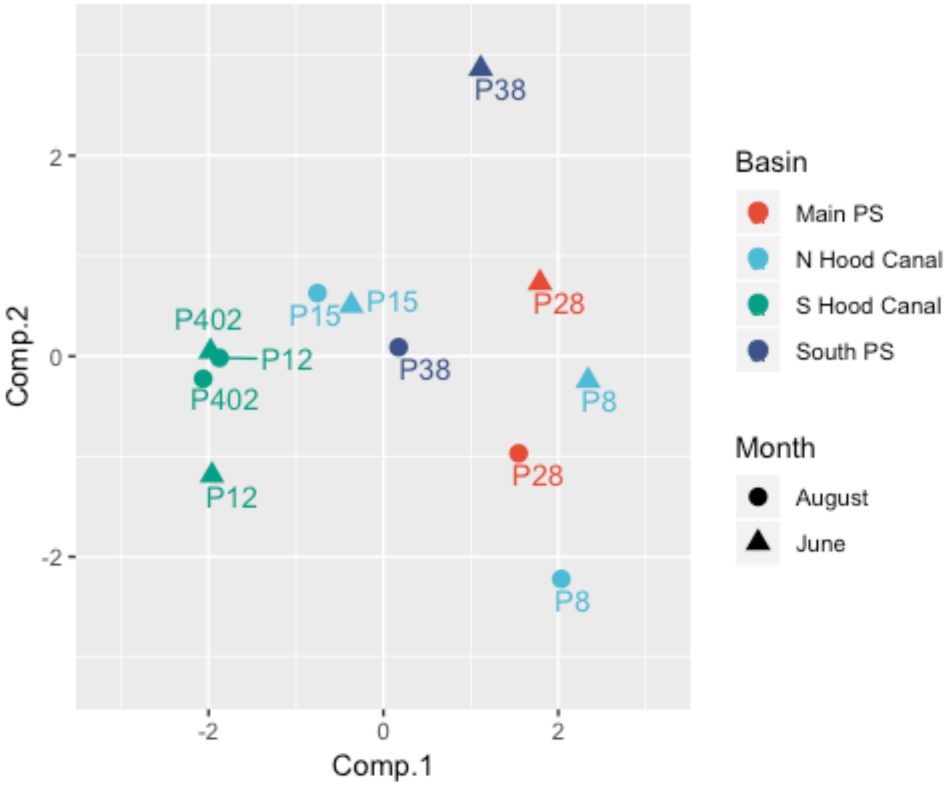


Figure 5. PCA ordination of the six PRISM stations based on six environmental parameters (minimum pH, maximum temperature, mean salinity, mean DO, maximum Chl a, and maximum Ω_{arg}) in June and August of 2017.

Zooplankton Analysis

Laboratory analysis of all 12 vertical tow samples revealed a total of 25 taxonomic groups of zooplankton (Appendix B). In June, maximum zooplankton density occurred at station P402 (approximately 7,997 individuals/m^3). Maximum zooplankton densities increased between June and August, with highest values recorded at stations P402 and P38 (approximately 9,801 individuals/m^3). The zooplankton community was characterized by a high abundance of copepods, which made up 70.2% of all recorded taxonomic groups. Noticeable larvacean blooms occurred at two sites in August (P38 and P402), rendering larvaceans the second most abundant group (14.8% of total abundance) across these samples. Taxonomic diversity varied among stations and sampling months, with the Shannon-Wiener diversity index (H) ranging from 0.48 at P38 to 1.32 at P8 in June and from 0.52 at P12 to 1.35 at P402 in August (Appendix E). Overall, the zooplankton assemblages were spatially and temporally variable, especially at the southernmost stations (Figure 6).

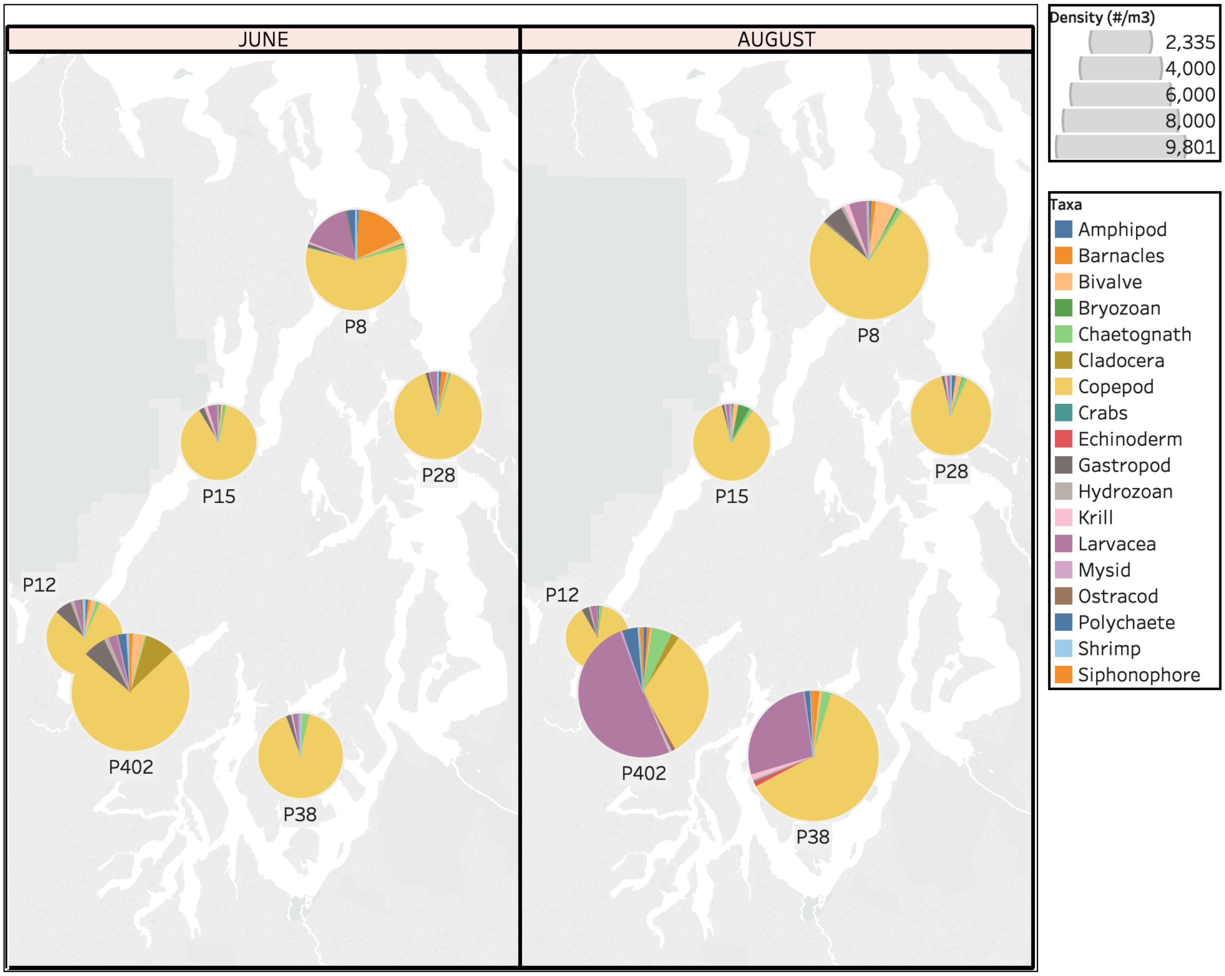


Figure 6. Total abundance of zooplankton by broad taxonomic groupings at six stations (P8, P12, P15, P28, P38, P402) in June and August of 2017. The size of each pie chart represents the density of zooplankton, or individuals per cubic meter. Fish, isopods, ctenophores, and arachnids were recorded 3 or fewer times and were omitted from analysis.

Bray-Curtis Analysis

Bray-Curtis analysis indicated that zooplankton community composition showed a high degree of dissimilarity between June and August at station P38, whereas dissimilarity between

June and August was small at station P15 (Figure 7). Moreover, dissimilarity was large between stations in South Hood Canal (P12 and P402) that are relatively close together. Dissimilarity was, on average, higher in August than in June. Wisconsin double standardization results indicate that more common species (e.g., copepods) make up the majority of the variation in this analysis.

