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 physiology and survival of zooplankton, with differential responses among taxa. To understand how sensitivity to ocean acidification varies among zooplankton taxa, I performed a meta-analysis of the published literature, focusing on eight taxonomic groups of zooplankton found in marine waters of the U.S. Pacific Northwest. According to the meta-analysis, pteropods are the taxon most sensitive to increasing levels of pCO_2 and calcification is the process most severely affected, while larvaceans and metabolism are the least sensitive taxon and process, respectively, examined in this study. I hypothesized that the relative sensitivities to pCO_2 reported in the literature would explain a significant amount of variation in zooplankton

abundance in Puget Sound, WA (USA), where pCO_2 is known to vary across short spatial and temporal scales. To test this hypothesis, I collected zooplankton samples and environmental data on two research cruises in June and August 2017. Copepods, a highly sensitive taxon according to the meta-analysis, were consistently the most abundant taxon, while larvaceans, the least sensitive taxon, reached high abundance in a few samples. 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 the abundance of sensitive taxa as revealed by the meta-analysis, calling into question the coherence between laboratory and field studies and suggesting that sensitivity to existing levels of ocean acidification may play a subordinate role in determining the abundance of some zooplankton taxa in this inland sea. The results of this study have important ramifications for long-term monitoring programs, especially with regard to the utility of measures of abundance as an indicator of response to environmental change.

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Introduction

Zooplankton are well-known indicators of ecosystem health, food web function, and water quality (Beaugrand, 2005) because their short life cycles allow them to respond to seasonal variations and abrupt changes in environmental conditions. While the response of zooplankton to ocean acidification (OA) is understudied compared with many benthic species, their critical importance to marine food webs underscores the need to better understand their sensitivities to changing seawater carbonate chemistry. Moreover, understanding the ecological context in which these responses occur is important, given the co-occurring environmental stressors (e.g, increasing temperature and oxygen stress) to which zooplankton are exposed.

Laboratory studies have shown that low pH and high pCO_2 associated with OA can significantly affect the survival, growth, calcification, development, and reproduction of planktonic organisms (Kroeker et al. 2013; Busch & McElhany, 2016). Sensitivity to current and projected levels of pCO_2 and pH varies substantially among zooplankton taxa. Field studies have established that pteropods and the planktonic stages of bivalves and crabs are sensitive to OA (Bednaršek et al. 2014, Bednaršek et al. 2017, Gimenez et al. 2018, Bednaršek et al. 2020a), presumably due to changes in energy allocation required to regulate pH-dependent biological process (Waldbusser et al. 2013). While laboratory studies can be indicative of zooplankton response to changing pH and pCO_2, *in situ* responses will ultimately determine the fate of individuals and populations. Hence, field studies that examine *in situ* effects are of value.

In the Pacific Northwest region of the U.S., the Salish Sea encompasses the inland waters of the Strait of Georgia, Strait of Juan de Fuca, and Puget Sound. The Salish Sea has known vulnerabilities to low pH and high pCO_2 (Feely et al. 2010; Bianucci et al. 2018; Evans et al. 2019) caused by a combination of factors that include respiration of naturally high levels of

biological productivity (Feely et al. 2010, 2012), relatively long residence times caused by constrained flow between sub-basins and outer coastal waters (Babson et al. 2006; Pawlowicz et al. 2007), seasonal stratification (Evans et al. 2019), and influx of CO_2-enriched waters associated with seasonal upwelling on the outer coast (Feely et. 2008). Contemporary values of pCO_2 in surface waters in parts of the Salish Sea can be as high as 1417 µatm and pH can be as low as 7.5 (Pelletier et al. 2018), with associated aragonite saturation state (Ω_{arg}) routinely less than 1. These conditions are expected to become more severe with time, as the infiltration of anthropogenic CO_2 proceeds and the buffering capacity of seawater declines (Fassbender et al. 2018; Khangaonkar et al. 2018).

Occupying the southern reaches of the Salish Sea, Puget Sound is a semi-enclosed estuarine fjord in Washington State and comprises several sub-basins. Oceanographic circulation between sub-basins is restricted by shallow sills, and populations of rockfish, Dungeness crab, and plankton all show evidence of local population genetic structure (Buonaccorsi et al. 2005; Jackson & O'Malley 2017; Rynearson & Armbrust 2004; Nuwer 2008), suggesting that movement of individuals and advection of holoplankton and meroplankton between basins is limited. The sub-basins vary in oceanographic conditions. For example, the Hood Canal sub-basin tends to be strongly stratified and is characterized by seasonally-severe hypoxia and elevated pCO_2, while the Central Basin is characterized by greater mixing and more moderate levels of dissolved O_2 and pCO_2. These contrasting conditions across a relatively short distance make Puget Sound suitable for studying the influence of OA on structuring zooplankton abundance and community composition.

Zooplankton communities in Puget Sound are spatially and seasonally variable, and are largely composed of copepods, fish larvae, and crab zoea (Kemp & Keister, 2015). While

research on the biological effects of changing carbonate chemistry on Puget Sound zooplankton is limited, diverse responses, such as shell dissolution in pteropods (Bednaršek et al. 2012), impaired development in euphausiids (McLaskey et al. 2016), and reduced survival of crab larvae (Miller et al. 2016) have been reported. Given the conditions to which zooplankton are routinely exposed in Puget Sound, local adaptation to low pH and high pCO_2 could exist, but the extent to which this occurs is not known.

To better understand zooplankton sensitivities to OA conditions in the Salish Sea, I performed a meta-analysis of laboratory studies reporting responses of zooplankton known to occur in Washington waters. I hypothesized that sensitivities as revealed by the meta-analysis would explain a significant fraction of the observed variation in zooplankton abundance across months and locations in Puget Sound. I chose abundance as my response variable because abundance or its co-variate, biomass, are commonly measured in long-term monitoring studies, including those that have been used to detect changes due to ocean condition (e.g., Hays et al. 2005; Mackas & Beaugrand 2010; McKinstry & Campbell 2018). I tested my hypothesis by investigating associations between zooplankton community composition and environmental factors (temperature, dissolved oxygen, chlorophyll a concentration, salinity, aragonite saturation state, and carbonate chemistry parameters) to determine the relative importance of seawater pH to zooplankton community composition in parts of the Salish Sea during the summer of 2017. Comparisons of zooplankton abundance and community composition across spatial gradients in pH can improve our understanding of biological responses to OA and inform expectations of what effects can be observed through the analysis of abundance measures alone.

Methods

Data Collection for Meta-analysis

I used Web of Science and Google Scholar to search the scientific literature for studies of zooplankton species that reported direct responses to carbonate chemistry as an experimental treatment variable. I focused the search on seasonally-abundant, holoplanktonic species known to occur in the nearshore and offshore waters of Washington and Oregon. Meroplanktonic larvae of shellfish, including crabs, were included due to their regional economic and ecological importance. Zooplankton species were categorized into one of eight taxonomic groups: pteropod, copepod, bivalve, shrimp, krill, crab, jellyfish, or larvacean.

Keywords included "ocean acidification", "pH", "carbonate chemistry", taxonomic group of interest (e.g., "pteropod"), and scientific name (e.g., "*Limacina helicina*"). Studies published in peer-reviewed journals over the 10-year period from January 2009 to February 2019 were included in the analysis to constrain the analysis to a reasonable and relevant time period. Data pertaining to indirect effects such as predation, habitat modification, and impacts to food quality were excluded from analysis. Qualifying 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, 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.

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 response to varying pCO_2/pH conditions as positive, negative, or not significant for each of six 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 recorded only if the author reported the change as statistically significant or provided a statistically significant p-value ($p<.05$). If an author did not provide this information, 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. If measures of statistical significance (or lack thereof) could not be discerned, the study was excluded from the analysis. For consistency among studies, ppm CO_2 was equated with pCO_2 because the differences between these two measures were unlikely to substantially influence the results of this analysis.

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 category	**Definition**	**Examples of response variable**
Survival	Measure of an organism's ability to stay alive	Mortality, hatching success, survival rate
Calcification	Biological precipitation of calcium carbonate	Amount of $CaCO_3$ precipitated, shell condition score, calcification rate
Development	Morphological transformation and progression through sequential life stages	Proportion to reach calyptopis I stage, percent stage II zoea, abnormality
Reproduction	Ability of adult organisms to produce offspring; reproductive output	Egg production rate, fertilization, buds produced per polyp
Metabolism	Physiological 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

Sensitivity Analysis

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

I modified the approach of Wittmann and Pörtner (2013) to determine the effect of increasing pCO_2 on major taxonomic groups and to compare sensitivity across groups. I created six bins of pCO_2 based on carbon chemistry conditions currently observed or projected for Puget Sound, Washington based on Busch et al. (2014), as follows: current summer surface water

conditions (200-550 μatm), current deep water conditions or surface conditions during periods of upwelling (550-950 μatm; 950-1300 μatm; 1300-1800 μatm), and surface and deep water conditions projected for the future (1800-3200 μatm). I included a final bin for extreme conditions (3200-10000 μatm) that are rarely observed in the field but capture results from some laboratory experiments.

I cleaned the dataset to eliminate duplicate response categories for each species, reducing the likelihood of over-representation of response categories by including only one experiment per response category per study. To select the representative experiment, I chose the study of the longest duration. If two experiments were of similar duration, I prioritized the experiment with the sharpest response. If there was no significant response or the direction of response was the same for several response variables, then I chose the variable that reported the more synthetic measure (e.g., shell area over shell length for the "growth" response variable). When responses among several life stages were reported for a single taxon, I included data for all life stages reported.

Per the methods of Wittmann and Pörtner (2013), data were interpolated and extrapolated to help compensate for missing values. When a significant response, either positive or negative, was reported at a low level of pCO_2, I extrapolated the same response to higher levels of pCO_2 in the absence of other data. If a response was reported at low and high levels of pCO_2 but no data were reported for intermediate levels of pCO_2, the response was interpolated to the intermediate levels.

I then quantified the percent significant negative response for each bin of pCO_2 for each taxon. I summed responses across all six bins of pCO_2 to estimate a relative sensitivity score for each taxon. If a taxon was positively affected at high pCO_2 (i.e., shrimp), the sum of significant

positive responses across pCO_2 bins was subtracted from the sum of negative responses. The sample sizes for several taxa (krill, copepods, shrimp, and larvaceans) are small, limiting inferences that can be made.

To compare sensitivities estimated from the meta-analysis to observations made *in situ*, I created categories of susceptibility to OA and grouped taxa into each category based their total percent significant negative response across the six bins of pCO_2. Taxa with responses greater than 300%, between 200-300%, and less than 200% were categorized as highly susceptible, susceptible, and least susceptible, respectively. I then applied this result to observational data collected on two cruises to determine whether relative abundance was consistent with sensitivities as revealed by the meta-analysis.

Zooplankton and Environmental Data Collection and Processing

In June and August 2017, I collected zooplankton and characterized water chemistry on two research cruises in Puget Sound, Washington aboard the R/V Clifford A. Barnes. 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 stations and dates were selected to cover a range of physical and chemical properties, including stations where high pCO_2 or low pH have been previously reported (Feely et al. 2010, McLaskey et al. 2016, Pelletier et al. 2018).

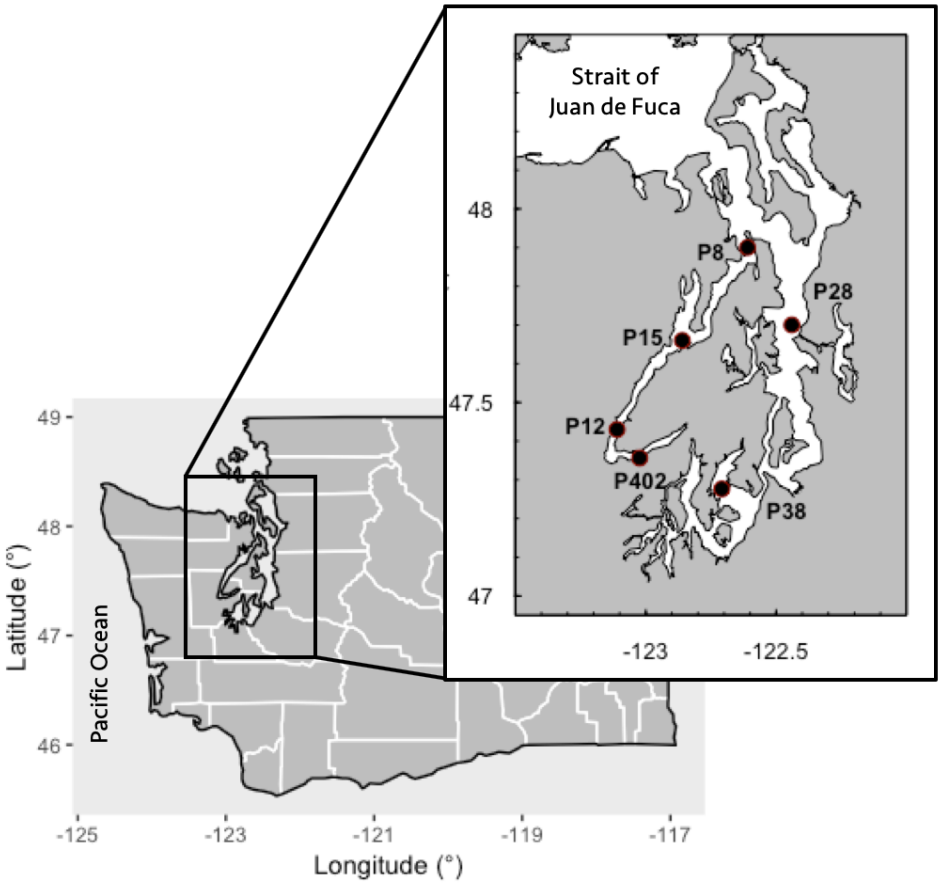

Figure 1. Approximate locations of the six PRISM stations (P8, P12, P15, P28, P38, and P402) sampled in Puget Sound, Washington, USA.

