EFFECTS OF OCEAN ACIDIFICATION ON PREDATOR-PREY INTERACTIONS IN ECHINODERMS

by

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Abstract

The need to understand future changes in marine ecosystems has become critically important as increasing atmospheric carbon dioxide (CO_2) drives rapid ocean acidification (OA). OA may improve or reduce the performance of marine species, and the relative impacts on interacting species will largely determine changes at the community level. The goal of this thesis was to determine the effects of acidification on predator-prey interactions between red sea urchins (*Strongylocentrotus franciscanus*) and sunflower stars (*Pycnopodia helianthoides*), a key predator-prey pair in Northeast Pacific kelp forest ecosystems. I tested this question using laboratory mesocosm experiments. Sea urchins were acclimated to ambient (pCO_2 ~ 500 μatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 μatm, pH ~ 7.7) conditions, with or without a caged sea star, for 22 weeks in a recirculating seawater system. In Chapter 2, I investigated the effects of OA on the growth, calcification, and feeding rate of *P. helianthoides*. High CO_2 had a significant positive effect on sea star growth, but no effect on calcified tissue mass. In addition, the consumption rate of turban snails (*Chlorostoma funebralis*) by sea stars was significantly higher in the high CO_2 treatment. In Chapter 3, I examined the effects of OA on the responses of *S. franciscanus* to sea star cues. Predator presence and high CO_2 negatively and additively affected sea urchin growth rates, but did not affect alarm responses to predator cues. Significantly higher grazing rates on kelp (*Macrocystis pyrifera*) were also observed in the presence of predators. Predators, but not CO_2, had a significant negative effect on urchin calcified mass. Urchin spine length was also significantly reduced under acidified conditions. Overall, these findings suggest *P. helianthoides* responds positively to ocean acidification, but *S. franciscanus* may suffer reduced fitness at seawater pCO_2 levels predicted for the end of the century. Differential effects

of ocean acidification on this predator-prey pair could increase the strength of the trophic interaction and lead to stronger top-down control in the future.

Preface

Chapters 2 and 3 were conceived by myself in collaboration with Chris Harley. I collected and analyzed the data, and wrote the manuscripts. Chris Harley contributed to interpreting the results and edited the manuscripts. Renee Bechmann at the International Research Institute of Stavanger also contributed to experimental design.

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List of Symbols and Abbreviations

~	approximately
[ ]	concentration
^{o}C	degree Celsius
$CaCO_3$	calcium carbonate
cm	centimetre
CO_2	carbon dioxide
$CO_{2[aq]}$	aqueous carbon dioxide
CO_3^{2-}	carbonate ion
DIC	dissolved inorganic carbon
HCO_3^-	bicarbonate ion
K^*_{sp}	stoichiometric solubility constant
L	litre
mm	millimetre
OA	ocean acidification
Ω	saturation state
pCO_2	partial pressure of carbon dioxide
ppm	parts per million
ppt	parts per thousand
SE	standard error
μatm	microatmosphere

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Dedication

I dedicate this thesis to my family: Charlotte, Matthew, Judy, and Carri

Chapter 1

Introduction

Increasing carbon dioxide (CO_2) emissions from human activities, such as deforestation and the burning of fossil fuels, poses a serious threat to biodiversity worldwide (IPCC, 2013). Atmospheric CO_2 concentrations have recently surpassed 400 parts per million (ppm), an increase of more than 100 ppm from pre-industrial levels (Tans and Keeling, 2014; Tyrell, 2007). Although CO_2 levels naturally oscillate between glacial and interglacial periods, the current rate of increase is unprecedented in the fossil record (Caldeira and Wickett, 2003; Pelejero *et al.*, 2010). Emission scenarios from the Intergovernmental Panel on Climate Change (IPCC) predict that, without considerable mitigation, atmospheric concentration of CO_2 will reach 1000 ppm by the end of the century (IPCC, 2013). This pervasive environmental change may have far-reaching and unexpected consequences for marine ecosystems. Approximately one-third of the CO_2 emitted from anthropogenic sources has been absorbed by the ocean (Sabine *et al*., 2004), which is driving rapid ocean acidification (OA).

1.1 Ocean Acidification and the Marine Carbonate System

The ocean is an important sink for anthropogenic CO_2, accounting for an estimated 30% of fossil-fuel emissions over the past two centuries (Sabine *et al.*, 2004). Carbon fixation and transport from surface waters to the deep ocean is facilitated by marine phytoplankton, which convert dissolved inorganic carbon (DIC) to organic carbon through the process of biogenic calcification (Raven *et al.*, 2005). The remains of dead phytoplankton fall through the water column and dissolve or are deposited in marine sediments (Feely *et al.*, 2004). Removal of carbon from surface waters *via* this ‘biological pump’ is arguably the most significant way in

which anthropogenic CO_2 is naturally sequestered from the atmosphere. However, atmospheric CO_2 is rising faster than the carbon pump can remove it, resulting in a build-up of CO_2 in both the atmosphere and the ocean (Raven *et al.*, 2005).

The accumulation of CO_2 in the ocean is causing a reduction in seawater pH and calcium carbonate ($CaCO_3$) saturation state (Orr *et al.*, 2005). Since the start of the industrial revolution, global ocean pH has decreased by 0.1 units, which amounts to an increase in acidity of approximately 30% (IPCC, 2013). Under the worst case climate change scenario from the IPCC, Representative Concentration Pathway (RCP) 8.5, it is predicted that seawater pH will decrease an additional 0.3-0.5 units by 2100 (IPCC, 2013). When CO_2 dissolves in seawater, it produces carbonic acid (H_2CO_3), which rapidly dissociates into hydrogen ions (H^+) and bicarbonate (HCO_3^-). The ocean has a natural carbonate buffer system, where additional H^+ produced from CO_2 dissolution react with carbonate ions (CO_3^{2-}) to form HCO_3^- (Raven *et al.*, 2005). As a result, an increase in the concentration of aqueous CO_2 [$CO_{2(aq)}$] leads to a simultaneous decrease in CO_3^{2-} concentration *via* the following reaction:

$$CO_2 + CO_3^{2-} + H_2O \leftrightarrow 2HCO_3^-$$

Decreased availability of CO_3^{2-} may impair the ability of shell-forming organisms to build calcium carbonate structures and also increase the rate of dissolution (Orr *et al.*, 2005).

The rates of calcification and dissolution in seawater are functions of the saturation state (Ω) of calcium carbonate (Feely *et al.*, 2004). The most commonly occurring forms of marine carbonates include calcite (e.g., coccolithophores and foraminifera), aragonite (e.g., corals and pteropod molluscs), and high-magnesium calcite (e.g., echinoderms and coralline algae) (Doney

et al., 2009). Calcium carbonate saturation state (Ω_{CaCO3}) is estimated by the product of calcium and carbonate ion concentrations divided by the stoichiometric solubility constant (K^*_{sp}), at a given temperature, pressure, and salinity (Feely *et al.*, 2004). The rate of dissolution will increase as a function of decreasing $CaCO_3$ saturation.

$$\Omega_{CaCO_3} = \frac{[Ca^{2+}][CO_3^{2-}]}{K^*_{sp}}$$

Although the ocean is typically supersaturated ($\Omega > 1$) with respect to $CaCO_3$, the degree of supersaturation has a direct effect on calcification rate (Kleypas *et al.*, 1999). Depending on the type of calcium carbonate used, important groups of calcifiers, such as phytoplankton and echinoderms, may be particularly vulnerable to the effects of OA as the solubility of aragonite and high-magnesium calcite is nearly double that of calcite in seawater (Mucci, 1983). Declining saturation states may lead not only to reduced calcification rates, but may also cause a shift in competitive dominance toward calcite secretors and non-calcifiers (Smith and Buddemeier, 1992; Connell *et al.*, 2013; Wootton *et al.*, 2008).

Saturation states and pH of the surface ocean vary both spatially and temporally, and are regulated by several biological and physiochemical factors (Raven *et al.*, 2005). The two primary drivers of spatial variation are sea surface temperature and the degree of upwelling. The solubility of CO_2 decreases with temperature and increases with pressure, so deep and high-latitude waters are typically less saturated with calcium carbonate compared to shallow, tropical waters (Feely *et al.*, 2004). Respiration and decomposition of organic matter by organisms below the photic zone can also cause accumulation of DIC and a reduction in pH at depth. In coastal upwelling zones, such as the northeastern Pacific, these corrosive waters are pushed onto the

continental shelf and can cause undersaturation of aragonite in the surface ocean (Feely *et al.*, 2008). The Indian and Pacific Ocean basins are particularly rich in CO_2 due to longer deep-water circulation pathways (Broecker, 2003), resulting in shallower $CaCO_3$ saturation horizons compared to the Atlantic Ocean. Furthermore, seasonal changes in temperature, productivity, and ocean mixing can also drive temporal variability in the concentration of CO_2 (Bates *et al.*, 1996).