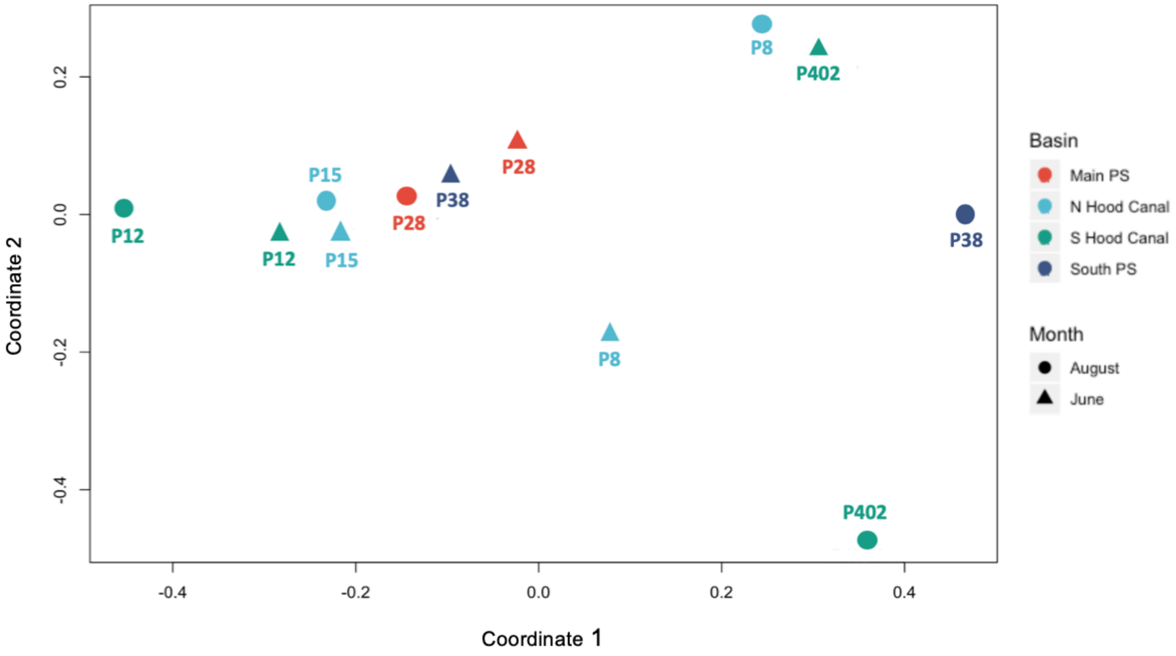


Figure 7. NMDS plot of zooplankton species composition by station and month in Puget Sound based on the Bray-Curtis dissimilarity ordination.

Linear Models

Linear model outputs showed that fluorescence (n=7) and salinity (n=4) were the two environmental parameters most often correlated with zooplankton abundance. Correlations with fluorescence were uniformly positive, while correlations with salinity were both positive and negative. Linear model outputs are shown in Appendix F.

After removing statistical outliers, I determined that single data points had largely driven several linear model results. Using the remaining models as the most robust results, they indicated that different taxa were most closely associated with different environmental variables. For example, larvaceans and polychaetes were positively correlated with mean fluorescence. Shrimp were positively correlated with maximum fluorescence, while negatively correlated with minimum salinity. Copepods were positively correlated with maximum temperature, while crabs were negatively correlated with maximum temperature. Bivalve larvae were negatively correlated with maximum pH, making them the only taxon that showed a response to carbonate chemistry parameters in this analysis.

BIOENV model

Analysis of environmental variables using the BIOENV function in R Studio indicated that temperature, maximum dissolved oxygen, and minimum fluorescence best explain the associations detected using the Bray-Curtis analysis (Table 2). Other models with more variables provided less good fit to the data.

Table 2. The top three combinations of environmental variables that best explain the biological matrices through the BIOENV procedure and their corresponding Spearman's correlation.

Variables	***Spearman's Correlation***
Temp.min, DO.max, Fluores.min	*0.4502*
Temp.min, DO.max, Fluores.min, Salinity.mean	*0.4465*
Temp.min, pH.max, DO.max, Fluores.min, Salinity.mean	*0.4409*

Comparison of Meta-analysis Results and Field Observations

Meta-analysis revealed that, when summing the percent of significant negative effect across the six bins of pCO_2, the total percent of negative effect was approximately 401% for gastropods, 392% for copepods, 360% for bivalve larvae, 271% for shrimp, 246% for krill, 134% for crabs, 74% for jellyfish, and 0% for larvaceans. Thus, gastropods, copepods, and bivalve larvae are the zooplankton groups most susceptible to OA, followed by shrimp and krill. Crabs, jellyfish, and larvaceans were least susceptible to OA. All other taxa were not represented in the literature and were categorized as insufficient data (Table 3). Figures with the insufficient data category can be found in Appendices G and H.

Table 3. The groupings of susceptibility to OA based on the sensitivity analysis. Taxonomic groups where data were exclusively collected for their planktonic larval lifestages are indicated.

Most Susceptible	Gastropods (including pteropods), Copepods, and Bivalves (larvae)
Susceptible	Shrimp and Krill
Least Susceptible	Crab (larvae), Jellyfish, and Larvaceans
Insufficient Data	Amphipods, Chaetognaths, Barnacles (larvae), Cladocerans, and Polychaetes

The distribution of differentially susceptible taxa varied across stations and sampling points (Figure 8). Taxa that the meta-analysis identified as highly susceptible were present at all stations and both months, and in many cases the most susceptible taxa were more abundant than other groups. This result is largely due to the influence of copepods, which are identified as highly susceptible to OA and are abundant in most samples. The effects of copepods on the results of the analysis can be seen by comparing Figure 8 (which includes copepods) with Figure 9 (which excludes copepods). P12 (South Hood Canal) had the lowest abundance of most susceptible taxa as well as the lowest mean pH between sampling periods. Overall, however, I detected no clear association between zooplankton abundance and sensitivity inferred from meta-analysis.

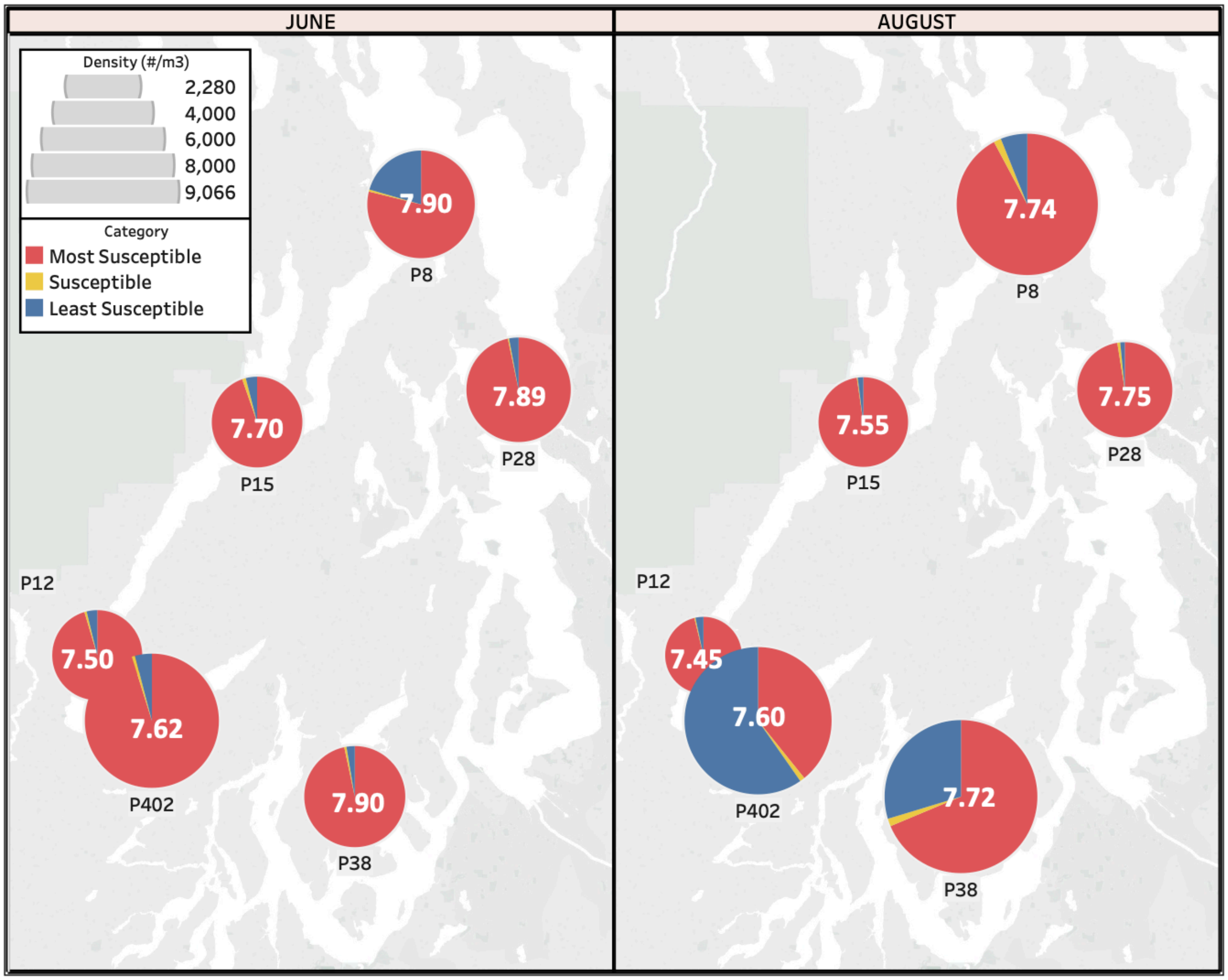


Figure 8. The abundance of zooplankton (individuals/m^3) in June and August 2017 based on estimated levels of susceptibility to ocean acidification. The mean pH for each station is recorded in white in the middle of each pie chart.