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), fluorometer (WET Labs ECO-AFL/FL), and Niskin bottles to quantify the physical and chemical characteristics of the station. 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). DO was analyzed using the modified Winkler titration method outlined in Carpenter (1965). When comparing the CTD's DO sensor with the Winkler titration samples, I determined that the sensor was correctly calibrated (percent error for low DO samples < 8 mg L-

1 averaged 6.7 ± 5.4 stdev; all samples 5.3 ± 12), so no correction to the profile was made. I estimated phytoplankton biomass by measuring chl a of water collected by Niskin bottles at the surface and fluorescence maximum layer as determined by the CTD. I quantitatively filtered this water onto a GF/F filter, wrapped the filter in foil, and froze it until it could be analyzed on shore (within 4-16 days). In the laboratory, I extracted the chl a in 90% acetone with sonification and measured its fluorescence with a fluorometer (Turner Designs TD-700).

All water samples collected for carbonate chemistry analysis were collected and analyzed according to Dickson et al. (2007). TA was measured by open-cell potentiometric titration and DIC was measured by acidification and quantification using a CO_2 coulometer (UIC model CM5015) at the University of Washington's School of Oceanography, using Certified Reference Materials to verify instrument calibrations. For each CTD cast, I calculated the offset between the CTD pH profile and the discrete bottle samples to create a corrected pH profile that assumes a constant offset with depth. I used the 'seacarb' package in R to calculate pH, Ω_{arg}, and pCO_2 from DIC and TA measurements for each station using constants from Lueker et al. (2000) and the total pH scale.

For all environmental analyses, minimum and maximum pH were calculated from discrete bottle samples and mean pH from the bottle-corrected CTD profile. Minimum and maximum DO were calculated from discrete bottle samples except at P402 in June, which was measured by CTD due to problems with the dosimat and inability to conduct oxygen titrations. Mean DO was calculated from the CTD profile because it did not require correction. Minimum and maximum Ω_{arg} were calculated from DIC and TA measurements. Temperature, salinity, and fluorescence were measured by CTD.

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 during dusk each day. A TSK flow meter was used to measure the volume of water filtered during each net tow. Zooplankton samples were preserved in a 5% buffered formalin and seawater solution and taken back to the laboratory for taxonomic identification.

In the laboratory, I diluted each of the 12 preserved samples 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 group (e.g., shrimp, krill, and chaetognath), and the remaining solution was quantitatively diluted, then subsampled 2-3 times with a 1 mL Stempel pipette. Additionally, one 10 mL Stempel pipette subsample was examined 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 taxonomic levels consistent with those used in the meta-analysis. Copepods were staged as nauplii or copepodites; barnacles as nauplii or cyprids; crabs as zoea or megalopae; and euphausiids as to nauplii, calypotpes, furcilias, or juveniles/adults.

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 performed using the 'princomp' function. Due to analytical requirements associated with small biological sample sizes, the number of environmental factors included in the analysis were

reduced by choosing the most ecologically important variables and eliminating variables for which a strong co-variate was included (e.g., minimum Ω_{arg} was excluded because it co-varies with minimum pH). Following these criteria, 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 per m^3 at each station was calculated from the zooplankton abundance in each sample divided by the volume of water filtered. Taxa that were observed three or fewer times in the laboratory (i.e., isopods, arachnids, ctenophores, and fish) were excluded from analyses.

The Bray-Curtis dissimilarity ordination (R package "vegan": Oksanen et al., 2019) was used to determine spatial and temporal differences in zooplankton community composition and to quantify dissimilarity between stations. To assess the role of more common zooplankton taxa in driving dissimilarity results, biological data were standardized using the Wisconsin double standardization function in the vegan package in R and input a second time into the Bray-Curtis ordination.

To determine whether Puget Sound zooplankton communities were spatially and temporally variable, I performed PERMANOVA tests using the adonis2 function (R package "vegan"; McArdle & Anderson 2001) with the Bray-Curtis dissimilarity index and 999 permutations. A linear model (R package "stats": R Core Team, 2018) was used to identify significant correlations between each taxon and each environmental parameter. As a check 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. Due to small sample sizes, only those 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 (R package "vegan": Clarke & Ainsworth, 1993) was used to estimate the combination of environmental variables most highly correlated with the biological matrix. For each combination, a Spearman's correlation indicated the association between the Bray-Curtis ordination and Euclidean distances. To eliminate covariates, pairwise comparisons, the 'PRcomp' function, and the 'rcorr' function in the Hmisc package in R were used to test for multicollinearity and correlation between environmental parameters. The most ecologically important variables were identified, with minimum and maximum values typically taking precedence over mean values. Minimum and maximum temperature, minimum and maximum pH, maximum Ω_{arg}, mean and maximum DO, minimum fluorescence, maximum chl 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, 36 were included in the sensitivity analysis (Appendix B). The duration of experiments reported in these studies ranged from 1 hour (Miller et al. 2014) to 122 days (Winans et al. 2010), with a mode of 7 days. Not every study reported data pertaining to life stage, 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 species included in the sensitivity analysis, pteropods were the taxon most frequently recorded (32%), while larvaceans were the least frequently recorded (3%). Summed across all taxonomic groups, significant negative responses in representative experiments increased with increasing levels of pCO_2 (Figure 2A). Pteropods and larval bivalves showed negative responses to low levels of pCO_2 (200-550 μatm), whereas shrimp, krill, and jellyfish showed no negative responses below 950-1300 μatm (Figure 2B).

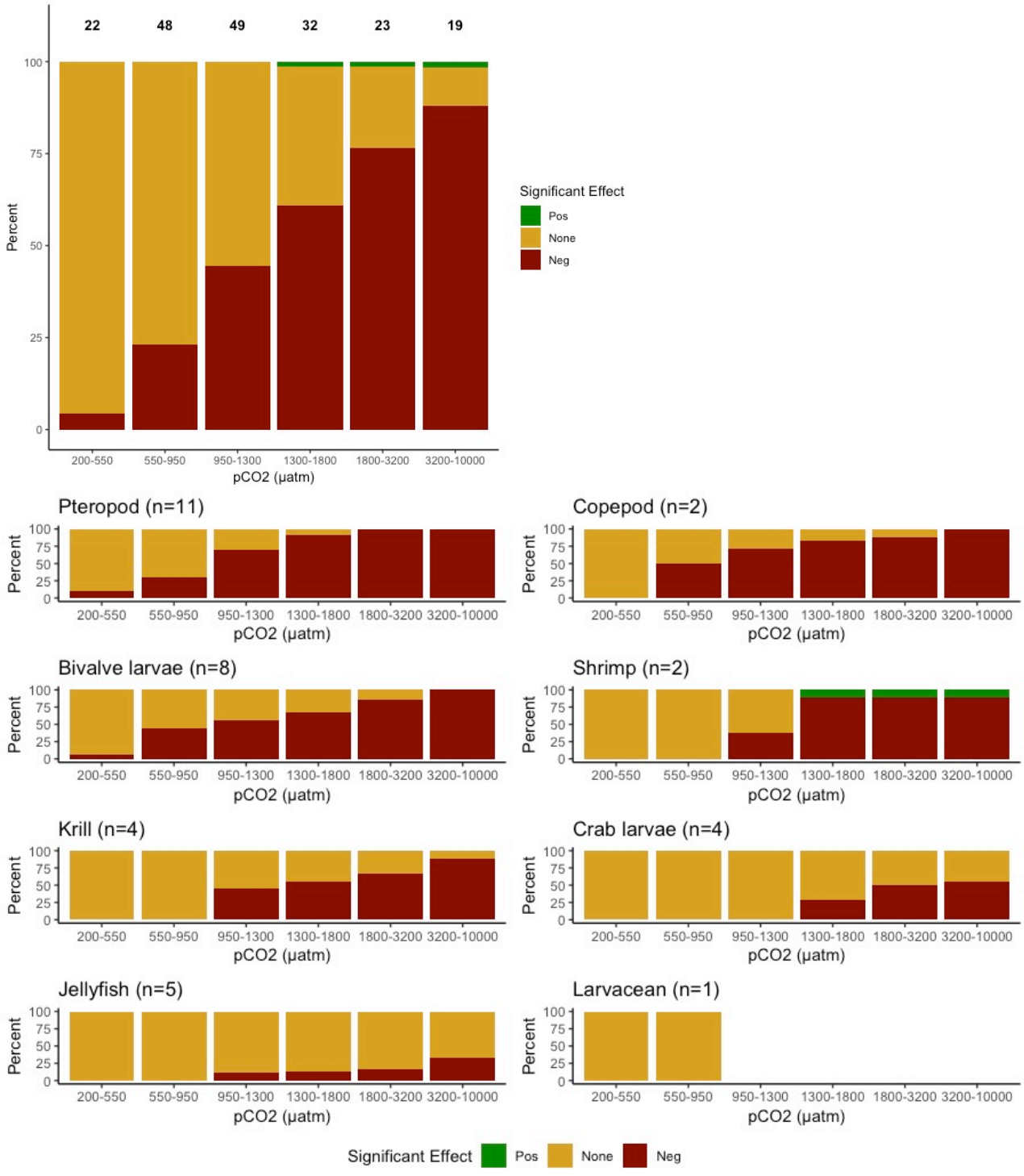

Figure 2A. Percent (%) of representative experiments across all taxonomic groups (pteropod, copepod, bivalve larvae, shrimp, krill, crab larvae, jellyfish, and larvacean) exhibiting positive (green), negative (red), or no significant response (yellow) to elevated levels of pCO_2 (µatm). The number above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

Studies indicate that among zooplankton, pteropods are the most sensitive to OA and are affected at pCO_2 levels as low as 530 µatm. Pteropod calcification, growth, survival, and metabolism are all negatively affected by increasing levels of pCO_2 (Appendix C-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 C-2). Larval bivalve calcification, growth, development, reproduction, and survival were affected, with calcification affected at pCO_2 levels as low as 545 µatm (Appendix C-3). Shrimp were the only taxon to show positive response to OA conditions, with one study reporting an increase in survival after 35 days at 1332 µatm. Shrimp development, however, was negatively affected at pCO_2 levels as low as 1237 µatm (Appendix C-4). Krill development, growth, metabolism, and survival were all negatively affected at pCO_2 levels as low as 956 µatm (Appendix C-5), while crab larvae showed tolerance up to pCO_2 levels of 1361 µatm (Appendix C-6). Jellyfish growth was affected at pCO_2 levels above 1000 µatm, but other response categories showed no effect (Appendix C-7). Larvaceans showed the least sensitivity, with no significant response to the levels of pCO_2 tested over the longest experimental duration (Appendix C-8). Notably, the meta-analysis included only one study of larvaceans.

Pooling data across all taxa revealed that calcification was the response category most sensitive to increasing pCO_2. Approximately 25% of experiments that tested for calcification at pCO_2 levels of 550 µatm or lower reported a significantly negative response. Metabolism was least sensitive, and the only response category unaffected at pCO_2 levels less than 1000 µatm (Figure 3).