1.2 Effects on Marine Organisms and Implications for Species Interactions

OA can negatively impact a wide range of physiological processes in marine organisms, such as calcification (Ries *et al.*, 2009), metabolism (Portner, 2008), acid-base regulation (Fabry *et al.*, 2008), and neurotransmitter functioning (Nilsson *et al.*, 2012). These mechanisms may underlie a variety of the responses observed under high CO_2 conditions, such as reduced growth and survival (Kroeker *et al.*, 2013a), delayed development (Kurihara, 2008), impaired olfactory cue detection (Leduc *et al.*, 2013), and reduced fertilization success (Havenhand *et al.*, 2008; Parker *et al.*, 2009). However, organismal responses to acidification are varied (Ries *et al.*, 2009), and fitness may be improved or reduced depending on species, population, and life stage (Kroeker *et al.*, 2013a; Parker *et al.*, 2011; Byrne, 2011).

The various pathways by which OA directly impacts marine organisms will likely prompt changes at the community level by altering species interactions. Gaylord *et al.* (2015) identify biotic interactions as a key "pressure point" by which OA may drive ecological change. However, a lack of experimental research testing the effects on species relationships presents a considerable barrier for predicting the potential consequences of rising ocean acidity. Understanding the effects of OA on species interactions may be particularly important in the context of consumer-resource relationships. Changes in predator-prey interactions, for example,

could cascade through communities, altering the structure and stability of marine food webs. In the following sections, I address a number of mechanisms by which ocean acidification may modify biotic interactions, and thus govern changes in marine ecosystems.

1.2.1 Effects of OA on calcification

Decreased rates of calcification in response to a reduction in seawater [CO_3^{2-}] have now been documented across a broad range of key taxonomic groups (Kroeker *et al.*, 2013a). However, the magnitude of effect varies among taxa, and OA may actually lead to increased calcification in some species (Ries *et al.*, 2009; Findlay *et al.*, 2011). Kroeker *et al.* (2013) show mean reductions in calcification are greatest in corals, molluscs, and coccolithophores (22-39%), but the responses of echinoderms and crustaceans are largely neutral. Ries *et al.* (2009) identify a number of mechanisms that may drive this variation in sensitivity to OA. First, calcifying organisms that are able to maintain elevated pH at the site of calcification are typically less negatively affected. In addition, the external layer of organic tissue produced by most calcifiers to protect their shell or skeleton from the surrounding seawater varies considerably in structure, composition, and extent of coverage among species. The solubility of the calcium carbonate mineral (i.e., calcite, aragonite, or high-Mg calcite) used by an organism is also an important driver of variation in sensitivity to OA. Finally, increasing pCO_2 may act as a fertilizer for photosynthesis, increasing the amount of energy available for calcification in some marine algae (Ries *et al.*, 2009).

Reduced calcification or dissolution in response to OA has been shown to decrease the strength and thickness of skeletal tissue (e.g., Holtmann *et al.*, 2013; Melatunan *et al.*, 2013; Gaylord *et al.*, 2011). As the shells and skeletons of marine invertebrates are often important for

protection and defense (e.g., sea urchin spines and pedicellariae; Moitoza and Phillips, 1979), changes in their mechanical integrity will likely impact species interactions. For example, acidification may disrupt the expression of inducible defenses – "phenotypically plastic traits that increase resistance to predators or competitors" (Leonard *et al.*, 1999) – such as shell thickening in response to a predator (Bibby *et al.*, 2007). Reduced morphological defense under acidified conditions could make some calcifying organisms more vulnerable to predators and weaker competitors, although changes in species interactions will likely be highly case-specific. For instance, the intertidal snail *Littorina littorea* is able to compensate for a thinner shell by increasing predator avoidance behaviour (Bibby *et al.*, 2007).

1.2.2 Effects of OA on growth

An increasing number of studies show that ocean acidification leads to a reduction in organismal growth rates, particularly among the more heavily calcified taxa such as corals, molluscs, and echinoderms (Kroeker *et al.*, 2013a). Decreased growth rates likely reflect elevated energetic demands associated with the increased cost of calcification and acid-base regulation (Dupont and Thorndyke, 2014). Conversely, the growth rates of non-calcified groups such as seagrasses and fleshy macroalgae, as well as some modestly calcified species (e.g. sea stars), have been shown to respond positively to high CO_2 exposure (Zimmerman *et al.*, 1997; Koch *et al.*, 2013; Gooding *et al.*, 2009; Dupont *et al.*, 2010b). This variation in performance under high CO_2 may lead to shifts in competitive dominance away from some calcified taxa (e.g. mussels and corallines) towards noncalcareous species (Wootton *et al.*, 2008). In addition, Connell *et al.*, (2013) predict CO_2 enrichment may drive dominance shifts among algal competitors, such as kelp and turf algae. Finally, increased growth rates of predators (e.g., sea

stars) in combination with decreased body size of prey (e.g., calcified herbivores) could result in higher *per capita* predation rates under future ocean conditions (Sanford *et al.*, 2014; Gooding *et al.*, 2009).

1.2.3 Effects of OA on olfaction

Chemical cues play a critical role in both intra- and interspecific communication in aquatic ecosystems, mediating behaviours such as foraging, reproduction, and predator avoidance (Brönmark and Hansson, 2000). Olfactory communication between predators and prey is particularly well studied and can strongly influence predator-prey interactions. Exposure to predator cues can invoke strong morphological and/or behavioural responses in prey (i.e., induced defenses), which serve to reduce vulnerability to predation (Brönmark and Hansson, 2000). Prey detect and assess predation risk *via* a variety of chemical stimuli: (1) predator-specific odours called kairomones, (2) disturbance cues, released from startled prey, (3) damage cues, released from injured conspecifics, and (4) dietary cues, released post-ingestion from predators feeding on conspecifics (Ferrari *et al.*, 2010). Given the importance of chemosensory cues for organism fitness, disruption of olfactory cue detection is likely an important mechanism by which OA could impact marine communities (Leduc *et al.*, 2013; Brönmark and Hansson, 2000).

The majority of research investigating the effects of acidification on olfactory-mediated behaviour has focused on fish (Leduc *et al.*, 2013). For example, a number of studies on larval clownfish (*Amphiprion percula*) show OA impairs detection of olfactory cues from adult habitats (Munday *et al.*, 2009) and predators (Dixson *et al.*, 2010). Chemosensory and cognitive impairment in clownfish exposed to high CO_2 may be a result of interference with

neurotransmitter functioning (Nilsson *et al.*, 2012). Research on invertebrates is considerably more limited, but a handful of studies show OA disrupts the detection of food odours in hermit crabs (De la Haye *et al.*, 2012) and also alters behavioural responses to predator cues in muricid snails (Manriquez *et al.*, 2013, Manriquez *et al.*, 2014) and conch snails (Watson *et al.*, 2014). Impaired predator cue detection and response behaviour could lead to increased prey mortality in the future (Nilsson *et al.*, 2012). The effect of OA on predator olfaction is poorly understood, but could lead to decreased feeding activity and prey capture success (Cripps *et al.*, 2011; Allan *et al.*, 2013). Changes in predator-prey interactions under future CO_2 conditions will likely depend on the degree to which both species are affected.

1.3 Thesis Overview and Objectives

As I have described above, organismal responses to ocean acidification are highly varied and often species-specific. This diversity of responses will almost certainly lead to changes in species interactions, and thus govern the larger-scale impacts of OA. However, our understanding of the indirect effects of acidification *via* altered species interactions is very limited. Therefore, predicting the ecosystem-level consequences of environmental change poses a complex challenge to the field of ocean acidification research. In this thesis, I begin to address this question by empirically testing the effects of OA on a key predator-prey pair in British Columbia kelp-forest ecosystems: the red sea urchin (*Strongylocentrotus franciscanus*) and the sunflower star (*Pycnopodia helianthoides*).

1.3.1 Study Species

Red sea urchins and sunflower stars are common to nearshore benthic habitats from Alaska to Baja, California (Shivji *et al.*, 1983; DFO, 2012). *P. helianthoides* is a voracious predator and scavenger, consuming a wide variety of prey species including mussels, clams, abalone, crabs, sea urchins, and other echinoderms (Shivji *et al.*, 1983; Lambert, 2000). One of the largest and fastest sea stars in the world, it can grow up to 90 cm in diameter and weigh approximately 5 kg (Lambert, 2000). *S. franciscanus* is the largest of five sea urchin species in British Columbia, growing up to 18 cm test diameter (DFO, 2012). It can occur at extremely high densities and consume vast quantities of macroalgae, forming areas known as urchin barrens (Dayton, 1985). *S. franciscanus* is also fished commercially in BC, although landings have been steadily declining since the mid-1990s (DFO, 2012).