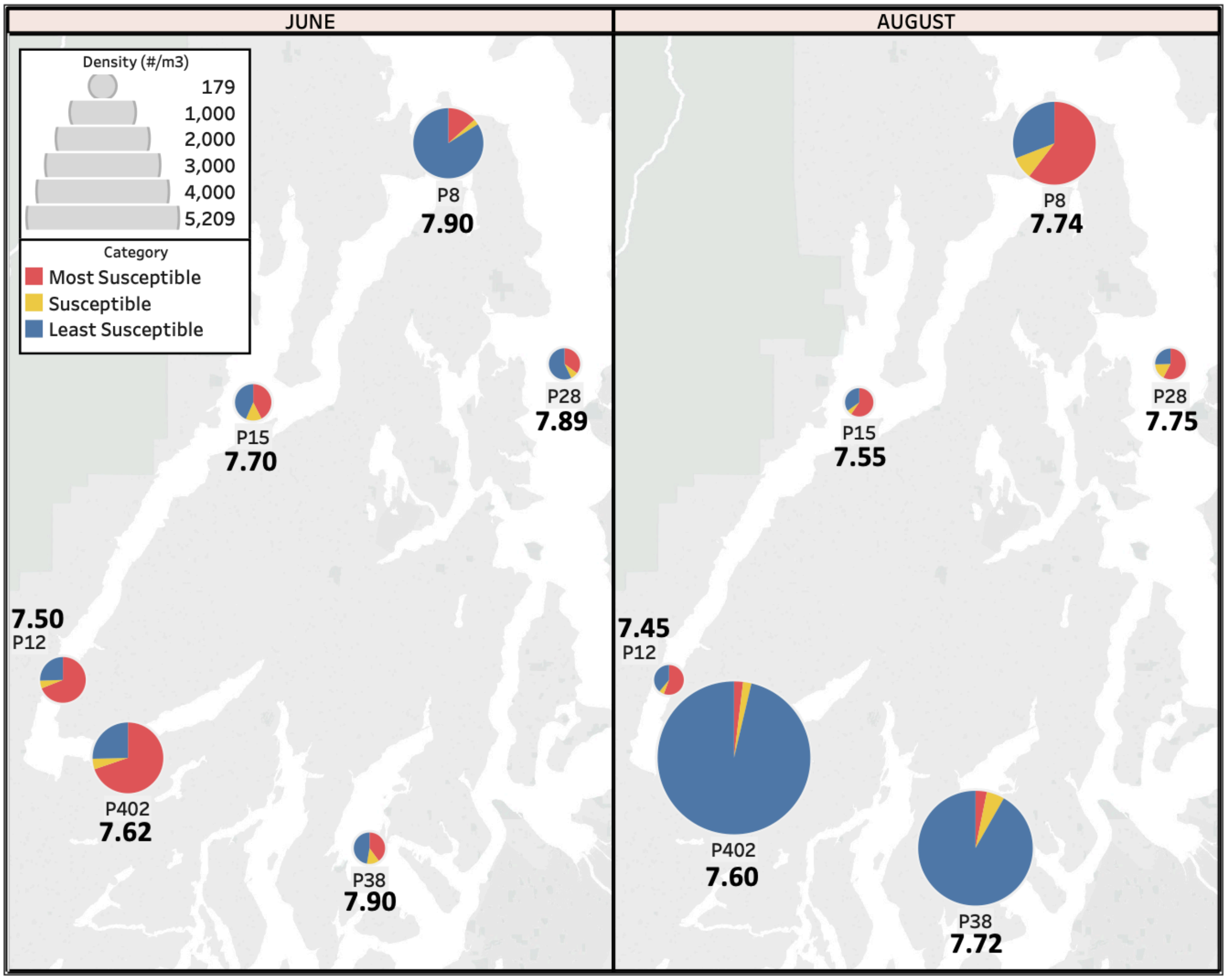


Figure 9. Total zooplankton abundances (individuals/m^3) divided into levels of susceptibility to OA. The mean pH for each station is recorded next to each station name. Copepods are excluded from this figure.

Discussion

Meta-analysis

The results of the meta-analysis reveal that calcification, metabolism, survival, development, reproduction, and growth are all impaired by OA across a variety of zooplankton taxa. Sensitivity varied widely across taxonomic groups, consistent with findings from prior meta-analyses of a broader range of taxa (Kroeker et al. 2013; Busch & McElhany, 2016). Soft, fleshy taxa such as larvaceans and jellyfish were the least susceptible to OA, whereas calcifying organisms such as pteropods and larval bivalves were the most susceptible. Calcification was identified as the process most sensitive to OA, rendering calcified zooplankton highly vulnerable. Note that most of studies used in the meta-analysis used organisms collected from outside of Washington and Oregon waters. This introduces the possibility that the results may not be perfectly representative of the response of local species to OA because of the potential for local population adaptation to conditions.

Moreover, changes in gene expression (not included in this analysis) have the potential to modify individual response to OA on short time-scales in way that are not reflected here. For example, Dineshram et al. (2012) found that Pacific oyster larvae (*Crassostrea gigas)* lost 18% of their expressed gene proteins after four days under a 0.44 unit decrease in pH. The authors suggested that both calcification and cytoskeleton proteins were severely suppressed. Koh et al. (2015) found that in pteropods (*Limacina helicina),* 579 and 469 out of 53121 genes were upregulated and downregulated, respectively, after four days under a 1.7 unit decrease in pH. Additional research on the underlying molecular mechanisms involved in physiological performances of zooplankton is a necessary component in accurately understanding sensitivity to OA.

The results of the meta-analysis contradict the perception that copepods are insensitive to ocean acidification; in fact, my analysis shows them to be the second-most sensitive taxonomic group. This may be due to life stage effects. The two copepod studies included in the meta-analysis consisted of predominantly younger lifestages, instead of adult females which are prominent in the literature. Although the adults may be relatively less sensitive to a variety of OA-related threats, these papers reported high sensitivity among reproductive processes such as egg production rate. However, evidence is limited, and the studies included in the meta-analysis did not analyze the responses of the most common copepod taxa in Puget Sound. Puget Sound copepods, such as the abundant *Pseudocalanus spp.,* are intermittently exposed to low pH and may have adapted to such conditions, with consequences for sensitivity that are not captured in this meta-analysis.

Biological response to changes in carbonate chemistry can be non-linear. For example, Lischka et al. (2011) found that pteropod mortality and shell growth showed nonlinear responses to increasing pCO_2. Consistent with this, sensitivity analysis of the assembled data did indicate a specific range in which zooplankton tolerance to OA is considerably affected. The largest percent increase in negative response occurred between 550-950 µatm and 950-1300 µatm, suggesting the existence of a threshold between these two ranges for some taxa. Due to small sample sizes and assumptions made during extrapolations, additional research is needed to verify where taxon-specific upper limits are exceeded.

For the purposes of this study, I followed the methods of Wittmann and Pörtner (2013) and assumed that responses were linear, extrapolating the available data to help fill data gaps. This could have had the effect of obscuring non-linear responses and underestimating or overestimating sensitivity. Moreover, I prioritized the duration of study over other attributes when selecting representative experiments, which could have eliminated some shorter studies with divergent responses.

Although a crucial component, laboratory studies alone are insufficient to understand susceptibility to OA. Most studies examine species response to abrupt exposure to experimental conditions. Experimental exposures typically do not replicate exposures in the field, which tend to vary over short time scales and change more gradually over long times scales. Consequently, the results of laboratory studies are not fully generalizable to the responses of individuals in nature and more data is needed to make accurate predictions of how taxon will diverge from the sensitivity derived from short-term exposure experiments.

Furthermore, OA does not exist in isolation. Response to OA needs to be evaluated in the context of multiple stressors, including changes in temperatures, DO, eutrophication, etc. To isolate the impacts of OA, I excluded studies addressing multiple stressors, which likely underestimates sensitivity because these stressors have been shown to exacerbate one another (Pörtner & Farrell, 2008).

Field Observations

The results of the linear models tended to agree with the BIOENV model outputs. Fluorescence was positively correlated with the abundance of several taxa and was a main variable explaining correlations between the environmental and biological matrices. Generally, as food availability increases, zooplankton abundance is likely to increase. This was especially true for larvaceans, a taxon highly correlated with fluorescence, that created a large bloom at P402 in August when the mean fluorescence more than doubled from values observed in June.

The results of the linear models were more equivocal for salinity, which had both positive and negative effects on zooplankton abundance. Research suggests that fluctuations in salinity can lead to variable effects on zooplankton communities by altering stratification, acting as a barrier to phytoplankton, and driving phytoplankton community composition (Mousing et al. 2016). Moreover, variations in salinity may have direct effects on zooplankton with small tolerances to certain saline conditions (PSEMP, 2013). Temperature was correlated with both copepods and crabs in the linear models, and was found to be a main driver in the BIOENV model as well. However, bivalve larvae were found to be negatively correlated with maximum pH, which cannot be explained in the context of these analyses. The small sample size may have led to statistical error, and more observations over a longer time could yield different results.

Although the South Hood Canal stations (P12 and P402) were similar in measured physical and chemical properties, their zooplankton communities were dissimilar. This suggests that the measured variables may not be the primary driver of zooplankton changes at these sites, and that the variation might be accounted for by life-cycle timing, predation, human disturbance, speed and direction of currents, or other parameters unexplored in this study.

Although the results of this study suggest that pCO_2 played only a marginal role in determining zooplankton abundance in the field, OA still has the capacity to push an organism over physiological thresholds. Tolerance to other stressors may be reduced under OA conditions. Moreover, small shifts in temperature in the long term have been shown to have significant impacts on organisms, and the same may be true for OA. Longer time series may illuminate finer-scale sensitivity to OA in the field.