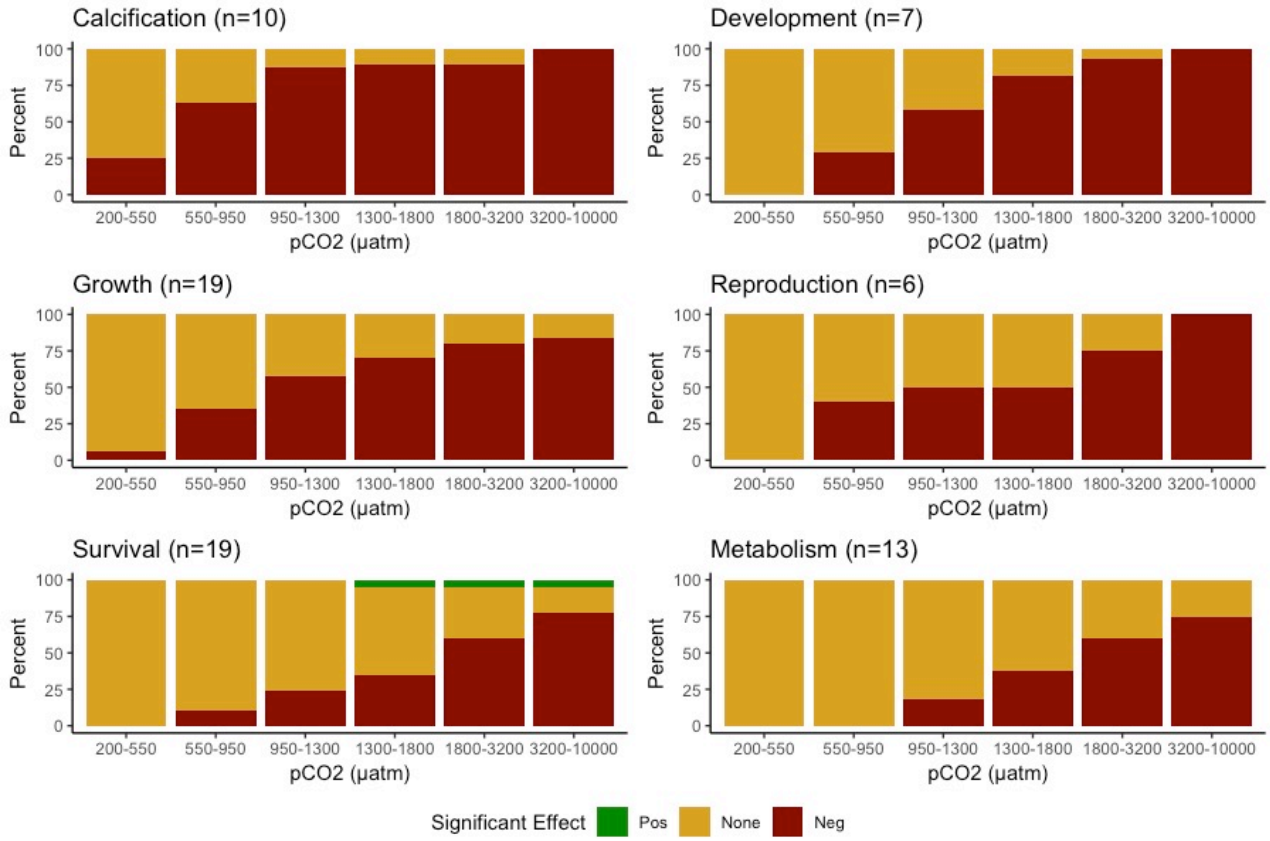

Figure 3. Summary of responses in representative experiments to varying levels of pCO_2 (µatm) pooled across taxonomic groups. N represents the number of studies included in the analysis.

Environmental Observations

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 DO was recorded in South Hood Canal with an average of 4.8 mg/L over the two sampling periods. The lowest recorded DO value was 1.9 mg/L at P12 (South Hood Canal) in August. Bottle-corrected CTD profiles showed lower mean pH values for all stations in August than in June (7.64 and 7.75 respectively), and pH was lower at depth in August than in June. The lowest pH in both June and August was observed at station P12 in South Hood Canal (7.32 and 7.30 respectively). Minimum Ω_{arg} ranged from saturated waters (2.77) at P38 in June to severely undersaturated waters (0.32)

at P12 in August. pH and Ω_{arg} were lower at stations in South Hood Canal than at stations in the other basins (Table 2).

Table 2. Mean (minimum-maximum) of each environmental variable at the six PRISM stations in June and August of 2017. Only minimum and maximum measurements are reported for pCO_2 and Ω_{arg}.

Parameter	Month	P8	P12	P15	P28	P38	P402
Temperature (°C)	June	11.1 (10.8-12.2)	9.8 (9.0-18.2)	10.42 (9.5-18.2)	10.9 (10.4-13.0)	11.8 (11.1-18.3)	10.7 (9.3-19.8)
	August	11.8 (11.3-13.2)	10.2 (9.3-19.6)	11.2 (9.8-18.7)	12.5 (12.2-14.6)	13.6 (13.2-17.2)	10.9 (9.4-20.4)
Salinity (psu)	June	29.5 (28.9-29.7)	29.0 (22.6-29.9)	29.0 (22.8-29.7)	29.1 (28.3-29.6)	28.4 (27.9-28.6)	28.1 (19.3-29.2)
	August	30.64 (29.8-31.0)	29.2 (25.3-29.7)	29.7 (27.1-30.0)	30.0 (29.5-30.4)	29.4 (29.9-29.5)	28.6 (23.5-29.2)
DO (mg/L)	June	7.8 (6.8-8.8)	5.4 (2.5-10.9)	6.2 (4.3-9.8)	7.6 (6.3-11.0)	8.18 (6.8-11.6)	5.0 (3.7-10.8)
	August	6.4 (5.5-7.0)	3.9 (1.9-12.4)	5.0 (3.4-8.5)	6.5 (5.6-8.4)	6.7 (5.3-9.8)	4.7 (2.7-9.9)
Fluorescence (µg/L)	June	3.6 (1.4-11.1)	1.3 (0.0-28.5)	1.2 (0.1-13.7)	0.9 (0.2-11.0)	0.6 (0.2-3.5)	2.3 (0.2-18.0)
	August	0.8 (0.5-4.6)	0.6 (0.1-8.5)	0.7 (0.1-7.6)	0.7 (0.2-5.7)	0.8 (0.2-8.6)	4.8 (0.5-24.7)
pH (total)	June	7.90 (7.85-8.04)	7.50 (7.32-8.02)	7.70 (7.54-8.18)	7.84 (7.78-8.17)	7.90 (7.85-8.29)	7.63 (7.48-8.08)
	August	7.74 (7.70-7.78)	7.45 (7.30-8.17)	7.55 (7.42-8.20)	7.75 (7.74-7.94)	7.73 (7.69-8.06)	7.61 (7.35-8.04)
pCO_2 (µatm)	June	(372-636)	(390-2306)	(244-1354)	(259-764)	(190-620)	(306-1567)
	August	(751-961)	(261-2435)	(237-1827)	(494-850)	(362-963)	(380-2129)
Ω_{arg}	June	(1.13-1.70)	(0.35-1.52)	(0.55-2.10)	(0.95- 2.22)	(1.07-2.77)	(0.45-1.58)
	August	(0.88-1.05)	(0.32-2.15)	(0.44-2.59)	(0.96-1.52)	(0.85-2.02)	(0.34-1.60)

PCA revealed substantial variation in environmental variables across stations and sampling points (Figure 4). Spatial variation among stations 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 Ω_{arg} 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 stations (P12 and P402) are tightly clustered and somewhat distinct from the other stations, which is consistent with basin-specific observations drawn from field-collected data (Feely et al. 2010).

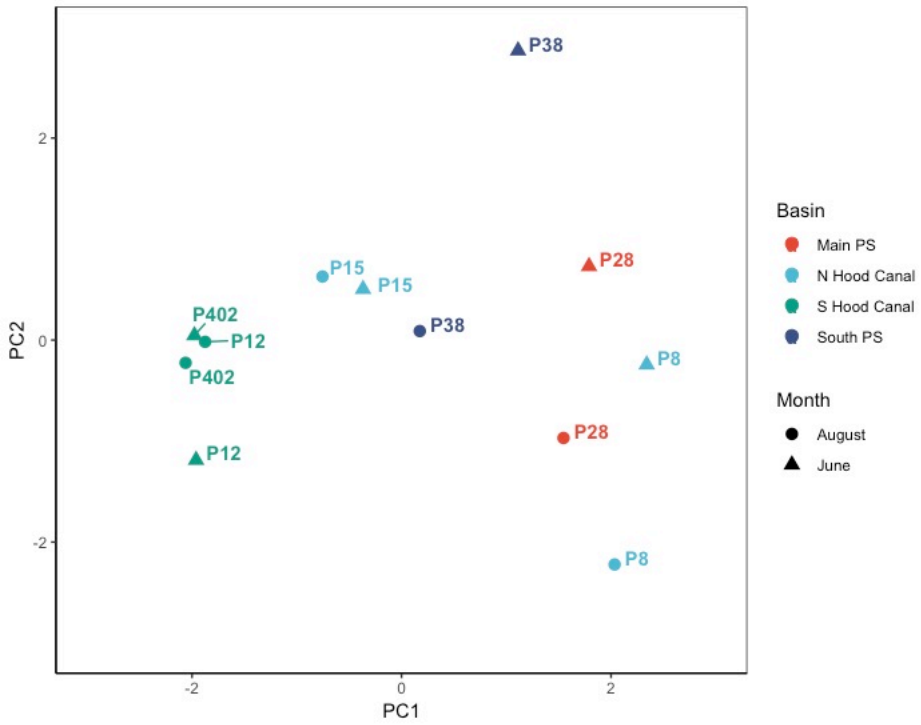

Figure 4. PCA ordination of the six PRISM stations based on six environmental parameters (minimum pH, maximum temperature, mean salinity, mean DO, maximum chl a, and maximum Ω_{arg}) in June and August 2017.

Zooplankton

Laboratory analysis of all 12 vertical-tow samples revealed a total of 25 taxonomic groups of zooplankton (Appendix D). In June, a maximum density of 7,997 individuals/m^3 was recorded at station P402, whereas in August, maximum densities had increased to 9,801 individuals/m^3 at stations P402 and P38. Copepods were abundant across stations, accounting for 70.2% of abundance across all samples. Blooms of larvaceans were observed at two stations in August (P402 and P38), consistent with the increase in total abundance at these stations in August (Figure 5).

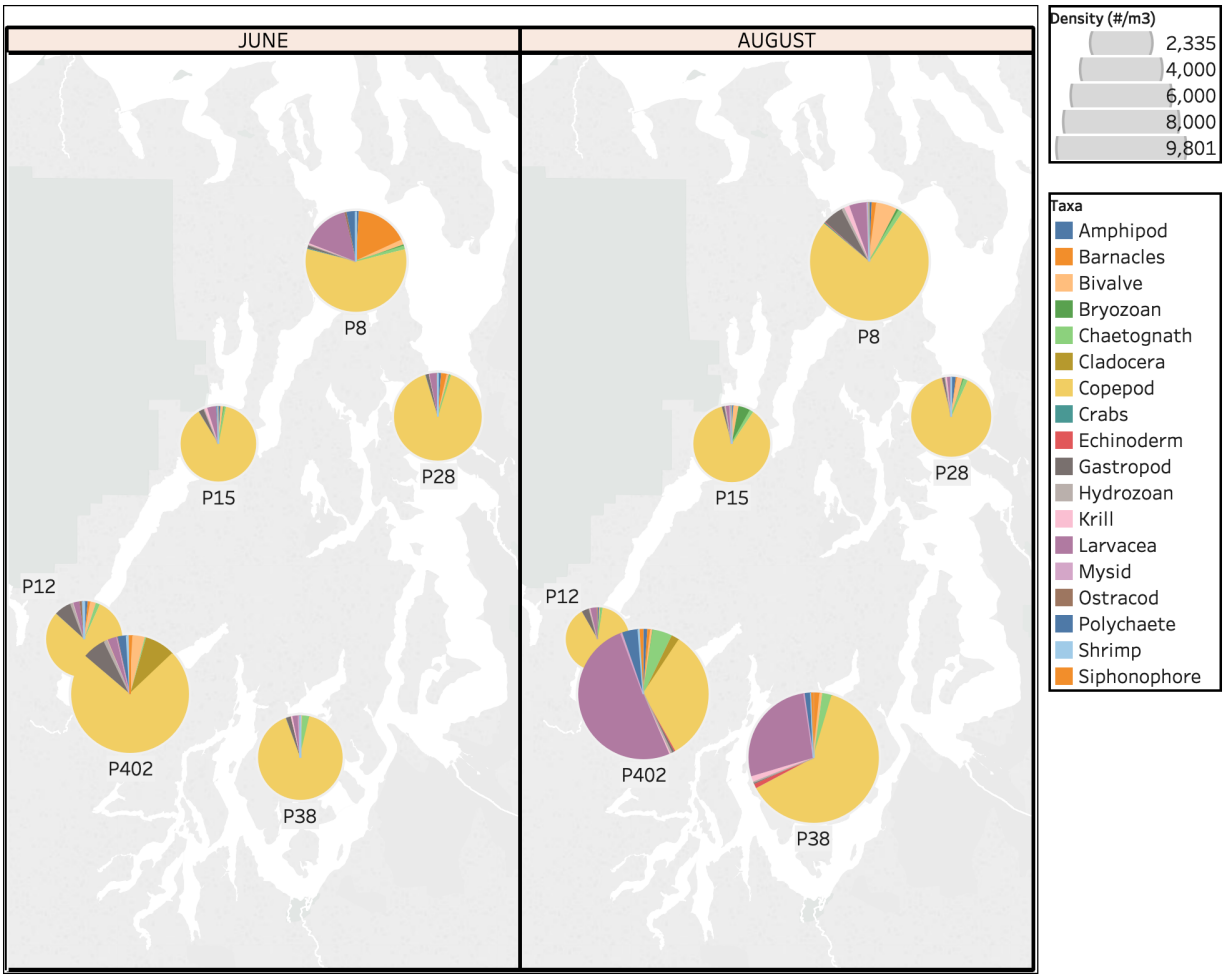

Figure 5. The abundance of zooplankton (individuals/m^3) by broad taxonomic groupings at six stations (P8, P12, P15, P28, P38, P402) in June and August 2017. Fish, isopods, ctenophores, and arachnids were recorded 3 or fewer times and were omitted.