Predation by *P. helianthoides* on *S. franciscanus* can play an important role in structuring kelp-dominated communities of the Northeast Pacific. *P. helianthoides* can facilitate algal recruitment by clearing large areas of urchins, which ultimately impacts species richness and primary productivity (Duggins, 1983). In addition, *P. helianthoides* predation can impact red urchin population structure. Tegner and Dayton (1981) found that the size-frequency distributions of *S. franciscanus* populations in southern California are bimodal in the presence of *P. helianthoides*. They suggest *P. helianthoides* preferentially feed on intermediate size classes (5-8 cm test diameter) of red urchins and hypothesize that small juveniles (< 4 cm) are protected by the spine canopies of adults, whereas large adults (> 9 cm) reach a size refuge. Given this size-specific predation by *P. helianthoides*, I used intermediate sized red urchins in this study.

1.3.2 Research questions

The broad research question I ask in this thesis is as follows: What is the effect of ocean acidification on the predator-prey interaction between *S. franciscanus* and *P. helianthoides*? This question can be broken down into three components:

1. What are the direct effects of OA on the behaviour, growth, and morphology of *P. helianthoides* (Chapter 2) and *S. franciscanus* (Chapter 3)?
2. What are the effects of predator presence on the behaviour, growth, and morphology of *S. franciscanus*? (Chapter 3)
3. How do OA and predator presence interact? In other words, how does OA modify the effects of *P. helianthoides* on *S. franciscanus*? (Chapter 3)

In answering these questions, I aim to predict how predator-prey relationships may change under future ocean pH conditions, and suggest how these changes may drive shifts in benthic subtidal communities of the Pacific Northwest.

Chapter 2

Effects of Ocean Acidification on the Growth, Feeding Rate, and Calcification of *Pycnopodia helianthoides*

2.1 Introduction

Ocean acidification has emerged as one of the most important environmental changes affecting marine species and ecosystems (Harley *et al.*, 2006). Research on the effects of OA has largely focused on marine calcifiers, such as corals, phytoplankton, molluscs, and echinoderms, as these may be some of the most vulnerable taxa (Kroeker *et al.*, 2010, 2013a). Echinoderms, for example, may be particularly sensitive as they build their skeletons with magnesium calcite, an amorphous and highly soluble form of calcium carbonate (Politi *et al.*, 2004). However, echinoderms show largely mixed responses to acidification. Although the direction of effect is predominately neutral or negative, there is considerable variation across species, life-stages, and biological processes (Dupont *et al*., 2010a).

In general, the more heavily calcified echinoderms (e.g., sea urchins) tend to be more negatively affected by acidification, particularly at larval and juvenile stages (for review, see Dupont *et al.*, 2010a; Dupont and Thorndyke, 2014). A range of sublethal effects have been documented, such as reduced calcification, slower growth, and delayed development, which could ultimately impact organism fitness (Dupont *et al.*, 2010a). Conversely, some echinoderm species appear less vulnerable to OA, or even do better under high CO_2 conditions (Clark *et al.*, 2009; Dupont *et al.*, 2010b; Gooding *et al.*, 2009; Ries *et al.*, 2009; Schram *et al.*, 2011; Wood *et al.*, 2008). For example, sea stars, which are typically less calcified, appear to be more resilient

to the effects of acidification. Studies have documented increased growth rates at both larval and adult stages (Gooding *et al.*, 2009; Dupont *et al.*, 2010b), as well as increased metabolism and calcification (Wood *et al.*, 2008) in sea stars exposed to high CO_2 conditions. However, responses to acidification are largely species-specific (Dupont *et al.*, 2010a) and variability within the sea star class (Asteroidea) is relatively under-studied. As sea stars can play key roles in structuring marine ecosystems (Paine, 1966), understanding this variation will be particularly important for predicting changes at the ecosystem level.

In this Chapter, I examine the effects of ocean acidification on the growth, feeding rate, and calcification of juvenile sunflower stars (*Pycnopodia helianthoides*). I use laboratory mesocosm experiments to test responses of *P. helianthoides* to long-term OA exposure. This ecologically important predator has relatively low amounts of calcified tissue and may be pre-adapted to OA due to the naturally high variation in pH found throughout its range (Feely *et al.*, 2008). Previous research on another NE Pacific sea star, *Pisaster ochraceus*, suggests *P. helianthoides* may respond positively to OA. Gooding *et al.* (2009) found increased rates of growth and predation on mussels (*Mytilus trossulus*), but decreased calcification, in *P. ochraceus* exposed to high CO_2. Therefore, I hypothesized that exposure to acidification (high CO_2/low pH) would have a positive effect on the growth and feeding rate of *P. helianthoides*, but a negative effect on calcification.

2.2 Methods

2.2.1 Collection site and experimental set-up

Juvenile sea stars (15 – 20 cm initial diameter) were hand collected using SCUBA between 5 and 10 m depth from Copper Cove, West Vancouver, British Columbia (49°22'42"N,

123°16'46"W) in May 2013. They were transported in coolers to the University of British Columbia and placed in independently recirculating 260 L seawater systems. The tanks were lit with Corallife 65W Actinic Compact Fluorescent bulbs (24″) for 14 hours per day. Seawater temperature and salinity were representative of average springtime conditions in southwestern British Columbia (DFO, 2013; see Appendix A, Table A-1 and Figure A-3). The sea stars were acclimated to lab conditions for one week prior to the experiment and fed turban snails (*Chlorostoma funebralis*) ad libitum.

2.2.2 Manipulation and measurement of seawater chemistry

Juvenile sea stars (103.15 g ± 5.69 SE) were randomly assigned to either the control (ambient; n=6) or acidified (high CO_2/low pH; n=6) seawater treatment. Sea stars were exposed to ambient (pCO_2 = ~500 µatm, pH = ~8.0) or high CO_2 (pCO_2 = ~1000 µatm, pH = ~7.7) conditions for 22 weeks. Each treatment was replicated six times with one sea star per replicate (i.e., 260 L tank). Sample size in the acidified treatment was reduced to five due to a seawater system failure. Seawater pCO_2 was manipulated by adding ambient air or air mixed with CO_2 gas to each tank using air stones. Flow rate was adjusted using mass flow controllers. Partial seawater changes were carried out at weeks 8 and 15 to ensure the seawater contained adequate concentrations of key ions (e.g., Ca^{2+}, Mg^{2+}, etc.). During week 9, CO_2 scrubbers were installed to help maintain seawater pCO_2 and pH at target levels. Temperature and pH were recorded once per week using an Oakton Acorn pH 5 handheld meter. The potentiometric glass electrode was accurate to ± 0.01 pH units and the ATC probe was accurate to ± 0.05°C. Salinity was measured using a refractometer. Seawater samples were also collected weekly for Dissolved Inorganic Carbon (DIC) analysis and preserved following methods from Dickson *et al.* (2007). A

Dissolved Inorganic Carbon Analyzer (Model AS-C3, Apollo SciTech Inc, Bogart, Georgia, USA) was used to measure DIC. Other carbonate system parameters were calculated using the software program CO2SYS (Pierrot *et al.*, 2006).

2.2.3 Measurement of sea star growth, calcification and feeding rate

Sea star wet mass and maximum diameter were recorded at weeks 0, 13, and 22. Wet mass was measured by weighing the sea stars on a scale after blotting dry with paper towel. Maximum diameter was measured by recording the maximum distance between arm tips on opposite sides of the central disk. Beginning in week 20, sea star feeding rate on *C. funebralis* was recorded over a 10 day period. Snails of similar size (~6 g; n=55) were randomly assigned to the control or acidified treatment and then placed in 40 x 15 cm plastic containers with mesh sides. The snails (inside the plastic containers) were placed in 260 L tanks without predators and exposed to ambient or high CO_2 conditions for two weeks prior to the experiment. Sea stars were starved for one week prior to feeding experiments to standardize hunger. Individual sea stars were provided with five randomly selected snails at the beginning of the feeding trial and the number of empty shells was recorded every 24 hours. At the end of the experiment, sea stars were blotted dry, weighed, and placed in separate plastic bags. The bags were then stored in a freezer at -20°C to euthanize the sea stars. To measure calcified mass, sea stars were placed in a drying oven at 70°C for 48 hours, weighed, and placed in 6% sodium hypochlorite (bleach) to remove soft tissue. The solution was then vacuum filtered on No. 1 Whatman filter paper. The calcified material was then rinsed with distilled water and dried at 70°C to a constant mass. Samples were weighed using a high precision scale (AB104-S Analytical Balance, Mettler Toledo, Switzerland).

2.2.4 Statistical Analysis

All analyses were carried out using R statistical software. Analysis of covariance (ANCOVA) was used to determine the effect of acidification on final sea star diameter and final wet mass. Initial diameter and initial wet mass were used as covariates, respectively, to account for differences in sea star size. ANCOVA was also used to determine the effect of CO_2 treatment on both total dry mass and calcified mass, using final wet mass and total dry mass as covariates, respectively. It was determined that model assumptions were met by examining diagnostic plots of the residuals. To assess the effect of acidification on snail mortality due to predation by sea stars, a survival analysis was conducted using the Cox Proportional Hazards Model. The model included sea star size as a covariate. The significance of each variable and 2-way interaction was tested using likelihood ratio tests. Diagnostic checks were performed and it was determined that model assumptions of proportional hazards, influential observations, and non-linearity (in the relationship between the log hazards and the covariates) were not violated.