Research indicates that zooplankton are already being negatively affected under current OA conditions in Puget Sound, including conditions that were observed in this study. My results, however, did not suggest that zooplankton assemblages were responsive to low pH or waters undersaturated with respect to Ω_{arg}. During this study, zooplankton in Puget Sound experienced a wide range of environmental conditions, including pH ranges of 7.30 to 8.29 and Ω_{arg} values as low as 0.32, and it is possible that impacts are present but were undetectable using this study's sampling design and methods. Moreover, zooplankton may be responding to decreased pH in sublethal ways that are not detectable through analysis of abundance alone, which may be unsurprising since I found survival to be one of the more robust response categories. Furthermore, field observations were able to capture only two snapshots of six stations in a highly dynamic ecosystem. Low statistical power is common among datasets as small as this, and the multicollinearity between oxygen and pH makes distinguishing and interpreting their influence on zooplankton communities challenging.

Caveats

Small sample size in both the meta-analysis and field samples limit the inferences that can be drawn from these data. The results reported here represent preliminary findings that will benefit from further examination that includes larger sample sizes from laboratory and field studies.

Conclusion

This analysis reinforced other studies' findings that taxa do not respond uniformly to conditions associated with OA, and that calcifying organisms have elevated sensitivity to OA (Hendriks et al. 2010). Pteropods and larval bivalves, the only organisms in this study tested for reductions in calcification, scored within the top three most sensitive taxa, consistent with the perception that calcifying species are at a greater disadvantage than non-calcifying species with respect to OA. By identifying the taxa most sensitive to OA and providing more detail on the spatial and temporal distributions of zooplankton, this paper has the potential to assist in management decisions and inform local policy.

A better understanding of taxon-specific sensitivities will contribute to a fuller evaluation of response to changes in multiple interacting stressors that will shape zooplankton communities as climate change intensifies and coastal urban areas grow.

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APPENDIX

Appendix A. The location data of the six PRISM sites in Puget Sound, Washington.

Station	Latitude (N)	Longitude (W)	Basin	Location
P8	47.90	122.61	North Hood Canal	Hood Head
P12	47.43	123.11	South Hood Canal	Hoodsport
P15	47.66	122.86	North Hood Canal	Dabob Bay
P28	47.70	122.45	Main Puget Sound	North of West Point
P38	47.28	122.71	South Puget Sound	Carr Inlet
P402	47.36	123.02	South Hood Canal	Sisters Point

Appendix B. The 25 taxonomic groups and their associated life history stage or classification (if applicable) identified in the 12 samples collected in Puget Sound in June and August of 2017.

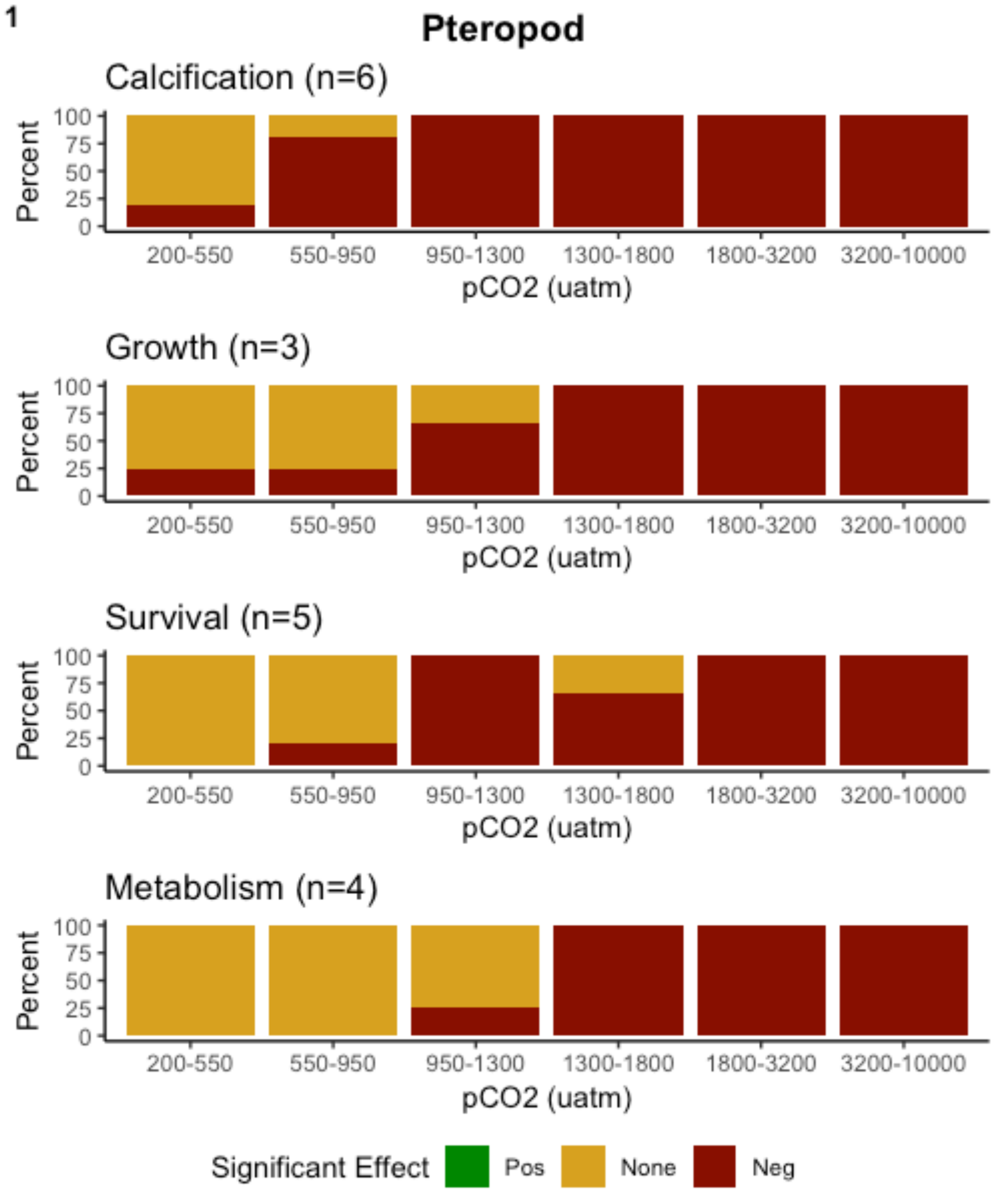
Taxonomic Group	Classification(s)
Amphipoda - Gammarid	N/A
Amphipoda - Hyperiid	N/A
Annelida - Polychaeta	Larva, adult
Bivalvia	Veliger
Bryozoa	Cyphonaut
Chaetognatha	N/A
Chordata - Fish	larva
Chordata – Larvacea	N/A
Cladocera	N/A
Cnidaria - Hydrozoa	Medusa
Cnidaria - Siphonophora	Nectophore, gonophore, bract, medusa
Copepoda	Nauplius, copepodite
Ctenophora	N/A
Decapoda - Brachyura	Megalopa, zoea, juvenile
Echinodermata	larva
Euphausiidae	Nauplius, calpytopis, furcilia, juvenile/adult
Gastropoda – Pteropoda	N/A
Gastropoda - Other	Unknown, veliger
Isopoda	N/A
Maxillopoda - Cirripedia	Nauplius, cyprid larva
Arachnidae	N/A
Mysida	N/A
Ostracoda	N/A
Phoronid	Actinitroch
Shrimp	N/A

Appendix C. Taxa and lifestages for the 39 studies that were included in the sensitivity analysis. When applicable, additional level of specificity is provided for lifestages.

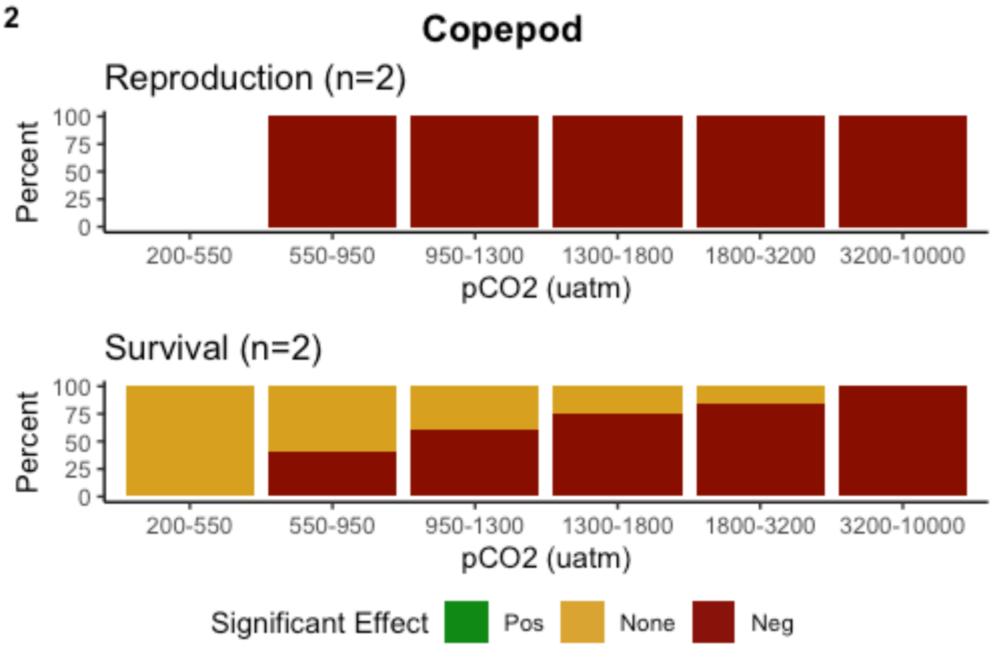
Taxon	Lifestage	Source(s)
Pteropod		
Limacina helicina	Juveniles and adults	1. Comeau et al. 2009 2. Comeau et al. 2009 3. Comeau et al. 2010 4. Lischka et al. 2011 5. Comeau et al. 2012 6. Lischka et al. 2012 7. Busch et al. 2014 8. Maas et al. 2016 9. Bednaršek et al. 2017 10. Lischka et al. 2017
Clione pyramidata	Adults	1. Maas et al. 2012 2. Maas et al. 2016
Copepod		
Acartia tonsa	Eggs, larvae (nauplii, copepodites), adults (male and female)	1. Cripps et al. 2014 2. Aguilera et al. 2016
Acartia clausi	Eggs, adult	1. Zervoudaki et al. 2014
Bivalve		
Mytilus edulis	Eggs, larvae (D-veliger)	1. Gazeau et al. 2010 2. Bechmann et al. 2011
Crassostrea gigans	Spermatozoa, eggs, larvae (D-veliger, umbonate, pediveliger, spat)	1. Havenhand et al. 2009 2. Parker et al. 2010 3. Gazeau et al. 2011 4. Dineshram et al. 2012 5. Timmins-Schiffman et al. 2013
Mytilus californianus	Larvae (veliger)	1. Gaylord et al. 2011
Ostrea lurida	Larvae	1. Hettinger et al. 2012
Shrimp		
Pandalus borealis	Eggs, larvae (S-1, S-2, S-3, S-4)	1. Bechmann et al. 2011 2. Arnberg et al. 2013