Bray-Curtis Analysis

Bray-Curtis analysis indicated temporal shifts in zooplankton community composition (Figure 6). Station P38 (South Puget Sound) shifted substantially between June and August on the horizontal axis (MDS1), while station P8 (North Hood Canal) and station P402 (South Hood Canal) showed notable shifts on the vertical axis (MDS2) between months. Three other stations (P12, P15, and P28) showed less substantial shifts between June and August. In both June and August, stations in South Hood Canal (P12 and P402) showed substantial dissimilarity on both the horizontal and vertical axes, despite their relatively proximity to each other. Data transformed using the Wisconsin double standardization indicate that shifts in the more common species (e.g., copepods) contributed the majority of the variation to this result. Despite the spatial and temporal shifts indicated by the NMDS plot, PERMANOVA tests based on the Bray-Curtis analysis failed to indicate significant spatial ($F=1.5$, $p=.224$) or temporal ($F=.07$, $p=.551$) variation between stations.

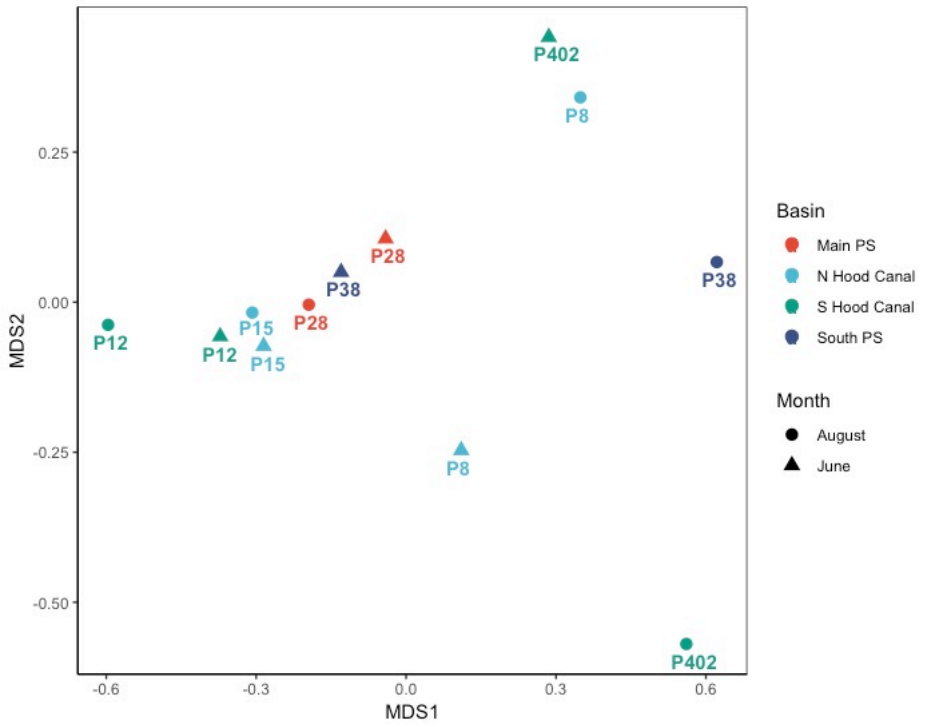

Figure 6. NMDS plot of zooplankton species composition by station and month in Puget Sound based on the Bray-Curtis dissimilarity ordination (stress = 0.04).

Linear Models

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

After removing statistical outliers, I determined that single data points had driven several linear model results. Using the remaining models as the most robust results, they indicated that taxa were most closely associated with different environmental variables (Appendix E). 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, and 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 indicated that temperature, maximum DO, and minimum fluorescence best explain the associations detected by the Bray-Curtis analysis (Table 3). While pH and DO often are correlated, the top two best-fit models identified maximum DO but not maximum pH as an explanatory variable. Other models with a larger number of variables provided less good fit to the data.

Table 3. 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	*0.450*
Temp.min, DO.max, Fluores.min, Salinity.mean	*0.447*
Temp.min, pH.max, DO.max, Fluores.min, Salinity.mean	*0.441*

Comparison of Meta-analysis Results and Field Observations

I used the taxon-specific sensitivities revealed by the meta-analysis to infer a hierarchical range of susceptibilities to pCO_2. Summed across all levels of pCO_2, total percent negative response 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 (for which experimental evidence was quite thin). I grouped taxa into the four categories of

susceptibility (Table 4) and compared the rankings against the abundance of specific taxa collected in the field (Appendices F and G).

Table 4. Susceptibility to OA based on the sensitivity analysis. Taxonomic groups where data were exclusively collected for their planktonic larval life stages are indicated.

Most Susceptible	Gastropods (including pteropods), Copepods, and Bivalves (larvae)
Susceptible	Shrimp and Krill
Least Susceptible	Crabs (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 7). Taxa that the meta-analysis identified as highly susceptible were present at all stations and both sampling 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.

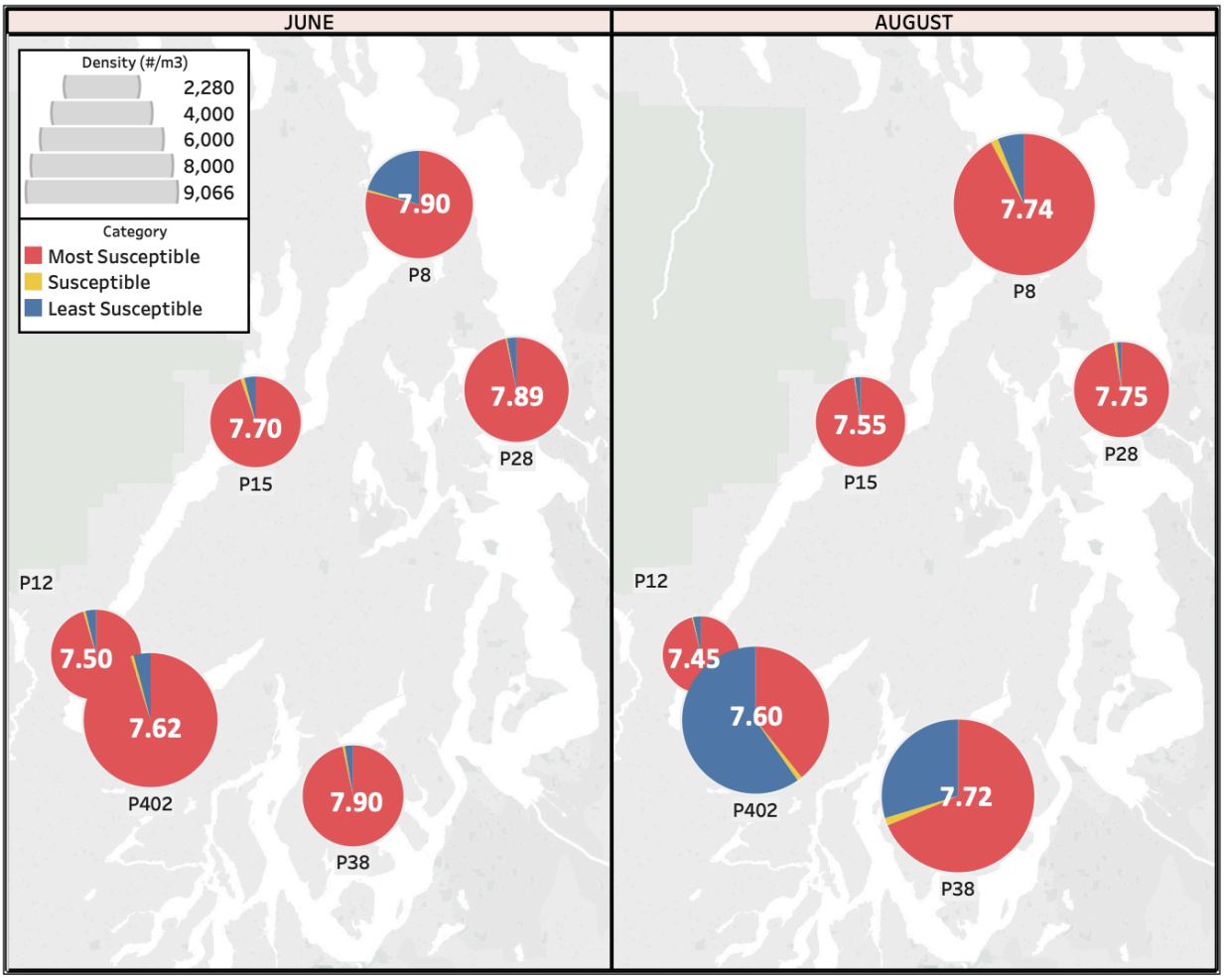

Figure 7. The abundance of zooplankton (individuals/m^3) in June and August 2017 based on estimated levels of susceptibility to ocean acidification. The mean pH for each station is recorded in white in the middle of each pie chart.

Removing copepods from the analysis reveals a different distribution of susceptible taxa across stations and sampling points (Figure 8). Interestingly, stations P12 and P402, both located in South Hood Canal, showed similarities with respect to water chemistry (low DO, low pH, and low Ω_{arg} compared with other stations) but differed in total zooplankton abundance, which was highest at station P402 in both June and August. Overall, I detected no clear association between zooplankton abundance and sensitivity as inferred from meta-analysis.