2.3 Results

Experimentally induced ocean acidification resulted in increased growth and feeding in juvenile *Pycnopodia helianthoides*. There was a significant effect of CO_2 and initial diameter on final sea star diameter (ANCOVA; CO_2, $F_{1,7} = 9.15$, $p = 0.01$, initial diameter, $F_{1,7} = 710.33$, $p < 0.0001$, interaction, $F_{1,7} = 0.29$, $p = 0.6$). Sea stars exposed to high CO_2 experienced accelerated growth over the 22-week period (Figure 2-1A). The relative growth (change in diameter/initial diameter) of sea stars in high CO_2 treatment was ~ 8 % higher compared to the sea stars in the control (Figure 2-1B). Relative change in wet mass also tended to be higher in the high CO_2 treatment (see Appendix B, Figure B-3), but the CO_2 effect was non-significant (ANCOVA;

CO_2, $F_{1,7}$ = 1.56, p = 0.25, initial wet mass, $F_{1,7}$ = 253.64, p < 0.0001, interaction, $F_{1,7}$ = 1.3, p = 0.29). Exposure to acidification also had no effect on sea star water content (approximated by the dry mass to wet mass ratio) or calcified tissue (Figure 2-2). There was no significant difference between CO_2 treatments in sea star dry mass (ANCOVA; CO_2, $F_{1,7}$ = 0.63, p = 0.45, final wet mass, $F_{1,7}$ = 41.47, p = 0.0003, interaction, $F_{1,7}$ = 0.001, p = 0.97) or calcified mass (ANCOVA; CO_2, $F_{1,7}$ = 1.02, p = 0.35, total dry mass, $F_{1,7}$ = 73.34, p < 0.0001, interaction, $F_{1,7}$ = 0.18, p = 0.69). Body shape (e.g., the diameter-height ratio) varied among individuals, but was not significantly affected by CO_2 (see Appendix B, Figure B-2).

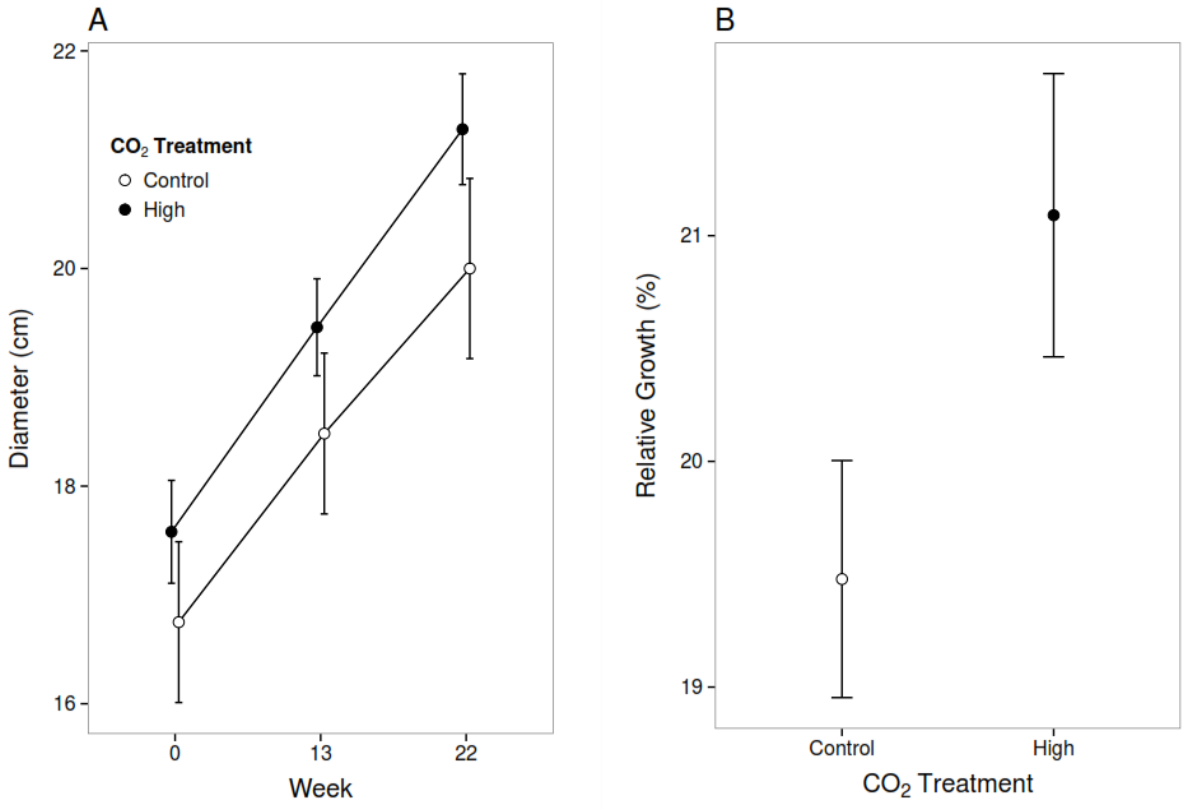

Figure 2-1. Effect of CO_2 manipulation on A) mean diameter (cm) and B) mean relative growth (percent change from initial diameter) of *Pycnopodia helianthoides*. Animals were exposed to ambient (pCO_2 ~ 500 µatm, pH ~ 8.0; n=6) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7; n=5) conditions for 22 weeks. Error bars represent 1 standard error (SE) of the mean. The effect of CO_2 was statistically significant (see text for details).

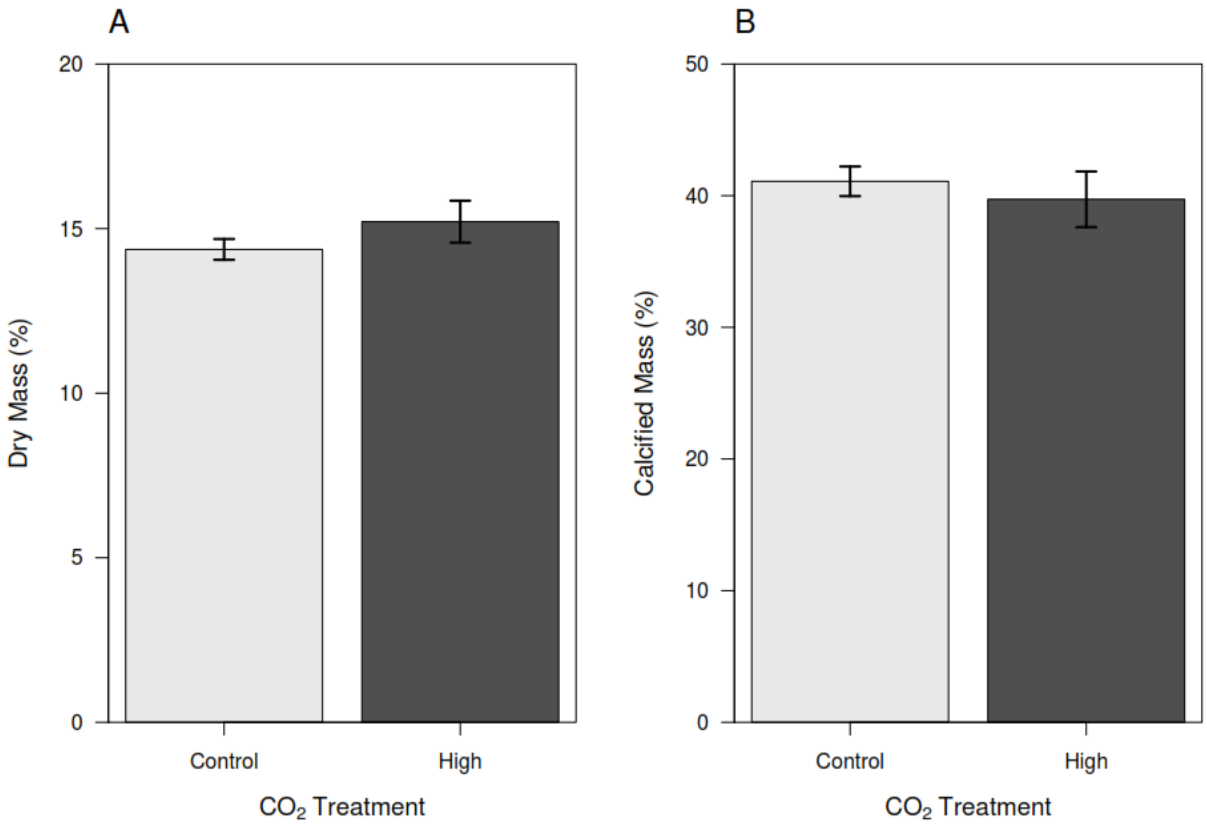

Figure 2-2. Effect of CO_2 manipulation on A) dry mass (as a percentage of total wet mass) and B) calcified mass (as a percentage of total dry mass) for *Pycnopodia helianthoides* (control: n=6; high: n=5). Error bars represent 1 standard error (SE) of the mean. Neither comparison was statistically significant (see text for details).