Appendix C (continued).

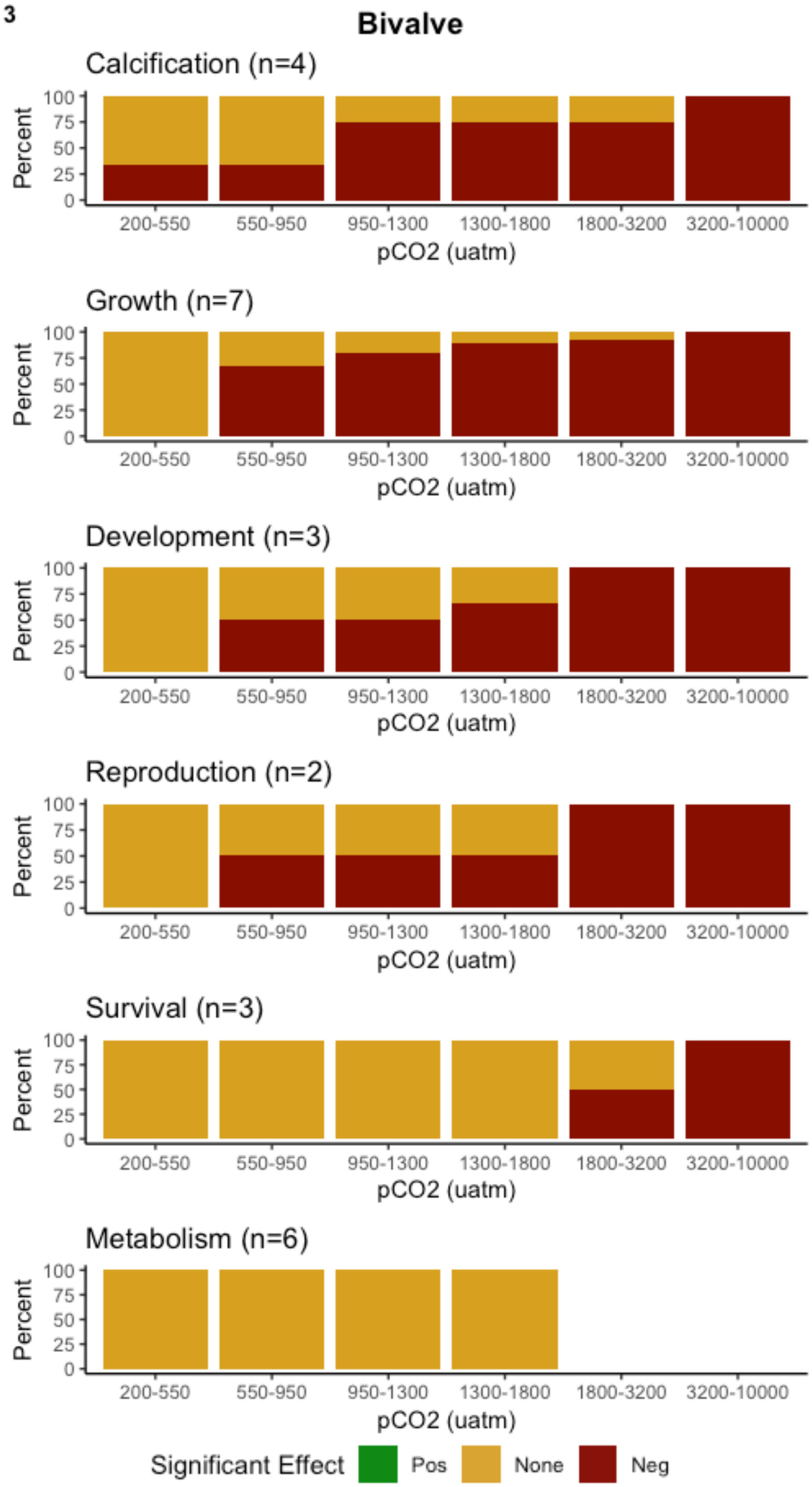
Krill		
Euphausia pacifica	Eggs, Larvae	1. Cooper et al. 2016 2. McLaskey et al. 2016
Thysanoessa inermis	Adults	1. Venello et al. 2018
Crab		
Cancer magister	Eggs, larvae (S-2, S-3, S-4)	1. Miller et al. 2016
Petrolisthes cinctipes	Eggs, larvae (S-1)	1. Carter et al. 2013 2. Ceballos-Osuna et al. 2013 3. Miller et al. 2014
Jellyfish		
Cyanea capillata	Polyps	1. Lesniowski et al. 2015 2. Lesniowski et al. 2015
Aurelia labiata	Polyps	1. Winans and Purcell 2010
Aurelia aurita	Polyps, larvae (ephyrae)	1. Tills et al. 2016 2. Algueró-Muñiz et al. 2016 3. Treible et al. 2018
Larvacean		
Oikopleura dioica	N/A	1. Troedsson et al. 2015



Appendix D-1. Summary of data across response categories for pteropods exhibiting positive (green), no response (yellow), or negative (red) response to elevated levels of pCO_2 (µatm). Data were extrapolated to compensate for missing values. n represents the number of studies included in the analysis.