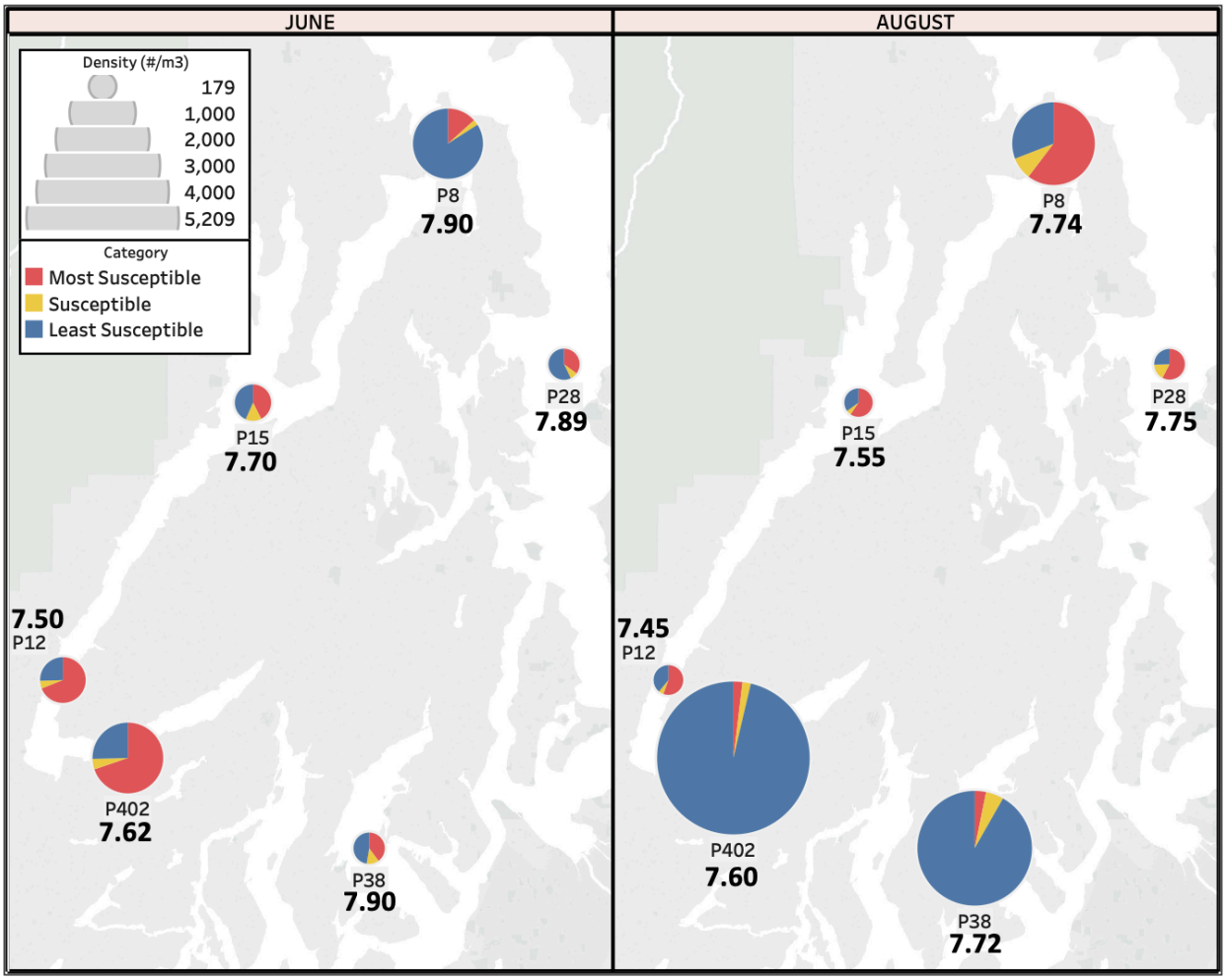

Figure 8. Total zooplankton abundances (individuals/m^3) divided into levels of susceptibility to OA. The mean pH for each station is recorded next to each station name. Copepod abundance is excluded from the most susceptible category (red) in this figure.

Discussion

Meta-analysis

The results of the meta-analysis showed that calcification, metabolism, survival, development, reproduction, and growth across a range of zooplankton taxa are negatively affected by OA. Sensitivity varied widely across taxonomic groups, consistent with findings from prior meta-analyses of a broader range of taxa and functional groups (Kroeker et al. 2013; Busch & McElhany, 2016). According to this meta-analysis, gelatinous taxa such as jellyfish and

larvaceans were the least sensitive to OA, whereas calcifying organisms such as pteropods and larval bivalves were the most sensitive. Unsurprisingly, calcification was the process most sensitive to OA. Note that while the taxa considered in this study all are found in Washington waters, most studies included in the meta-analysis used specimens collected from outside the study area. Consequently, the influence of local adaptation cannot be evaluated, including changes in gene expression that can act on short timescales, on these findings.

The results of the meta-analysis revealed copepods to be among the most sensitive taxa, which is at odds with some published results (Kurihara & Ishimatsu, 2008; Weydmann et al. 2012) but consistent with others (Cripps et al. 2014) and may reflect factors including life stage effects. The copepod literature included in the meta-analysis consisted predominantly of studies on earlier life stages, which may be more sensitive to OA (Lewis et al. 2013, Cripps et al. 2014). Moreover, studies included in the analysis did not test the responses of copepod taxa that are abundant in Puget Sound, where species such as *Pseudocalanus* spp. are intermittently exposed to low pH and may have developed adaptations to such conditions, with consequences for sensitivity that are not captured in this meta-analysis. Nor did the meta-analysis include a recent study, published after this analysis was complete, demonstrating that early life stages of *Calanus pacificus* from Puget Sound are generally tolerant to short-term direct effects of OA (McLaskey et al. 2019). Consequently, the results of this study may overestimate copepod sensitivity in Puget Sound.

Sensitivity of larval crabs to OA, however, may be underestimated in this study. The meta-analysis revealed larval crabs to be in the lowest susceptibility category, but a recent study (Bednarsek et al. 2020a) examined field-collected specimens of Dungeness crab (*Metacarcinus magister*) larvae from Washington and Oregon and found exoskeleton dissolution resulting in

deformities and damaged or missing mechanoreceptors. The field exposures modeled by Bednarsek et al. (2020a) were lower (910 μatm pCO_2 or less) than those used in the laboratory studies I analyzed, which reported crab tolerance up to 1361μatm, suggesting larval crabs may be more sensitive than indicated by laboratory studies.

Notably, biological responses to changes in carbonate chemistry can be non-linear. Lischka et al. (2011) found that pteropod mortality and shell growth showed nonlinear responses to increasing pCO_2. For the purposes of this study, I followed the methods of Wittmann and Pörtner (2013) and assumed that responses were linear, interpolating and extrapolating from the available data to help fill data gaps. This could have had the effect of obscuring non-linear responses and underestimating or overestimating sensitivity.

Laboratory studies alone are insufficient to understand organismal sensitivity to OA *in situ*. Most laboratory studies examine species response to abrupt perturbations, but experimental exposures cannot adequately replicate exposure in the field. Consequently, the results of laboratory studies are not fully generalizable to the responses of individuals in nature. Coupling the results of laboratory studies with field studies can improve understanding of biological response to OA for key species, environmental co-variables, and regions.

Furthermore, OA is only one of multiple factors influencing organismal response and is most realistically evaluated in the context of factors such as temperature, DO, and eutrophication. In this study, however, I purposely excluded from the meta-analysis experiments that included multiple stressors in order to isolate the effects of OA, with the consequence that my findings may underestimate sensitivity to OA among organisms exposed to multiple simultaneous stressors.

Environmental Observations

The linear models and the BIOENV model both identified chlorophyll fluorescence and salinity as leading variables associated with zooplankton abundance. In particular, fluorescence was positively correlated with the abundance of several taxa and was a leading variable explaining correlations between the environmental and biological matrices. Zooplankton abundance tends to be positively associated with primary production (or its correlate, chlorophyll fluorescence), and my observations confirm the importance of primary production to zooplankton abundance in Puget Sound. This was especially the case for larvaceans, which were strongly correlated with fluorescence in my analysis. A large bloom of larvaceans was detected in August at station P402 coincident with mean fluorescence that more than doubled from values observed in June.

The results of the linear models were more equivocal for salinity, which showed both positive and negative effects on zooplankton abundance. In other regions, zooplankton community structure has been found to be strongly correlated with salinity (e.g., Smoot & Hopcroft, 2017), although some have suggested that salinity serves as a descriptor, but not a driver, of zooplankton communities (Helenius et al. 2017). My data do not allow me to discriminate between salinity as a driver versus a descriptor of zooplankton abundance. Linear models indicated that temperature was correlated with the abundance of both copepods and crabs, and this was supported by the BIOENV model, which identified temperature as a leading driver. Curiously, bivalve larvae were found to be negatively correlated with maximum pH, suggesting that other factors outweighed the influence of pH on the abundance of bivalve larvae in my samples. Note that I did not assess the condition of bivalve larvae or any other taxa, so cannot comment on condition as a function of site-specific seawater pH.

Although the South Hood Canal stations (P12 and P402) were similar in the physical and chemical properties I measured, their zooplankton communities differed, especially in the samples from August. Notably, the stations differ in depth (Appendix A). According to results of the linear models, depth could account for some of the differences observed in zooplankton community composition, although depth did not appear as a significant factor in runs of the BIOENV model. Factors that I did not measure, such as life-cycle timing, competition, and predation, could also contribute to this difference.

The results of this study suggest that pCO_2 and its covariate, pH, played a subordinate role in determining zooplankton abundance at these stations on these sampling dates, or that their effects, if present, were not detectable. The strong positive effect of fluorescence on zooplankton abundance is consistent with evidence that food availability is important in mediating organismal response to OA (Thomsen et al. 2013, Pansch et al. 2014, Ramajo et al. 2016). The strong association between temperature and zooplankton abundance could suggest that increasing ocean temperatures coupled with secular trends in OA have the potential to force an organism beyond physiological thresholds, reducing tolerance to OA (Crain et al. 2008; Kroeker et al. 2013). Longer time series that pair chemical and biological observations and distinguish the effects of seawater carbonate chemistry from factors such as temperature and DO can facilitate attribution of biological effects among co-occurring stressors (Doo et al. 2020).

Research indicates that zooplankton are negatively affected by pCO_2 conditions that currently occur in Puget Sound, including conditions that were observed in this study (Barton et al. 2012, McLaskey et al. 2016, Bednaršek et al. 2017, Bednaršek et al. 2020b). This study's results, however, did not suggest that zooplankton assemblages were responsive to low pH or waters undersaturated with respect to Ω_{arg}; if effects were present, they were not detectable by

my methodology. This is remarkable because the mean values of pH observed ranged from 7.5-7.9. For comparison, the change predicted for the surface ocean by the end of this century is 7.75 (IPCC, 2014).

Over the course of this study, zooplankton at the stations sampled 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 negative effects occurred but remained undetected. For example, zooplankton may respond to pH or Ω_{arg} in sublethal ways that are not detectable through analysis of abundance alone. This is consistent with the finding from the meta-analysis that survival is among the least sensitive response categories. Even so, acute or persistent sublethal effects that affect individual fitness should eventually cause changes in abundance, suggesting that sublethal effects, if operative in these populations, were not of sufficient duration or magnitude to cause measurable declines in abundance via changes in individual fitness. This observation is important in the context of long-term monitoring programs that use measures of zooplankton abundance as an indicator of environmental conditions. The strength of inferences made from monitoring data may be limited by the duration, frequency, and intensity of sampling.

The expression of variable life history and behavioral traits likely plays important but unstudied roles in determining differential sensitivity to ocean acidification in natural habitats. Some zooplankton species exhibit behaviors that can influence their ability to adapt to or avoid stressful conditions, such as low pH. Copepods, for example, have been shown to actively adjust their position or motility in response to a variety of stimuli, including turbulent flow (Michalec et al. 2017), light (Martynova & Gordeeva, 2010), pollutants (Michalec et al. 2013), and hypoxia (Keister & Tuttle, 2013), while echinoderm larvae have been shown to alter their swimming behavior to avoid low pH (Maboloc et al. 2020). I used mean water column pH to characterize

pH at each station, and this almost certainly obscured any behavioral responses that might have occurred. Moreover, I cannot estimate the duration of individual exposure to specific conditions of pH or other stressors. While seawater pH varies seasonally in Puget Sound (Pelletier et al. 2018), shorter-term and smaller-scale variations also occur. Variation in the duration of exposure caused by environmental and behavioral factors could partially explain why the meta-analysis revealed copepods to have high sensitivity to ocean acidification but field observations did not. Also, zooplankton species and life stages with stronger vertical migration have been associated with higher tolerance of low pH because they naturally experience a range of pH in the course of daily migrations (Lewis et al. 2013). The decision to identify samples to higher-order taxonomic levels (i.e., not to species or genus levels) allows broad assessment across taxa but constrains the ability to detect species-specific differences in response to environmental conditions, including behavior.

It's important to note, however, that changes in abundance can serve as an indicator of zooplankton response to OA. For example, Smith et al. (2016) used natural gradients in CO_2 to show significant reductions in zooplankton biomass and abundance under high CO_2 conditions. Other taxa have shown similar declines in abundance in response to increasing pCO_2 or acidity (Hall-Spencer et al. 2008, Cigliano et al. 2010, Kroeker et al. 2011). Moreover, measures of abundance are commonly used for zooplankton observations and in time series and have been used extensively to examine zooplankton responses to climate (e.g., Mackas et al. 2007; Peterson et al. 2017). Consequently, abundance is a logical first factor to examine zooplankton response to OA. Significant effects of low pH or high pCO_2 on development, reproduction, and other traits tied to individual fitness, for example, should be reflected in measures of abundance. Zooplankton abundance is a common metric used in long-term environmental monitoring

programs (Mackas et al. 2010) and is used as input to models of food web and ecosystem response to OA (Busch et al 2013, Marshall et al. 2017). Abundance data can also capture shifts in a species center of abundance, informing the directionality and magnitude of responses to climate change (Chivers et al. 2017). Hence, understanding the utility and limitations of abundance measures to broader ecological inquiries is essential to interpretation of empirical studies and to natural resource management. Importantly, the results of this study suggest that the relationship between measures of zooplankton abundance and seawater pH is not simple, emphasizing the utility of intensive sampling over longer time periods to elucidate underlying relationships.