Acidification tended to have a positive effect on sea star feeding rates (Figure 2-3), although statistical support for this effect was marginal ($p = 0.055$; Table 2-1). A difference between CO_2 treatments in the rate at which *Chlorostoma funebralis* were consumed by sea stars did not appear until day 4 of the predation trial. There was also a significant effect of sea star size, where larger sea stars ate snails more quickly.

Table 2-1. Cox Proportional Hazards analysis testing the effect of CO_2 treatment (control/high) on the survival of turban snails (*Chlorostoma funebralis*) fed to sunflower stars (*Pycnopodia helianthoides*). Sea star size (diameter) was included as a covariate. The significance of a covariate was tested by comparing the log likelihood of a submodel (excluding the covariate) to the log likelihood of the full model. (*) indicates a significant effect.

Parameter	Est.	SE	Likelihood-ratio χ^2	*df*	*p*
CO_2	1.489	4.835	3.68	1	0.055
Size	0.214	0.106	4.49	1	0.034*
CO_2 × Size	-0.056	0.229	0.06	1	0.809
Full model			8.23	3	0.042*

N_{snails} = 55 $N_{seastars}$ = 11

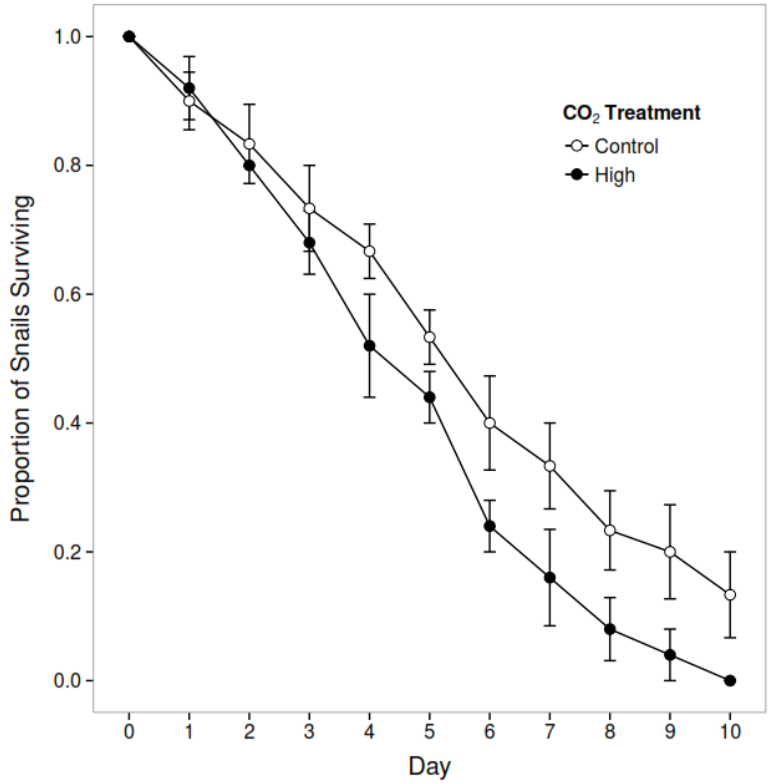

Figure 2-3. Survival curves of turban snails (*Chlorostoma funebralis*; n=55) fed to *Pycnopodia helianthoides* (control: n=6; high: n=5) under ambient (pCO_2 ~ 500 µatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7) conditions. Error bars represent 1 standard error (SE) of the mean. The effect of CO_2 was marginally non-significant (p = 0.055; see Table 2-1 for details).

2.4 Discussion

The results were consistent with my hypothesis that ocean acidification would have a positive effect on the growth and feeding rate of *Pycnopodia helianthoides*. However, I found no evidence that high CO_2 had an effect on calcification. This contrasts with the results of Gooding *et al.* (2009), who showed a reduction in relative calcified mass in *Pisaster ochraceus* exposed to high CO_2. *P. helianthoides* may be less sensitive to acidification as a smaller fraction of their total mass is made up of calcified components (~6%, compared to 10-12% in *P. ochraceus*). However, Appelhans *et al.* (2014) found calcification in *Asterias rubens*, a more heavily calcified species, was not affected by acidification. In addition, highly calcified brittle stars (*Amphiura filiformis*) have been shown to upregulate calcification under acidified conditions, although this is associated with significant muscle wastage (Wood *et al.*, 2008). It is important to note, however, that the heavily calcified endoskeleton found in brittle stars is likely an adaptation for predator defense (Wood *et al.*, 2008). As *P. helianthoides* is a top predator, investment in calcification may be less critical for survival.

Relative growth rate of *P. helianthoides* in the high CO_2 treatment was significantly higher than the control with respect to diameter, but not with respect to wet mass, although a similar trend was observed. Wet mass is likely a less reliable measure of growth due to variability in water absorption, as well as variation in measurement techniques (e.g., the degree to which surface water was removed prior to recording weight). Changes in body shape could be another potential source of variation (M. Vaughan, personal observation), but this did not appear to differ between CO_2 treatments. Increased growth rates in response to high CO_2 have been found in other juvenile sea stars, such as *P. ochraceus* (Gooding *et al.*, 2009) and *Crossaster papposus* (Dupont *et al.*, 2010b). The increase in the growth of *P. helianthoides* may, in part, be

a result of higher feeding rates, but CO_2 also has direct effects on physiological processes (Portner, 2008). For example, Dupont *et al.* (2010b) hypothesized that improved performance in *C. papposus* was driven by a direct positive effect on metabolism. In addition, Gooding *et al.* (2009) suggest that acidification may aid in digestion, making feeding less energetically costly. Low levels of environmental stress have also been shown to induce hormetic responses in many organisms, particularly during early life (Costantini, 2010).

Increased feeding rate under acidified conditions is likely driven by a complex interplay between effects on both predator energetic requirements and prey susceptibility (Gaylord *et al.*, 2015). As regulation of calcification and acid-base balance become more costly in the future, sea stars may increase *per capita* consumption in order to meet elevated energetic demands (Sanford *et al.*, 2014). Changes in prey susceptibility due to altered predator avoidance behaviour (Bibby *et al.*, 2007; Watson *et al.*, 2014), impaired cue detection (De la Haye *et al.*, 2012), reductions in tissue mass (Gaylord *et al.*, 2011), and weakened skeletal defenses (Kroeker *et al.* 2014) may also contribute to higher *per capita* predation rate by decreasing search time, handling time, and satiation (Kroeker *et al.*, 2014). However, it may not be possible to maintain higher feeding rates over the long-term, particularly if prey (e.g., calcified herbivores) become less abundant. Gaylord *et al.* (2015) suggest generalist predators may respond to declines in prey abundance or energetic content by shifting prey preference or dietary breadth. These changes in predator-prey dynamics could propagate through marine food-webs, altering species composition and community structure.

The improved performance of juvenile *P. helianthoides* under high CO_2 observed in this study differs from the larger body of work on OA, which suggests species-level responses will be largely neutral or negative (reviewed in Kroeker *et al.*, 2010, 2013a). However, the results shown

here, in combination with some other studies (e.g., Dupont *et al.*, 2010b; Gooding *et al.*, 2009), suggest the positive effects of OA may be more pervasive than previously assumed. Still, additional research will be needed to determine how *P. helianthoides* will respond to future ocean conditions. The short-term effects of high CO_2 on sea star growth and feeding could affect longer-term processes related to persistence if they translated to differences in population dynamics or fitness. Elevated growth rates could, for example, indirectly affect population size and recruitment by decreasing time to reproductive maturity. In addition, acidification could potentially impact other stages of the life cycle, such as fertilization and larval development (Gonzalez-Bernat *et al.*, 2013), which could magnify or reverse these short-term positive effects. Future research should investigate how short-term effects on individual performance may translate to changes at the population or community level.

Chapter 3

Nonconsumptive Effects of a Predatory Sea Star on Red Sea Urchins (*Strongylocentrotus franciscanus*) under Acidified Conditions

3.1 Introduction

The need to predict future changes in marine ecosystems has become critically important as increasing atmospheric carbon dioxide (CO_2) drives rapid ocean acidification (Feely *et al.*, 2004). Declining ocean pH and calcium carbonate ($CaCO_3$) saturation states may have major consequences for marine species, particularly calcifying invertebrates (Orr *et al.*, 2005). Our knowledge of the direct effects of OA on species has greatly improved in recent years (reviewed in Kroeker *et al.*, 2010, 2013a). However, research examining the indirect effects of acidification *via* altered species interactions is considerably more limited (Allan *et al.*, 2013; Connell *et al.*, 2013; Keppel *et al.*, 2015; Kroeker *et al.*, 2013b; Landes and Zimmer, 2012; Sanford *et al.*, 2014). Most studies are species-specific, which is not sufficient to predict changes in species interactions and thus the larger-scale impacts of OA (Landes and Zimmer, 2012). The relative impacts of environmental change on interacting species will largely determine changes at the ecosystem level (Harley, 2011; Kroeker *et al.*, 2013b).