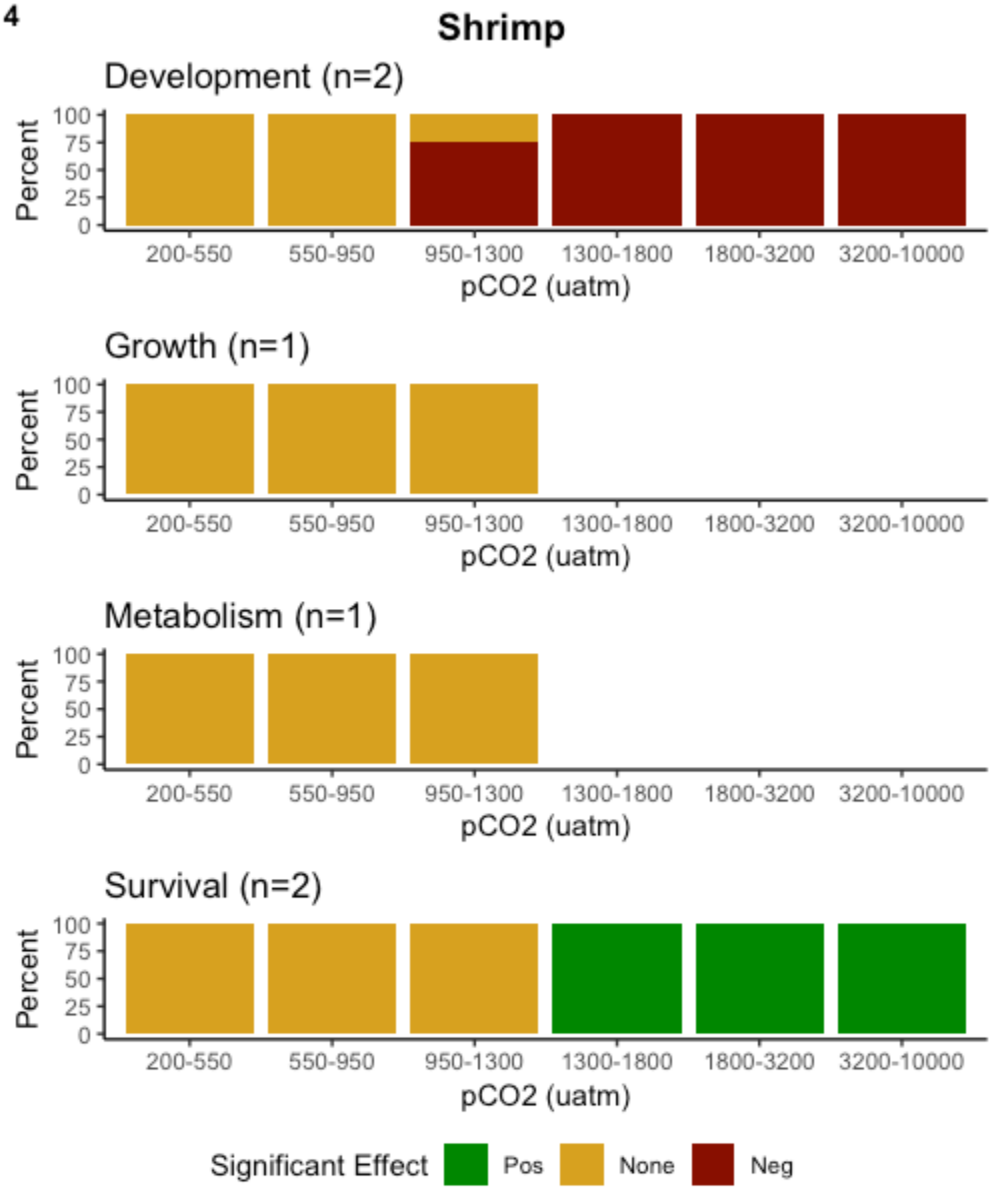


Appendix D-2. Summary of data across response categories for copepods exhibiting positive (green), no response (yellow), or negative (red) response to elevated levels of pCO_2 (µatm). Data were extrapolated to compensate for missing values. n represents the number of studies included in the analysis.

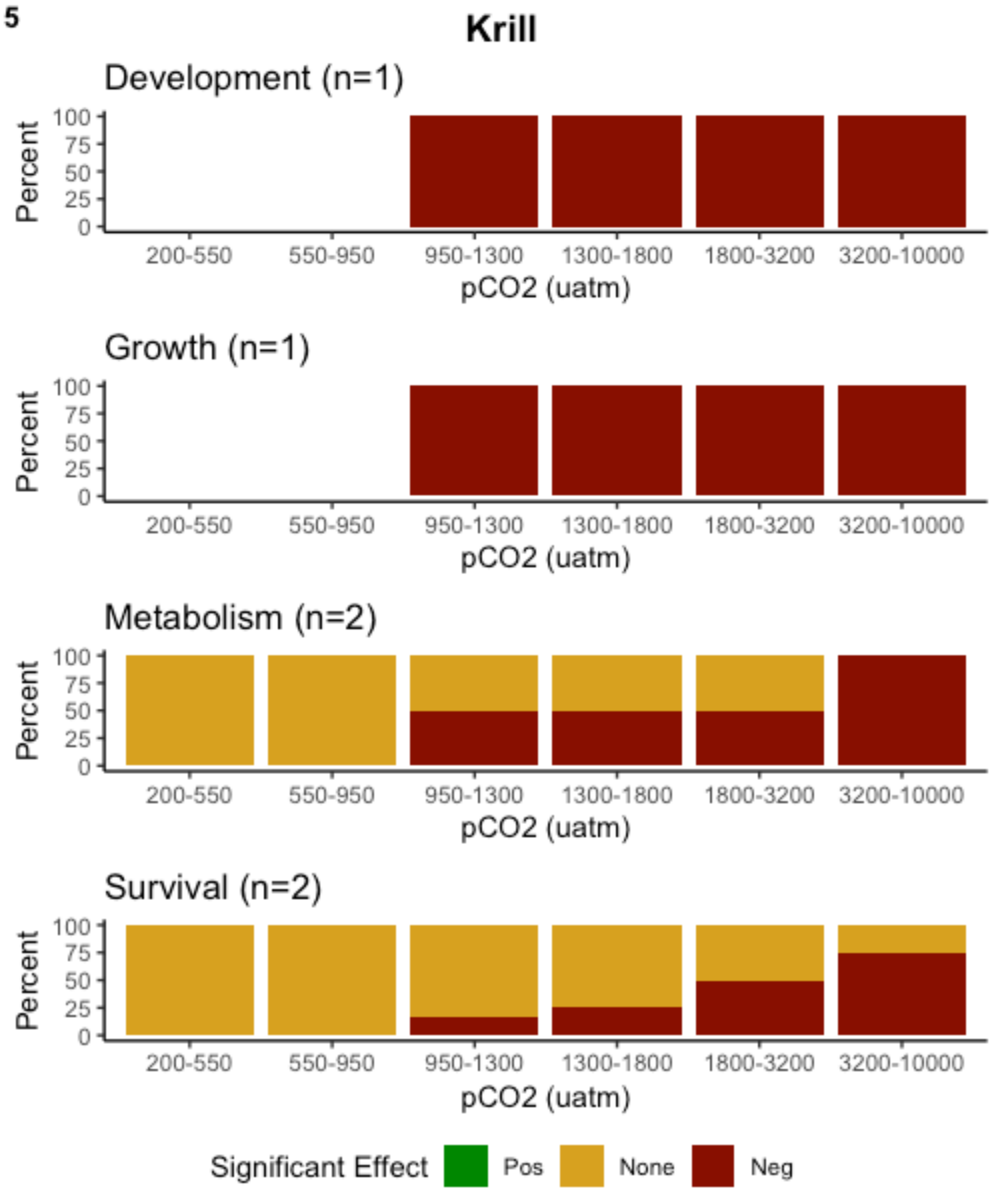


Appendix D-3. Summary of data across response categories for bivalve larvae exhibiting positive (green), no response (yellow), or negative (red) response to elevated levels of pCO_2 (µatm). Data were extrapolated to compensate for missing values. n represents the number of studies included in the analysis.