This study's field observations captured only two snapshots of six stations in a highly dynamic system. 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. Interpretation of these results is limited by the existing literature and density of observations made, and thus represent preliminary findings that will benefit from further elaboration that includes larger sample sizes from laboratory and field studies.

In conclusion, I found little correspondence between sensitivities estimated from controlled experiments and the abundance of zooplankton taxa observed in the field. This finding suggests that zooplankton abundance at these stations and times was most responsive to factors other than pH or pCO_2. In field settings, sublethal effects of low pH or high pCO_2 may not be captured by coarse measures of abundance, although such measures are routinely used in long-term monitoring programs. The use of more intensive field sampling, more sensitive measures of

biological response, and more realistic parameterization of laboratory experiments should improve our ability to detect biological response to changing ocean conditions.

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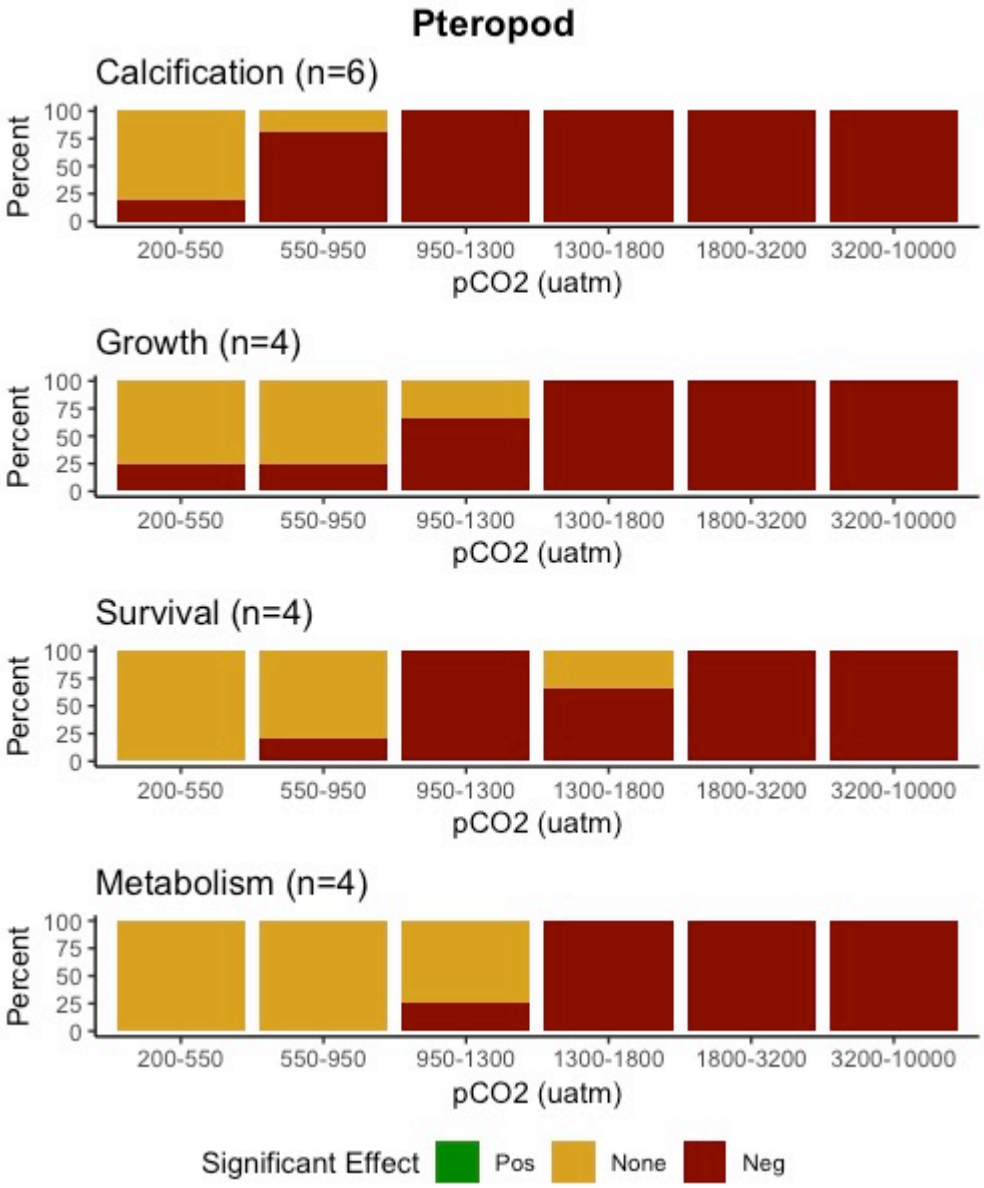
APPENDIX

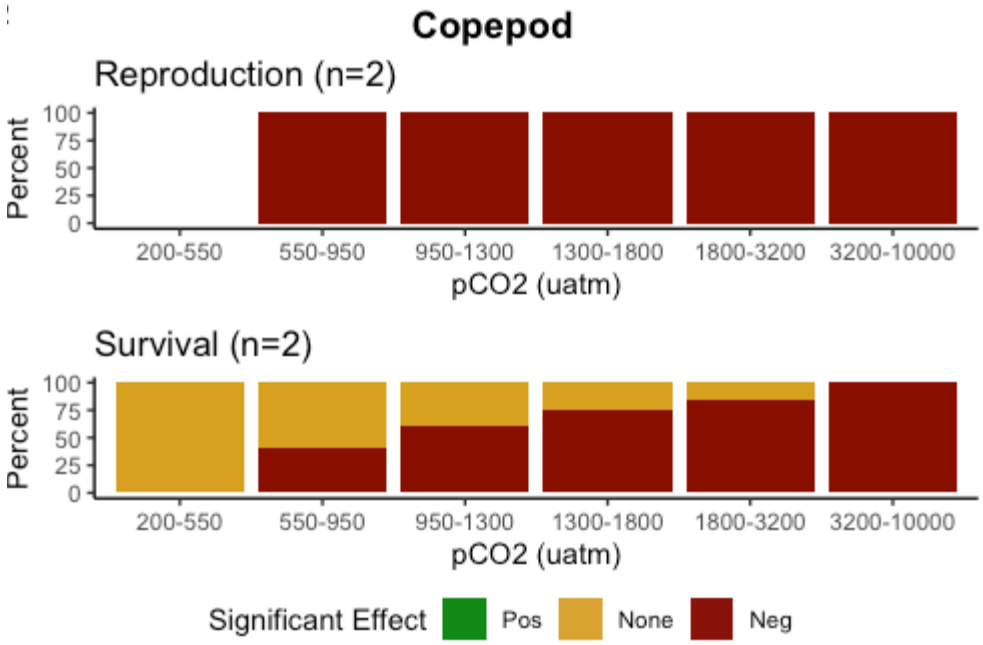
Appendix A. The location and approximate depth of the six Puget Sound Regional Synthesis Model (PRISM) stations sampled in Puget Sound, Washington, USA.

Station	Latitude (N)	Longitude (W)	Approximate depth (m)	Basin	Location
P8	47.90	122.61	133	North Hood Canal	Hood Head
P12	47.43	123.11	125	South Hood Canal	Hoodsport
P15	47.66	122.86	132	North Hood Canal	Brinnon
P28	47.70	122.45	165	Central Puget Sound	North of West Point
P38	47.28	122.71	103	South Puget Sound	Carr Inlet
P402	47.36	123.02	53	South Hood Canal	Sisters Point

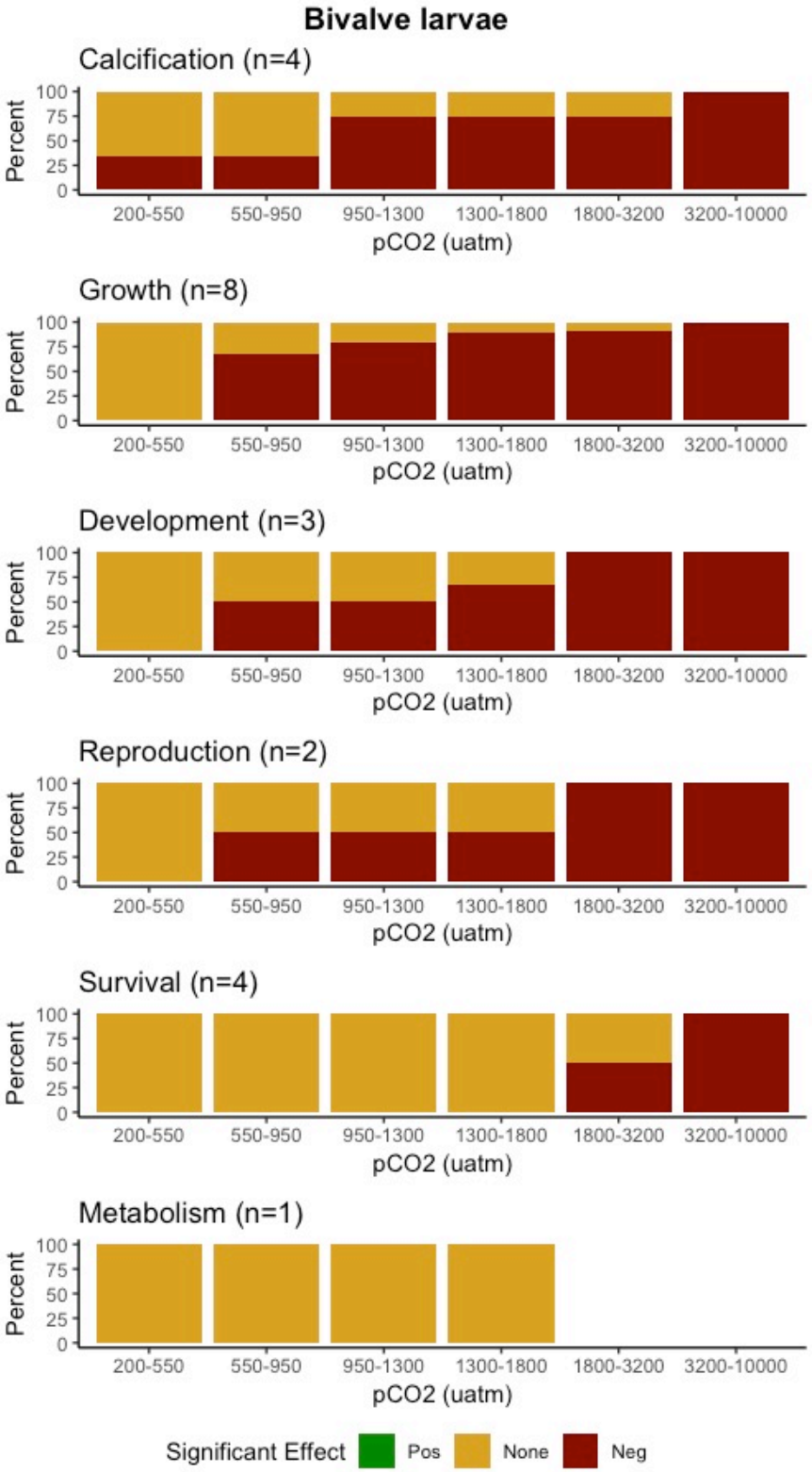
Appendix B. Studies included in the sensitivity analysis.