Of the many types of species interactions, predator-prey interactions can have a disproportionately large effect on marine community structure (Estes and Palmisano, 1974). The phenomenon of keystone predation, for example, was classically demonstrated in the marine intertidal (Paine, 1966, 1969). In his seminal study, Paine (1966) showed predation by the sea

star *Pisaster ochraceus* on mussels (*Mytilus californianus*) indirectly increases species diversity by allowing inferior competitors, such as barnacles and limpets, to persist in the community. Despite the importance of predator-prey interactions for governing community dynamics, surprisingly little is known about how acidification may drive shifts in these relationships.

The impact of predators on prey populations can take the form of changes in abundance due to direct consumption, as well as changes in prey traits, such as behaviour, growth, and development (i.e., nonconsumptive effects; Peckarsky *et al.*, 2008). Both consumptive and nonconsumptive effects can translate to changes in the community *via* indirect effects on other species (Peckarsky *et al.*, 2008). For example, sea otter predation in kelp forests directly impacts sea urchin populations by reducing abundance, but also drives shifts in distribution by causing urchins to disperse away from damaged conspecifics (Watson, 1993). By altering both urchin abundance and distribution, otters reduce herbivory and indirectly promote kelp recruitment (Estes and Palmisano, 1974). When predators are absent, sea urchins can overgraze kelp and form low-diversity barrens (Dayton, 1985). In this classic example of a trophic cascade, the nonconsumptive effects of predators may be equally important for regulating community structure (Peckarsky *et al.*, 2008; Watson, 1993). Understanding the effects of OA on these nonconsumptive interactions will likely be critical for predicting how predator-prey dynamics may be altered in the future.

There are a variety of pathways by which ocean acidification may alter predator-prey interactions, such as changes in behaviour, energy allocation and demand, olfactory cue detection, skeletal integrity, palatability, predator-prey size ratios, and population sizes (Arnold *et al.*, 2012; Gaylord et al., 2015; Kroeker *et al.*, 2014; Munday *et al.*, 2010; Sanford *et al.*, 2014). In echinoderms, the effects of OA are largely negative (Dupont *et al.*, 2010a; Dupont and

Thorndyke, 2014), although some taxa (e.g., sea stars) appear to be less vulnerable (Gooding *et al.*, 2009; Dupont *et al.*, 2010b; Chapter 2). For example, exposure to acidification has been shown to reduce the growth and feeding rates of adult urchins, likely due to increased maintenance costs associated with acid-base regulation (Dupont and Thorndyke, 2014). In addition, OA may impair calcification of skeletal defense structures, such as spines and pedicillariae (Holtmann *et al.*, 2013). In some invertebrate taxa, ocean acidification has also been shown to disrupt the development of induced defenses (e.g., skeletal thickening in response to predator cues; (Bibby *et al.*, 2007), as well as impair predator cue detection and escape behaviour (De la Haye *et al.*, 2012; Manriquez *et al.*, 2013; Watson *et al.*, 2014), but this remains inadequately tested in echinoderms. As a result of decreased body size, reduced skeletal defense, and impaired predator response, sea urchins may become more vulnerable to predation under high CO_2 conditions. A decrease in predator handling time, search time, and satiation could lead to an increase in *per capita* predation rate (Kroeker *et al.*, 2014).

In this Chapter, I investigate how ocean acidification alters the nonconsumptive effects of sunflower stars (*Pycnopodia helianthoides*) on red sea urchins (*Strongylocentrotus franciscanus*), an important predator-prey pair in benthic subtidal communities of the Pacific Northwest (Duggins, 1983). I used factorial laboratory mesocosm experiments to test the combined effects of predator presence and acidification on the growth, calcification, and grazing rate of sea urchins exposed to high CO_2. I also examined the effects of predator presence and OA on urchin behavioural responses to predator cues. I hypothesized that: (1) predator presence and high CO_2 would result in decreased sea urchin growth and grazing rates; (2) the development of defensive traits (i.e., induced defenses) in response to predator cues would be reduced in sea

urchins exposed to acidification; and (3) urchin alarm response to sea star cues would be impaired under acidified conditions.

3.2 Methods

3.2.1 Collection site and experimental set-up

Juvenile red sea urchins (4.18 cm ± 0.11 SE test diameter) were collected near the Bamfield Marine Sciences Centre (BMSC), on the West Coast of Vancouver Island, British Columbia, Canada (48^{o} 51.141'N, 125^{o} 06.839'W) between May 7th - 9th, 2013. Collection was carried out by hand using SCUBA at subtidal depths ranging from 5 – 10 m. Urchins were stored in flow-through seawater tables at BMSC during the collection period. They were then placed in coolers with ice packs and damp paper towels and transported to the University of British Columbia (UBC). Juvenile sea stars (15 – 20 cm diameter) were collected subtidally (5 – 10 m) on May 19th, 2013 from Copper Cove, West Vancouver, British Columbia, Canada (49°22.706'N, 123°16.783'W) and transported to UBC in coolers. The animals were placed in 24 independently recirculating 260 L seawater systems and allowed to acclimate to laboratory conditions for 1 week.

The sea urchins were divided into groups of four similarly-sized animals, and urchins from within each size-matched group were randomly assigned to one of four treatments: (1) ambient CO_2, no predator, (2) ambient CO_2, predator, (3) high CO_2, no predator, (4) high CO_2, predator. Each treatment was replicated six times (randomized block design) with three urchins per replicate (N=72). Predator treatments contained one caged sea star (N=12). However, two seawater tanks failed during the experiment (treatments 1 and 4), resulting in a final sample size of 66 urchins and 11 sea stars. Sea urchins were fed dried kelp (*Macrocystis pyrifera*) and sea

stars were fed turban snails (*Chlorostoma funebralis*) *ad libitum*. The tanks were lit with Coralife Actinic Fluorescent bulbs (24″) for 14 hours per day. Seawater temperature and salinity was representative of average springtime conditions in southwestern British Columbia (DFO, 2013; see Appendix A, Table A-1 and Figure A-3). Urchins were exposed to both control (pCO_2 = ~500 μatm, pH = ~8.0) and acidified (pCO_2 = ~1000 μatm, pH = ~7.7) conditions for 22 weeks. Note that the data for Chapters 2 and 3 were collected during the same experiment. For a description of the methods used to manipulate and measure seawater chemistry, refer to section 2.2.2.

3.2.2 Measurement of sea urchin growth and grazing rate

Sea urchin wet mass and test diameter were recorded at weeks 0, 13, and 22. Wet mass was measured by weighing the urchins on a scale after blotting dry with paper towel. Test diameter was measured using calipers. On week 13, urchin grazing rate on *M. pyrifera* was measured. Dried kelp (9 ± 0.2 g) was added to each tank. After 36 hours, the kelp was removed from the tanks, re-dried, and weighed. To test for a potential difference in kelp degradation rate between treatments, an additional 0.1 g of dried kelp, placed in a small plastic container with mesh sides and bottom, was added to each tank.

3.2.3 Measurement of sea urchin alarm response to predator cues

The purpose of this experiment was to measure the alarm response of *S. franciscanus* to waterborne chemical stimuli from a predator, *P. helianthoides*, under both control and high CO_2 conditions. The experimental design was adapted from Hagen *et al.* (2002). I exposed urchins to both a control stimulus (plain seawater) and a predator stimulus. The predator stimulus was

prepared by placing two ~20 cm sea stars in 2 L of seawater for 30 minutes. Urchins from the control treatment were exposed to cues from sea stars acclimated to control conditions, and vice versa for the high CO_2 treatment. The experiment was carried out by placing a single urchin in the center of a test arena (Figure 3-1) containing 5 L of control or high CO_2 seawater. The urchin was allowed to choose a direction of movement (10 cm in either direction) and then the stimulus barrier (5 mL of seawater or predator cue) was applied 5 cm ahead of the moving urchin using a disposable pipette. Urchin behavioural responses were categorized as: (1) response (i.e., stop, reverse, and/or crawl out) or (2) no response (i.e., no change in speed or in direction of movement).

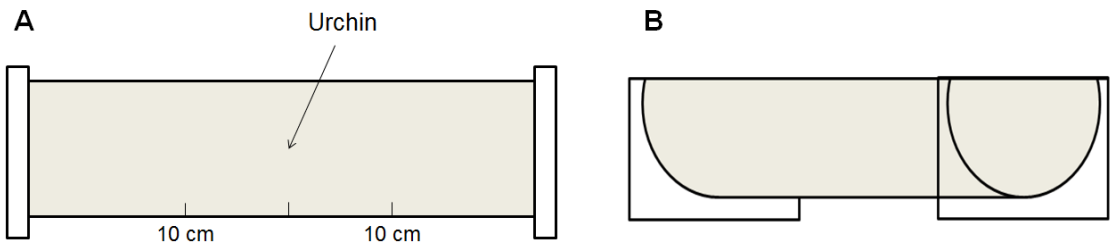

Figure 3-1. Schematic showing **A)** top and **B)** three-quarter side views of the Plexiglas experimental arena used for measuring the alarm response of red sea urchins (*Stronglyocentrotus franciscanus*) to waterborne chemical stimuli from a predator.