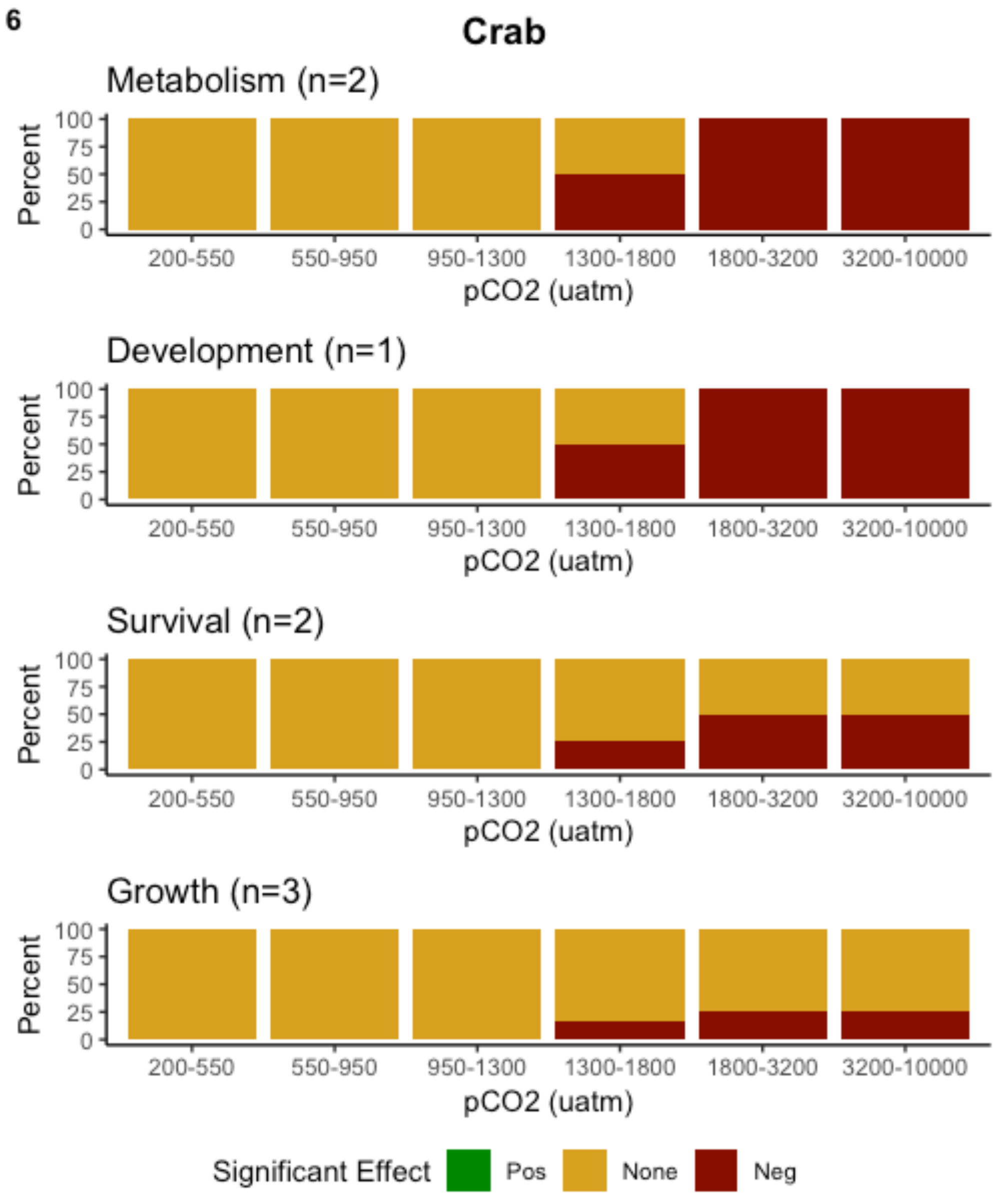
4



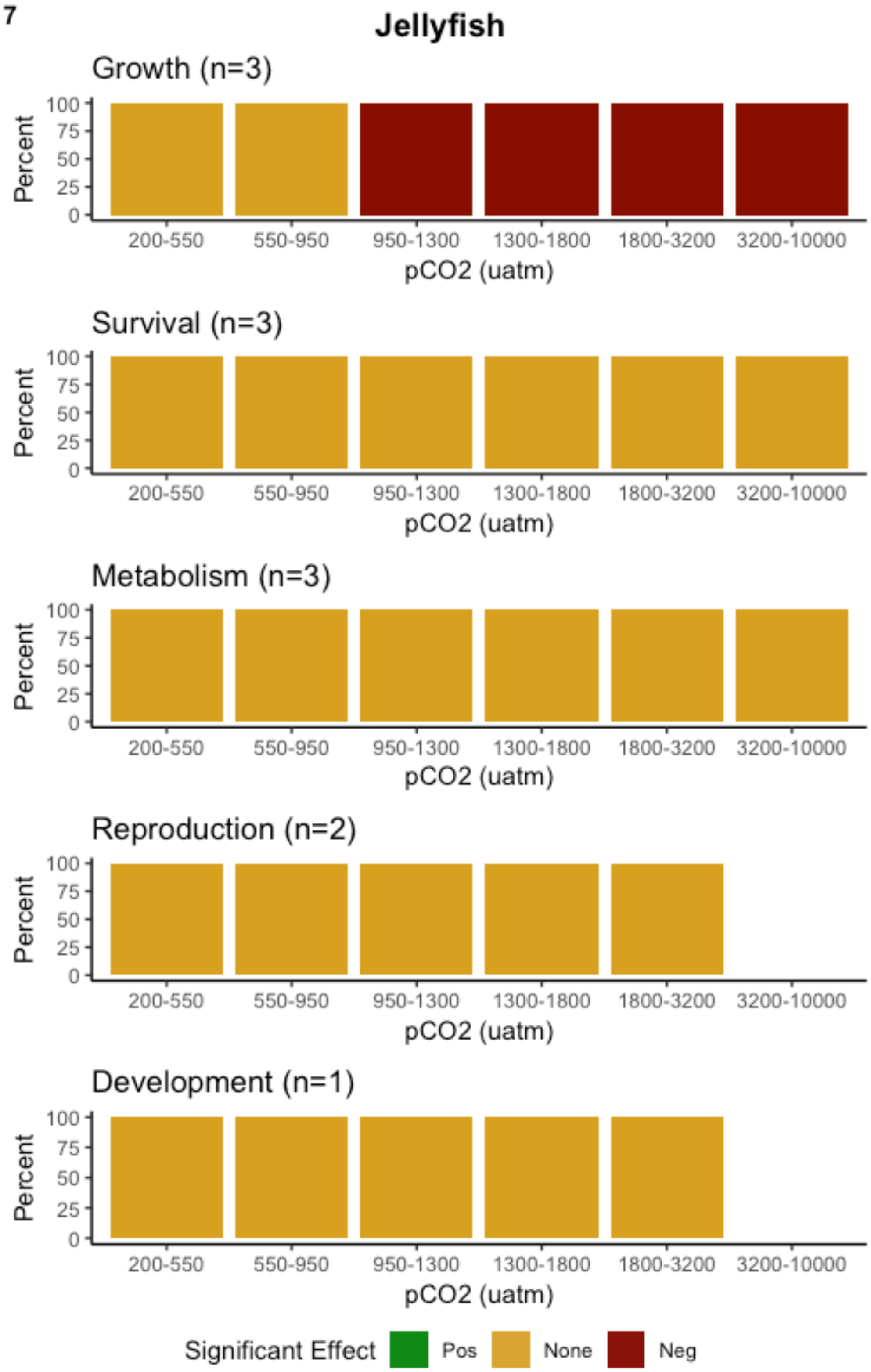
Appendix D-4. Summary of data across response categories for shrimp exhibiting positive (green), no response (yellow), or negative (red) response to elevated levels of pCO_2 (µatm). Data were extrapolated to compensate for missing values. n represents the number of studies included in the analysis.



Appendix D-5. Summary of data across response categories for krill exhibiting positive (green), no response (yellow), or negative (red) response to elevated levels of pCO_2 (μatm). Data were extrapolated to compensate for missing values. n represents the number of studies included in the analysis.

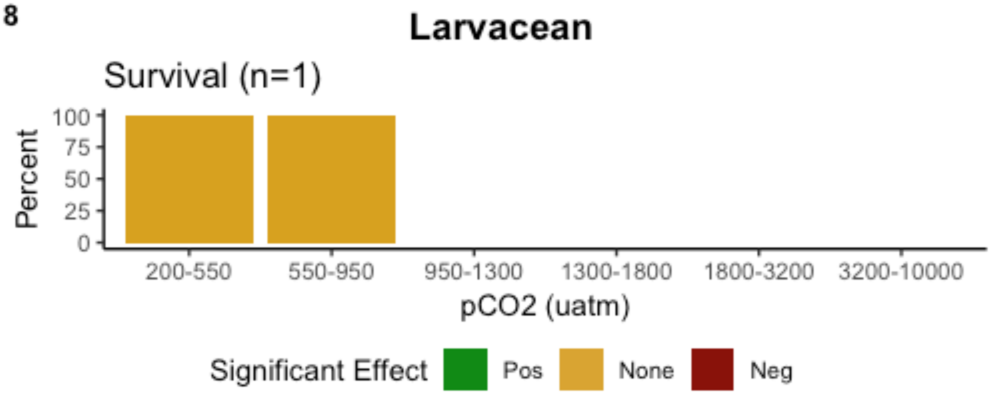


Appendix D-6. Summary of data across response categories for crab larvae exhibiting positive (green), no response (yellow), or negative (red) response to elevated levels of pCO_2 (µatm). Data were extrapolated to compensate for missing values. n represents the number of studies included in the analysis.

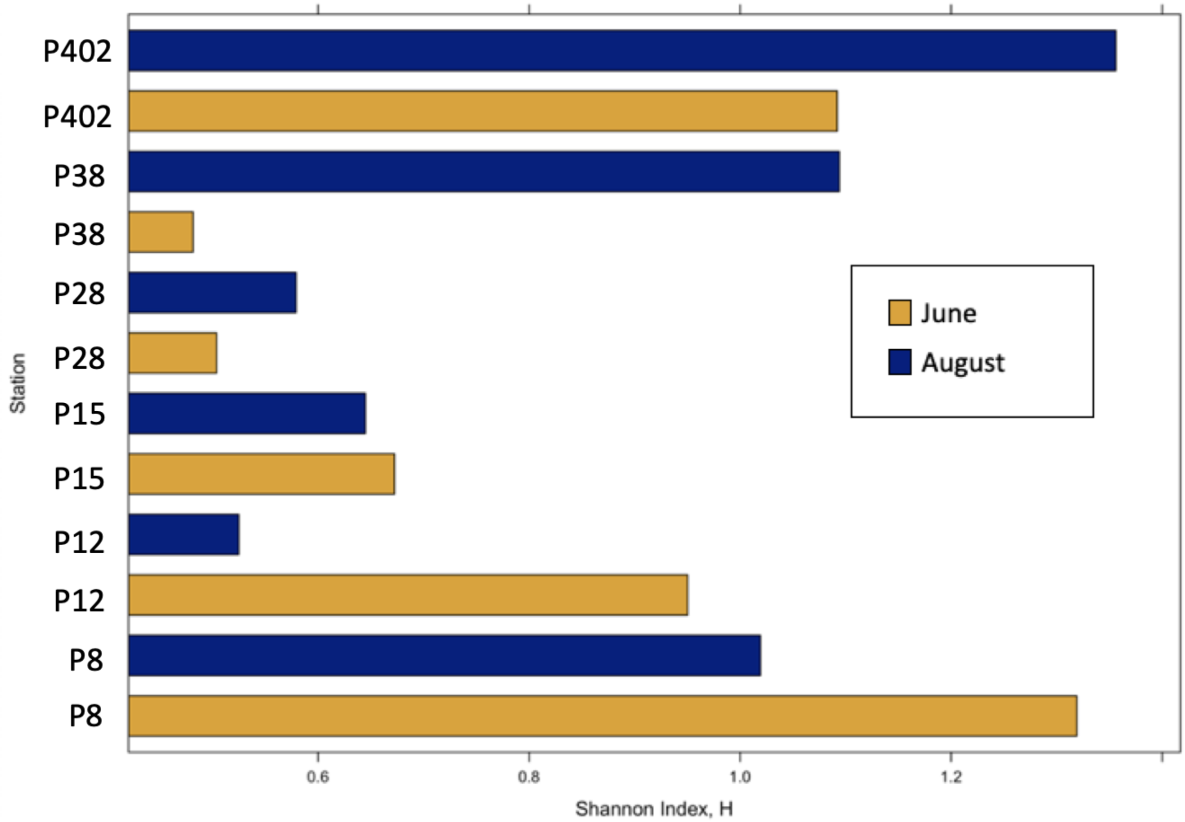


Appendix D-7. Summary of data across response categories for jellyfish exhibiting positive (green), no response (yellow), or negative (red) response to elevated levels of pCO_2 (µatm). Data were extrapolated to compensate for missing values. n represents the number of studies included in the analysis.