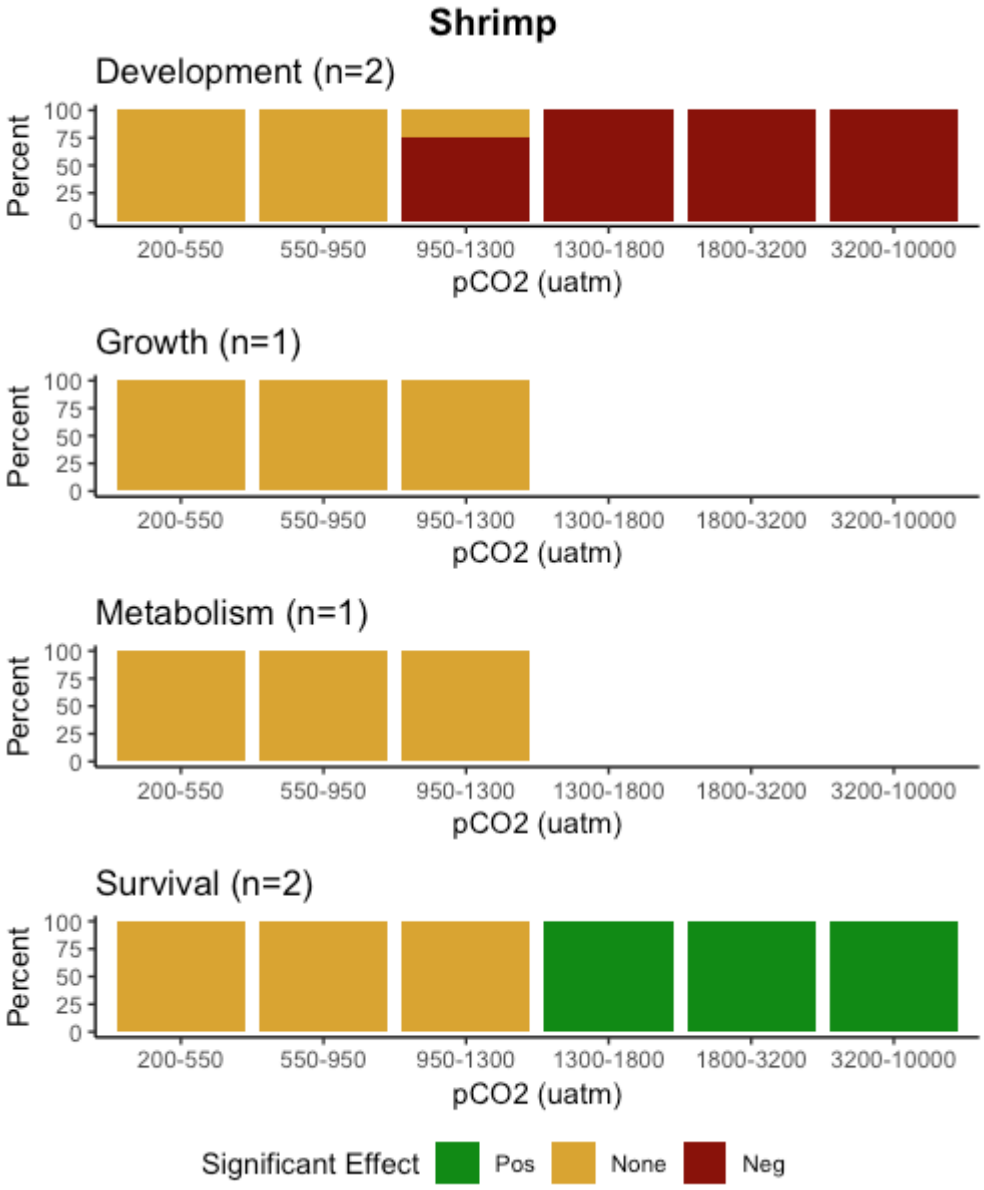
Appendix C-1. Percent (%) of representative experiments across response categories for pteropods exhibiting positive (green), no response (yellow), or negative (red) response to elevated levels of pCO_2 (µatm). Data were interpolated and extrapolated to compensate for missing values. n represents the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



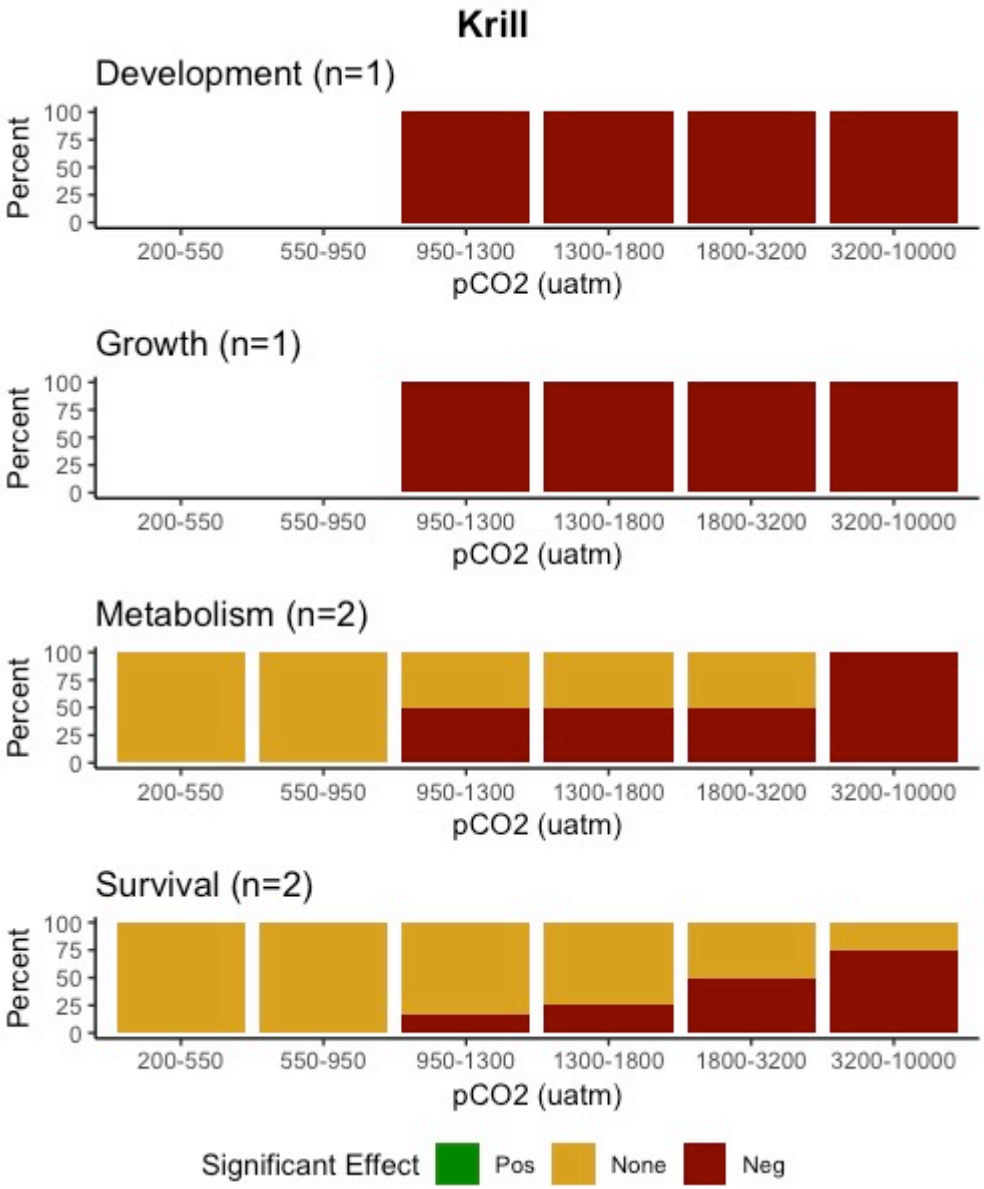
Appendix C-2. Percent (%) of representative experiments across response categories for copepods exhibiting positive (green), no response (yellow), or negative (red) response to elevated levels of pCO_2 (µatm). Data were interpolated and extrapolated to compensate for missing values. n represents the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



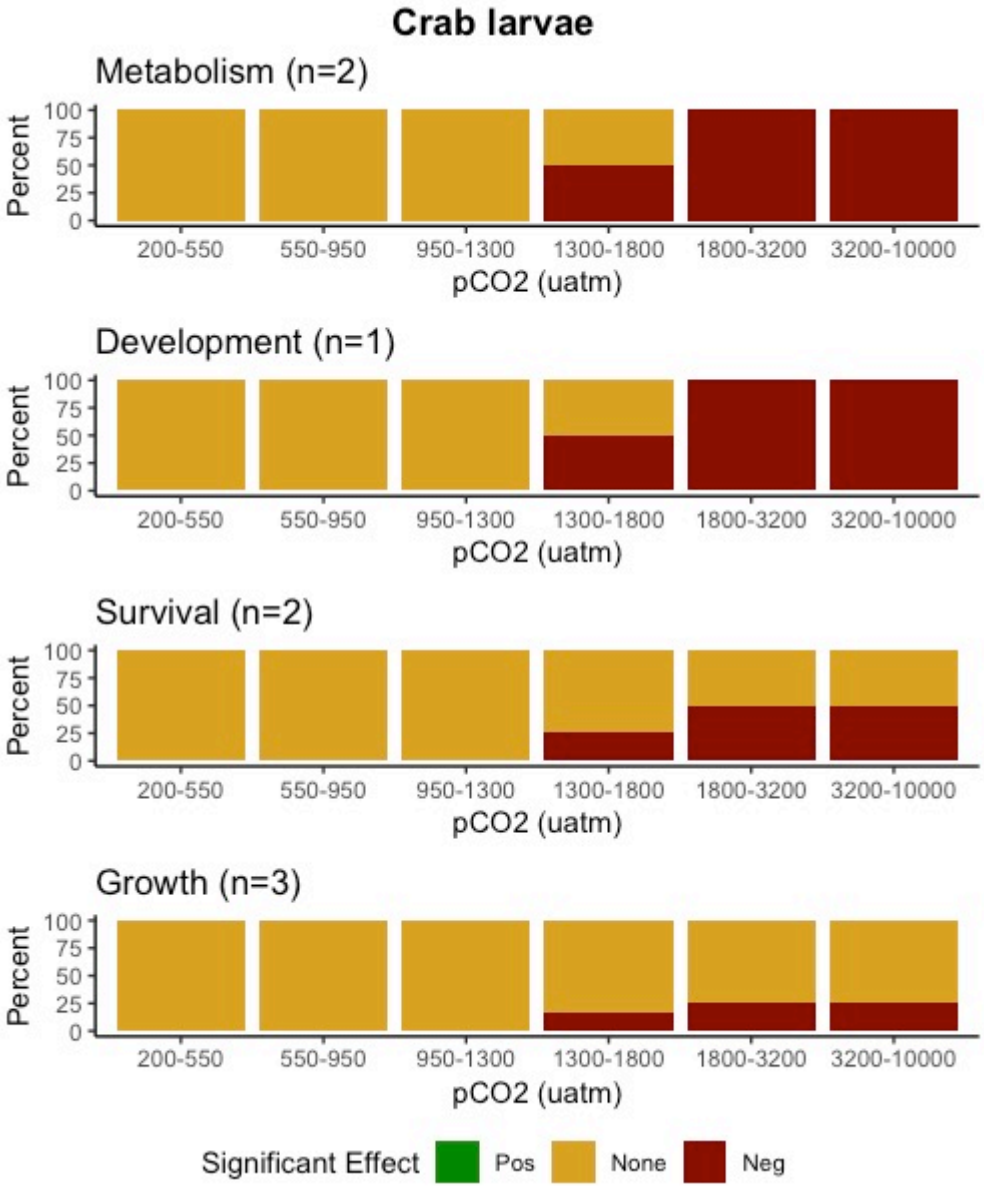
Appendix C-3. Percent (%) of representative experiments across response categories for bivalve larvae exhibiting positive (green), no response (yellow), or negative (red) response to elevated levels of pCO_2 (µatm). Data were interpolated and extrapolated to compensate for missing values. n represents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



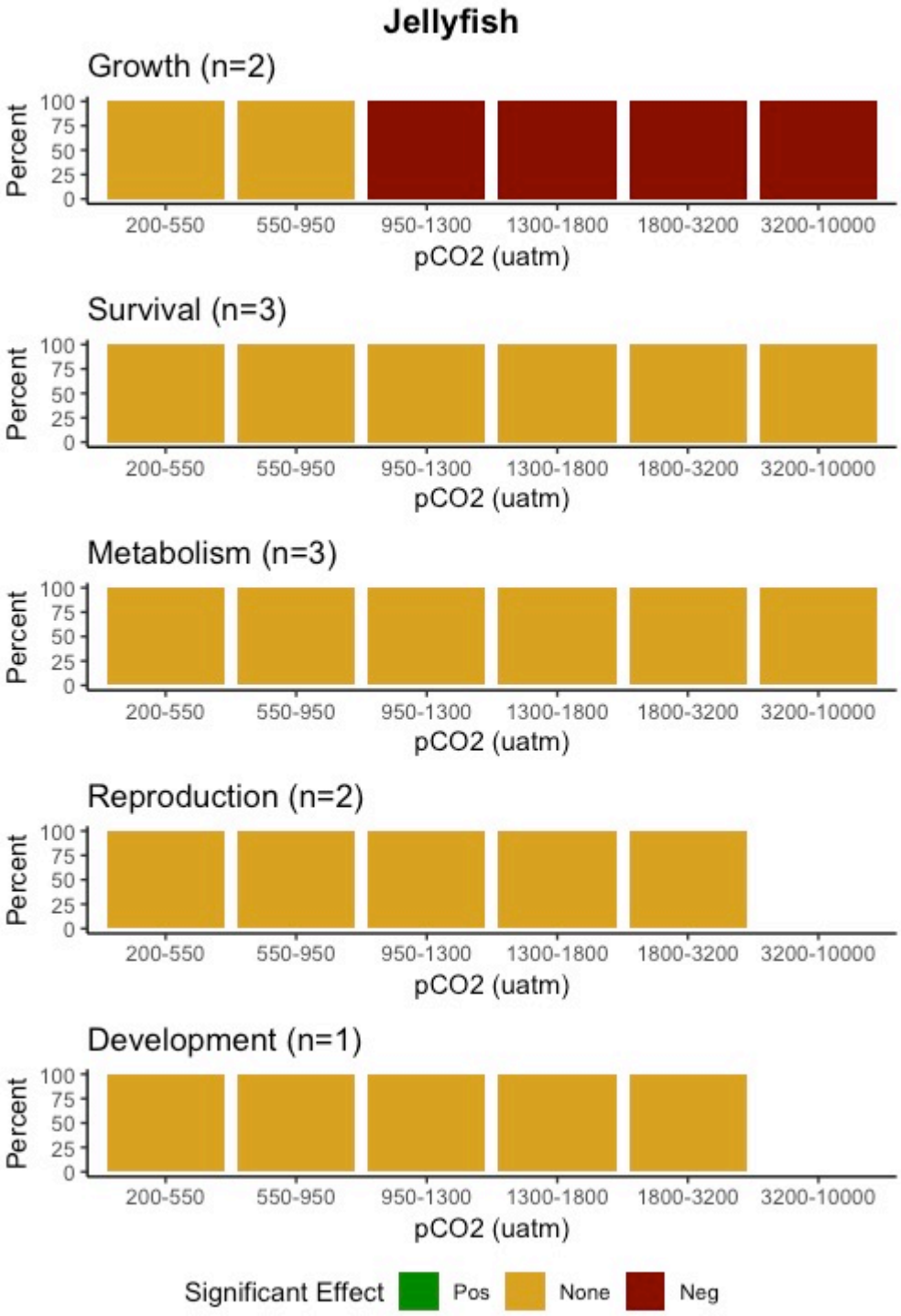
Appendix C-4. Percent (%) of representative experiments across response categories for shrimp exhibiting positive (green), no response (yellow), or negative (red) response to elevated levels of pCO_2 (µatm). Data were interpolated and extrapolated to compensate for missing values. n represents the number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