3.2.4 Measurement of sea urchin calcified tissue

At the end of the experiment, the ten longest spines were removed from each urchin and air dried. Soft-tissue was then dissolved by submerging the urchins in 6% sodium hypochlorite (bleach). Urchin tests, spines, and jaws were then rinsed with water and dried at 70°C to a constant mass. Test diameter and spine length were measured using calipers. Samples were weighed using a high precision balance (AB104-S Analytical Balance, Mettler Toledo, Switzerland).

3.2.5 Statistical analysis

All analyses were carried out using R statistical software. Analysis of Variance (ANOVA) was used to test for differences in urchin grazing rate (dry mass of kelp consumed per gram urchin) between the CO_2 and predator treatments. It was determined that model assumptions were met by examining diagnostic plots of the residuals. A linear mixed effects model (LME) was used to determine the effect of CO_2 and predators on final urchin diameter, using initial diameter as a covariate and tank as a nested random effect. LME was also used to determine the effect of CO_2 and predators on total calcified mass, spine and jaw calcified mass, and mean spine length, all using final test diameter as a covariate. Likelihood ratio tests were used in model comparison. Model fit was assessed by examining diagnostic plots of the residuals. Final diameter, total calcified mass, and spine and jaw calcified mass were log transformed in order to meet model assumptions. Differences in urchin responses to predator cues were determined using mixed effects logistic regression. The model included CO_2 treatment, predator presence, and cue type as fixed effects and tank as a random effect.

3.3 Results

Sea urchin growth and feeding were affected by acidification and by the presence of a predator. The results suggest that high CO_2 and predator presence have an additive negative effect on urchin growth. Urchin growth rates were significantly reduced in the high CO_2 treatment, compared to the control (Figure 3-2; Table 3-1A). A significant decrease in growth was also observed in urchins exposed to predator cues, but there was no interaction between predator acclimation and CO_2. In addition, relative growth rates decreased with increasing urchin size (Appendix C, Figure C-1). Conversely, high CO_2 and predator presence had an additive

positive affect on urchin grazing (Figure 3-3). Kelp consumption rate was significantly higher in the presence of a predator, but the effect of CO_2 was not significant (ANOVA; CO_2: $F_{1,18} = 2.36$, $p = 0.14$, predator:, $F_{1,18} = 6.09$, $p = 0.02$, interaction: $F_{1,18} = 0.02$, $p = 0.88$).

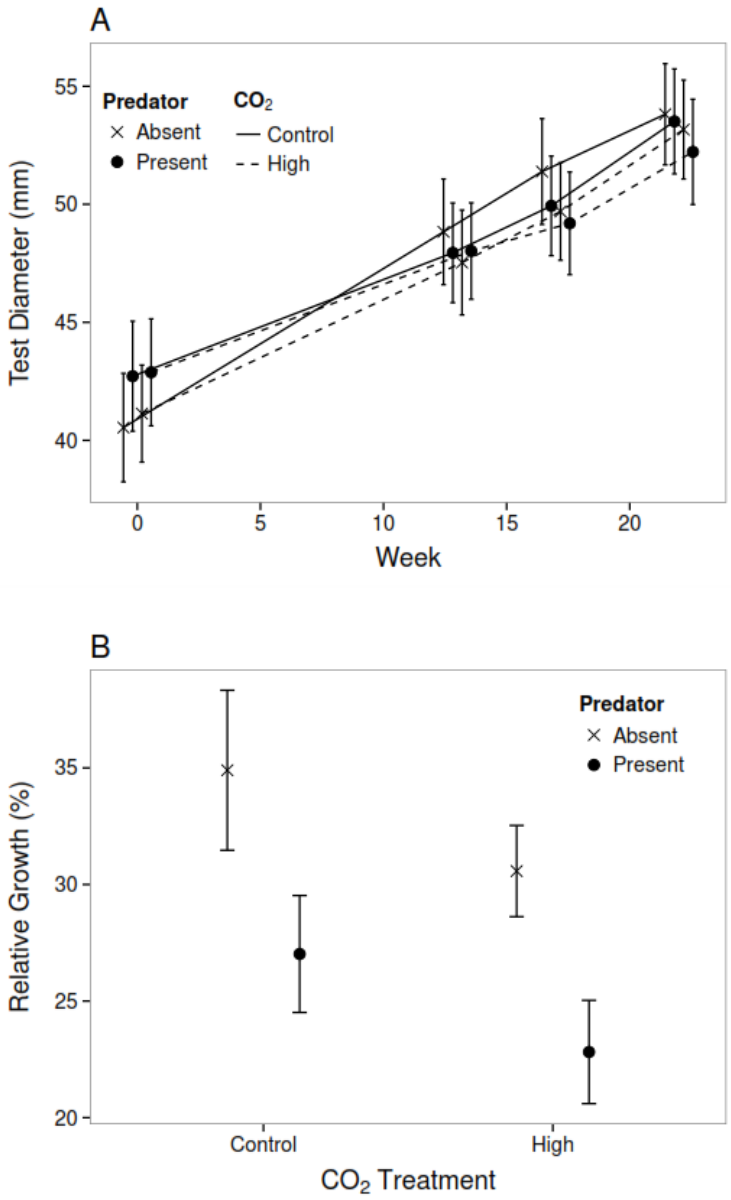

Figure 3-2. Effect of CO_2 and predator presence on A) test diameter (mm) over time and B) mean relative growth (percent change from initial diameter) for red sea urchins (*Strongylocentrotus franciscanus;* N=66). Urchins were exposed to ambient (pCO_2 ~ 500 µatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7) conditions for 22 weeks, with and without a caged predator (sunflower star, *Pycnopodia helianthoides*). Error bars represent 1 standard error of the mean. The effect was statistically significant for both treatments (see Table 3-1 for details).

Total calcified mass was significantly reduced by the presence of predators, but not by acidification (Figure 3-4A; Table 3-1B). In addition, the results suggest an interaction between predator treatment and urchin size. Smaller urchins had less calcified tissue in the presence of a predator, but this effect decreased with increasing test diameter (Appendix C, Figure C-4). Predators, but not acidification, also had a significant effect on spine and jaw calcified mass (Figure 3-4B; Table 3-1C), but there was no effect of either treatment on calcification in the test (Appendix C, Figure C-6). Mean spine length was significantly shorter in the high CO_2 treatment, but there was no effect of predators (Figure 3-5; Table 3-1D). There was no significant interaction between predator acclimation and CO_2 in any of the above comparisons.

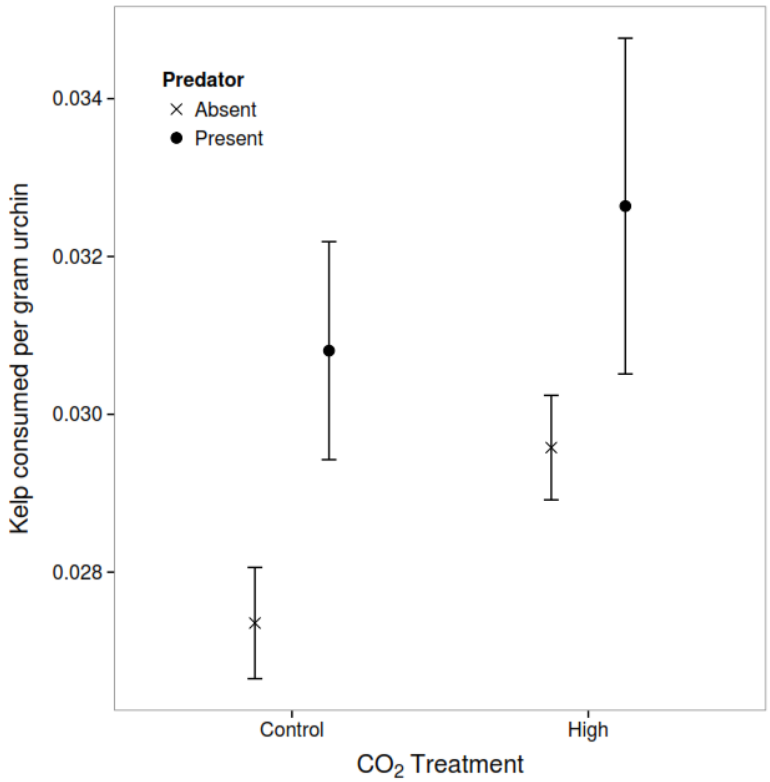

Figure 3-3. Mean kelp eaten per gram urchin for red sea urchins (*Strongylocentrotus franciscanus;* N=66) exposed to ambient (pCO_2 ~ 500 µatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7) conditions for 22 weeks, with and without a caged predator (sunflower star, *Pycnopodia helianthoides*). Error bars represent 1 standard error of the mean. The effect of predator presence, but not CO_2, was statistically significant (see text for details).