8



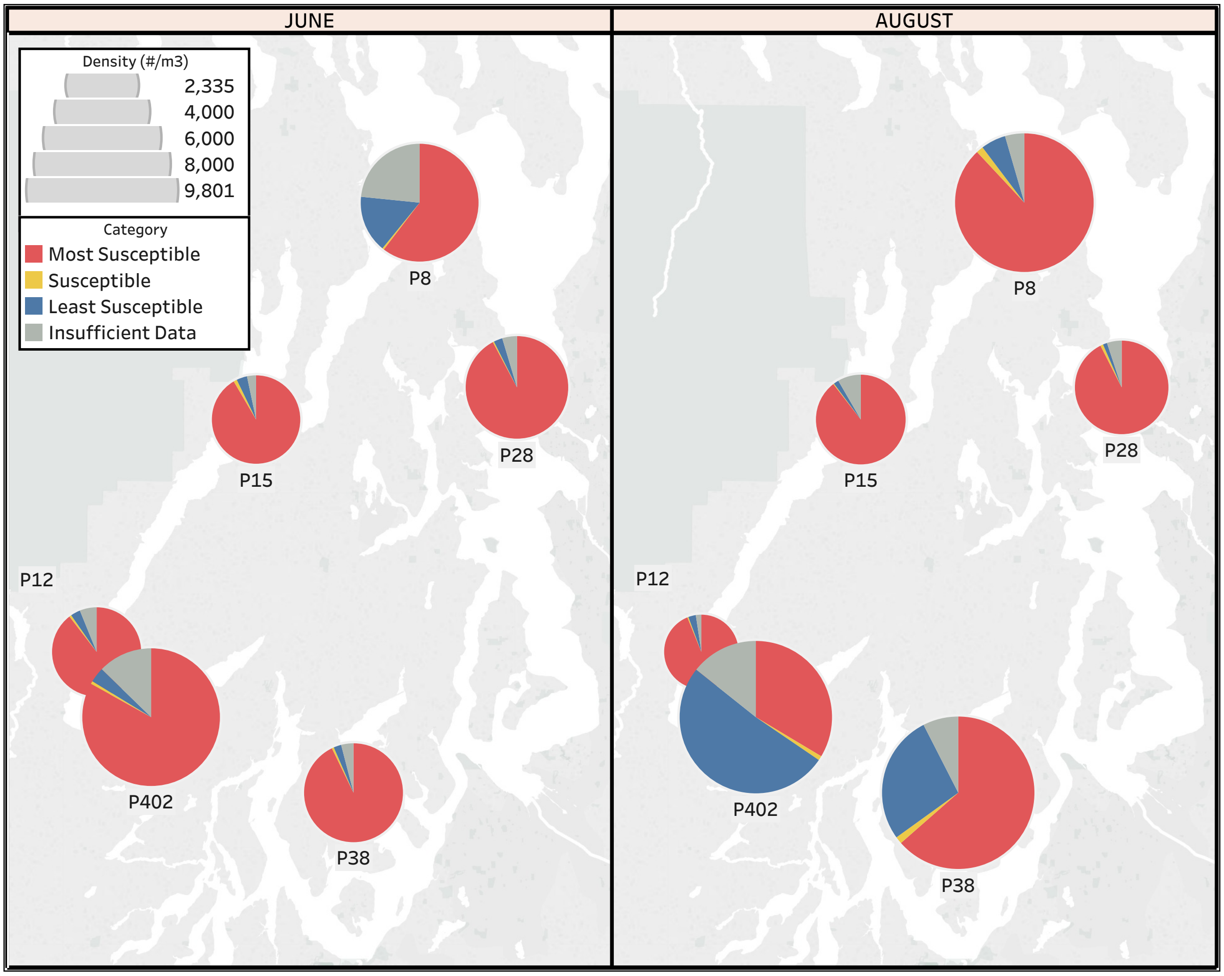
Appendix D-8. Summary of data across response categories for larvaceans exhibiting positive (green), no response (yellow), or negative (red) response to elevated levels of pCO_2 (µatm). Data were extrapolated to compensate for missing values. n represents the number of studies included in the analysis.



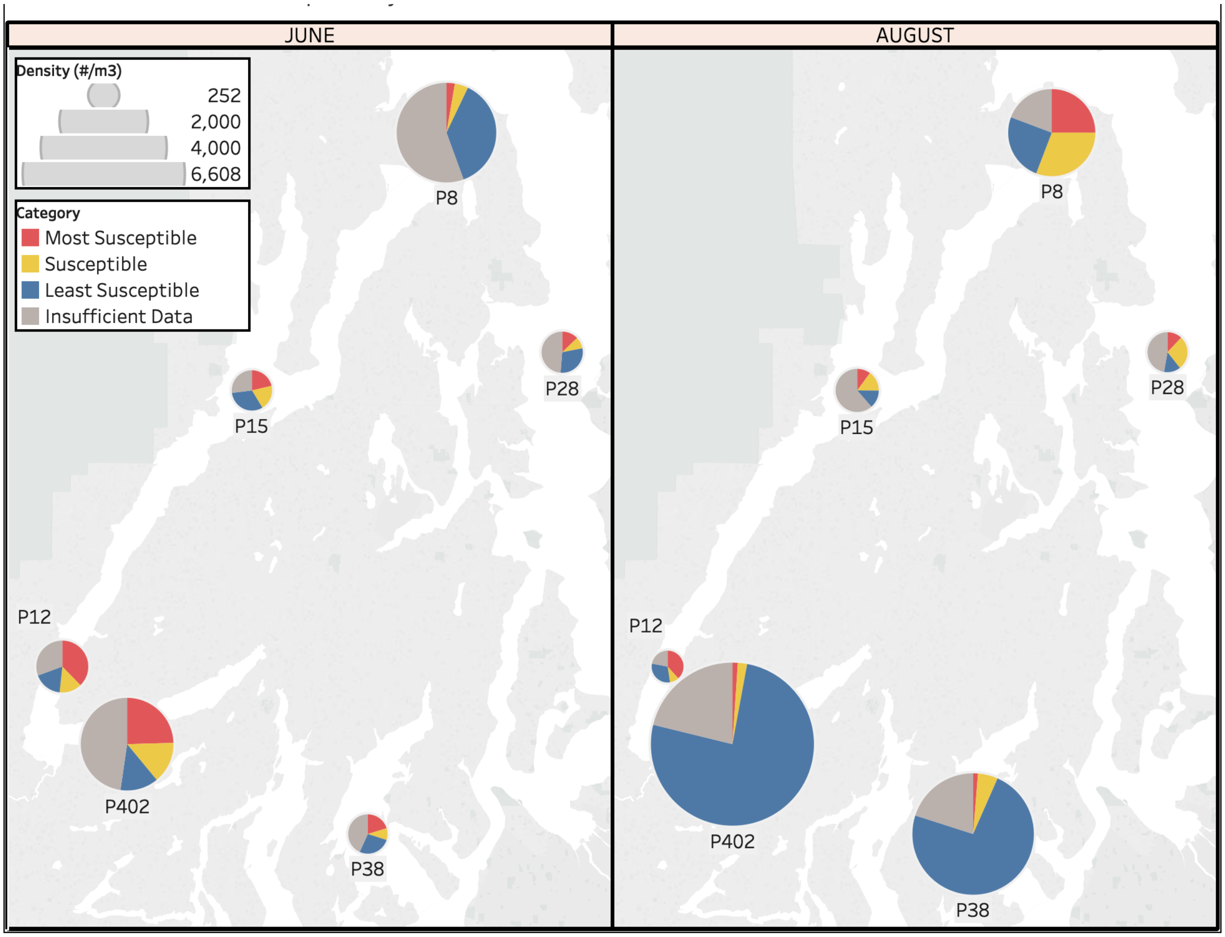
Appendix E. Shannon-Wiener diversity index values for the zooplankton community at each of the six stations over June and August of 2017.

Appendix F. Linear model results displaying only the significant (p<.05) environmental parameters, with their R-squared coefficient. * indicates p-value <0.05, ** indicates p-value <0.01, and *** indicates p-value <0.001. Green boxes indicate a positive correlation, whereas red boxes indicate a negative correlation.

Species	Temp.mean	Temp.max	Salinity.min	Salinity.mean	Salinity.max	DO.max	pH.max	Ω_{arg} max	Fluores.min	Fluores.mean	Fluores.max
Amphipod											
Mysid										0.045	
Bryozoan											
Larvacea										0.481*	
Polychaete										0.797***	
Copepod	0.338*										
Hydrozoan								0.416*			
Chaetognath										0.361*	
Shrimp			0.502**	0.352*							0.469*
Barnacles									0.907***		
Pteropod				0.454*	0.573**		0.494*				
Gastropod (others)			0.471*	0.396*							
Krill											
Bivalve							0.532**	0.452*			
Siphonophore											
Ostrocod											
Crabs		0.533*		0.389*		0.401*	0.398*		0.340*		



Appendix G. The abundance of zooplankton (individual/m^3) in June and August 2017 based on calculated levels of susceptibility to ocean acidification and taxa with insufficient data.



Appendix H. Total zooplankton abundances (individual/m^3) based on calculated levels of susceptibility to ocean acidification and taxa with insufficient data. Copepods are excluded from the most susceptible category to better display the distribution of less abundant taxa.