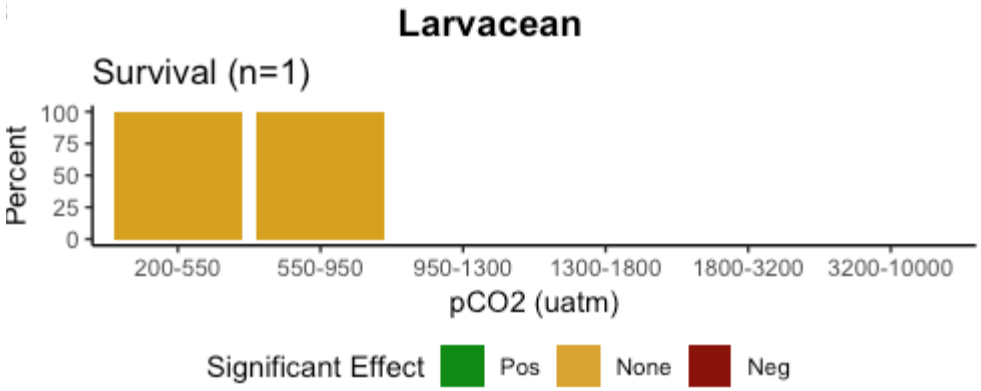
Appendix C-5. Percent (%) of representative experiments across response categories for krill exhibiting positive (green), no response (yellow), or negative (red) response to elevated levels of pCO_2 (µatm). Data were interpolated and extrapolated to compensate for missing values. n represents the number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



Appendix C-6. Percent (%) of representative experiments 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 interpolated and extrapolated to compensate for missing values. n represents the number of studies included in the analysis.



Appendix C-7. Percent (%) of representative experiments 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 interpolated and extrapolated to compensate for missing values. n represents the number of studies included in the analysis.



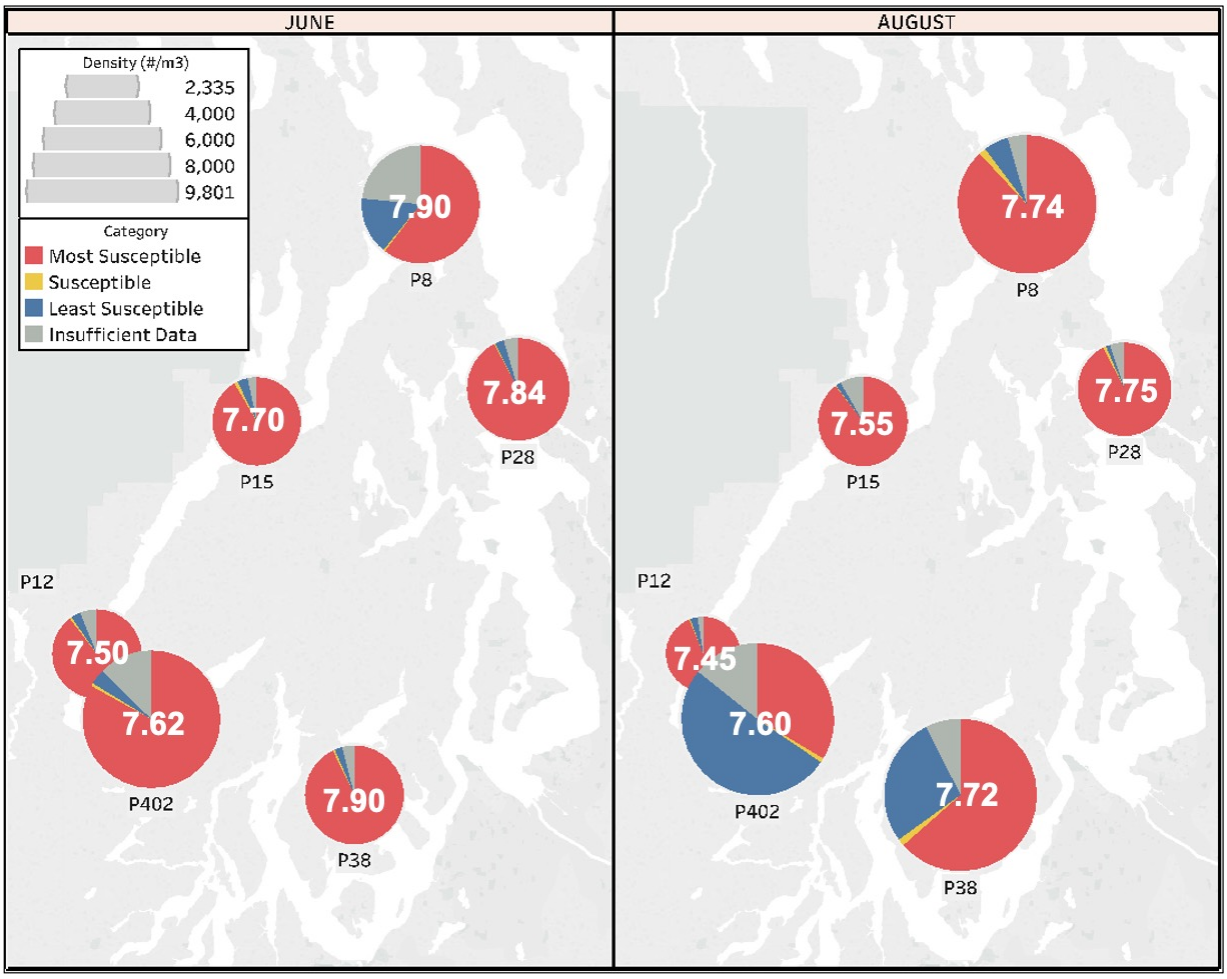
Appendix C-8. Percent (%) of representative experiments across response categories for larvaceans exhibiting positive (green), no response (yellow), or negative (red) response to elevated levels of pCO_2 (µatm). Data were interpolated and extrapolated to compensate for missing values. n represents the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

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

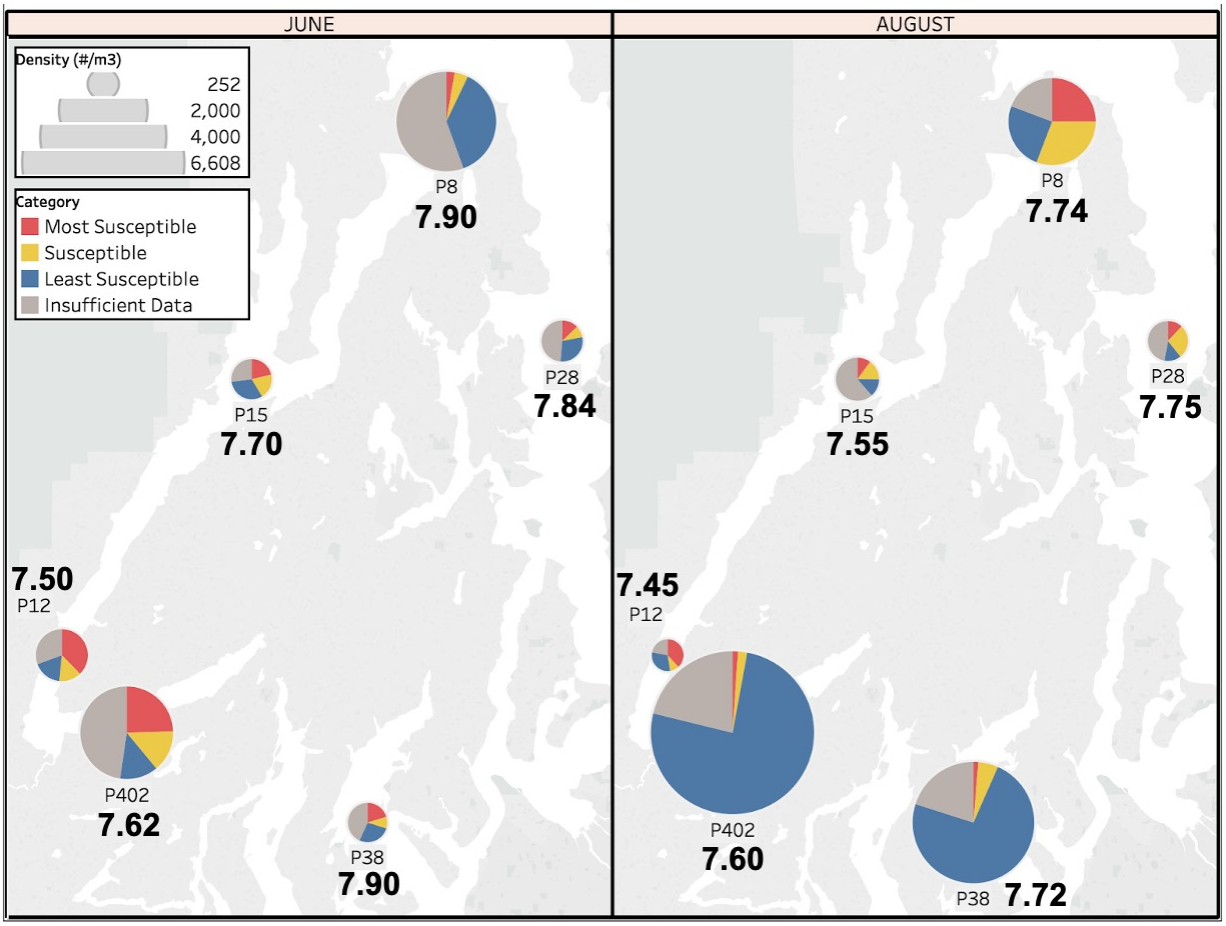
Taxonomic group	Classification(s)
Amphipoda - Gammarid	N/A
Amphipoda - Hyperiid	N/A
Annelida - Polychaeta	Larva, Adult
Bivalvia	Veliger
Bryozoa	Cyphonaut
Chaetognatha	N/A
Chordata - Fish	Larva
Chordata – Larvacea	N/A
Cladocera	N/A
Cnidaria - Hydrozoa	Medusa
Cnidaria - Siphonophora	Nectophore, Gonophore, 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 E. 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 F. The abundance of zooplankton (individuals/m^3) in June and August 2017 based on estimated levels of susceptibility to ocean acidification, including taxa for which data were insufficient (e.g., amphipods and other taxa excluded from the meta-analysis). The mean pH for each station is recorded in white in the middle of each pie chart.



Appendix G. The abundance of zooplankton (individuals/m^3) in June and August 2017 based on estimated levels of susceptibility to ocean acidification, including taxa for which data were insufficient (e.g., amphipods and other taxa excluded from the meta-analysis). Copepod abundance is excluded from the most susceptible category (red) in this figure to better display the distribution of less abundant taxa. The mean pH for each station is recorded next to each station name.