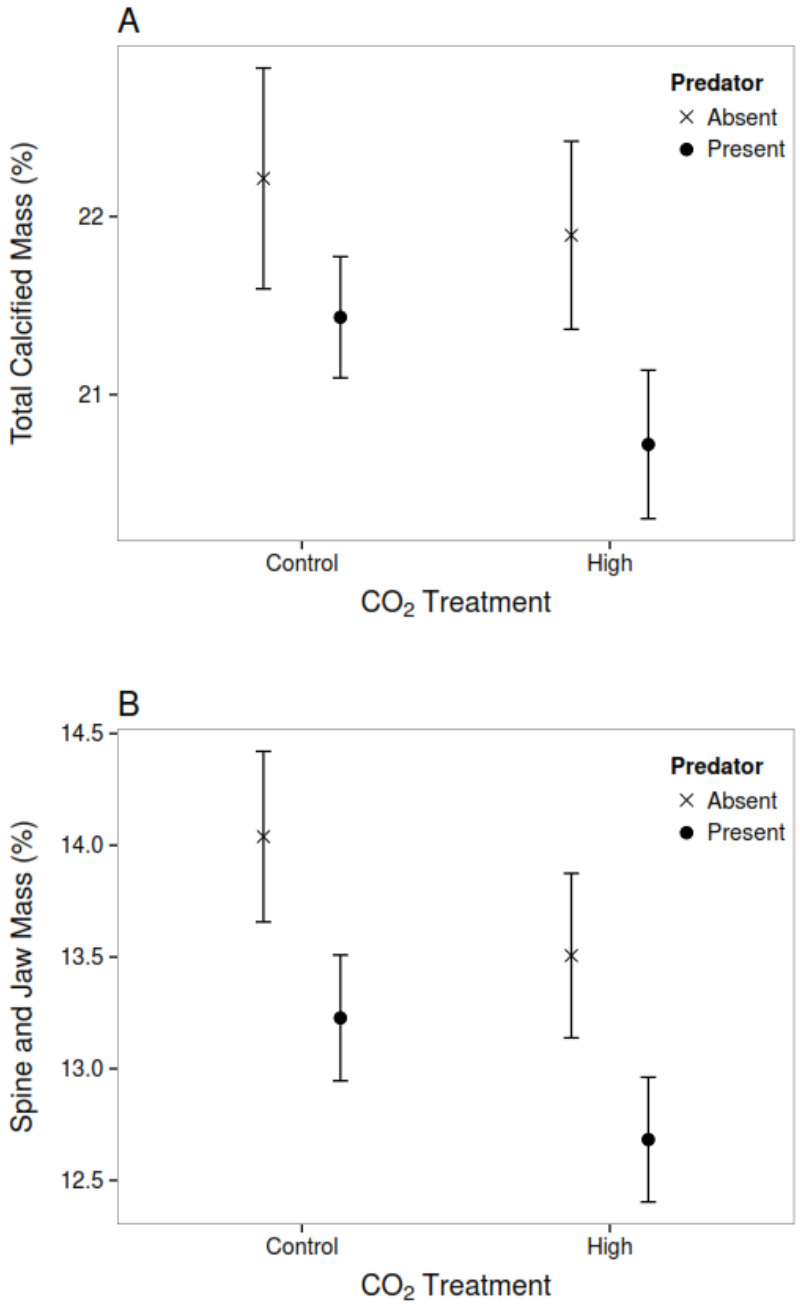

Figure 3-4. Effect of CO_2 and predator presence on A) total calcified mass (as a percentage of total wet mass) and B) spine and jaw calcified mass (as a percentage of total wet mass mass) for red sea urchins (*Strongylocentrotus franciscanus;* N=66). Error bars represent 1 standard error of the mean. The effect of predator presence, but not CO_2, was statistically significant (see Table 3-1 for details).

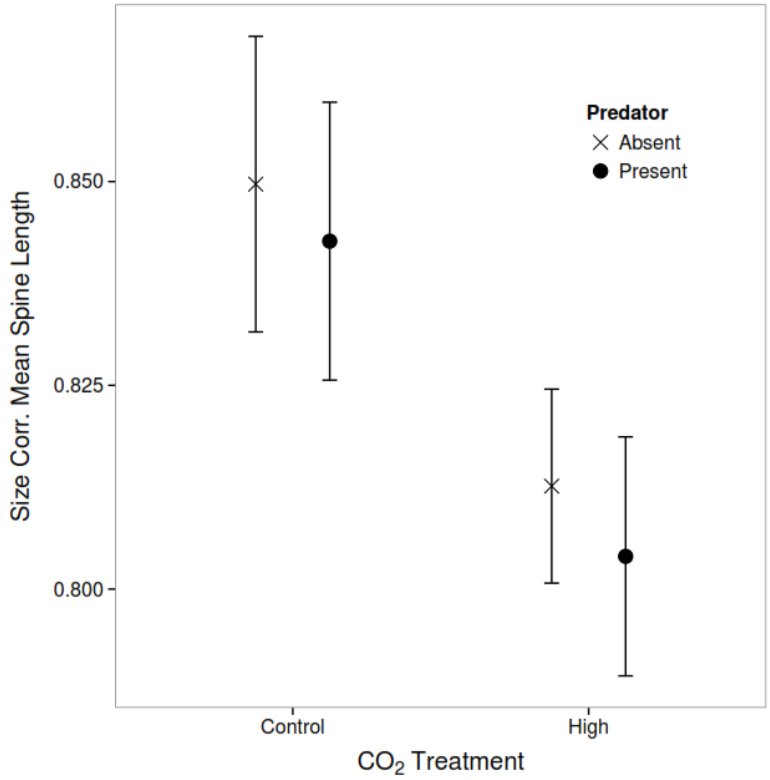

Figure 3-5. Mean size-corrected spine length for red sea urchins (*Strongylocentrotus franciscanus;* N=66) exposed to ambient (pCO_2 ~ 500 µatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7) conditions for 22 weeks, with and without a caged predator (sunflower star, *Pycnopodia helianthoides*). Error bars represent 1 standard error of the mean. The effect of CO_2, but not predator presence, was statistically significant (see Table 3-1 for details).

Exposure to chemical stimuli from *P. helianthoides* elicited a significantly higher avoidance response in *S. franciscanus*, relative to the seawater sham (Figure 3-6; Table 3-2). The percentage of urchins responding tended to decrease with acidification and with prior predator acclimation, but this effect was not significant. There were also no significant interactions between factors.

Table 3-1. Linear mixed effects models testing the effect of CO_2 treatment (control/high) and predator treatment (absent/present) on A) log final test diameter, B) log calcified mass, C) log spine and jaw mass, and D) mean spine length of red sea urchins (*Stronglyocentrotus franciscanus*). Urchin size (test diameter) is included as a covariate. (*) = significant effect

A) Log-transformed final test diameter

Parameter	Est.	SE	Likelihood-ratio χ^2	*df*	*P*
CO_2	-0.132	0.087	4.196	1	0.041*
Predator	-0.134	0.088	11.276	1	<0.001*
log(Initial Size)	0.674	0.055	150.64	1	<0.001*
CO_2 × Predator	-0.008	0.026	0.093	1	0.761
CO_2 × log(Initial Size)	0.077	0.062	1.524	1	0.217
Predator × log(I. Size)	0.064	0.061	1.064	1	0.302

B) Log-transformed calcified mass

Parameter	Est.	SE	Likelihood-ratio χ^2	*df*	*P*
CO_2	0.102	0.164	0.103	1	0.748
Predator	-0.415	0.166	7.114	1	0.008*
Size	0.050	0.003	183.6	1	<0.001*
CO_2 × Predator	0.006	0.059	0.012	1	0.915
CO_2 × Size	-0.002	0.003	0.515	1	0.473
Predator × Size	0.006	0.003	4.029	1	0.045*

C) Log-transformed spine and jaw mass

Parameter	Est.	SE	Likelihood-ratio χ^2	*df*	*P*
CO_2	0.083	0.184	0.560	1	0.454
Predator	-0.437	0.186	8.022	1	0.005*
Size	0.051	0.033	173.01	1	<0.001*
CO_2 × Predator	0.025	0.065	0.147	1	0.702
CO_2 × Size	-0.002	0.003	0.457	1	0.499
Predator × Size	0.006	0.003	3.153	1	0.076

D) Mean spine length

Parameter	Est.	SE	Likelihood-ratio χ^2	*df*	*P*
CO_2	-1.027	4.537	5.889	1	0.015*
Predator	11.427	4.603	0.810	1	0.368
Size	0.901	0.076	111.99	1	<0.001*
CO_2 × Predator	-0.590	1.516	0.151	1	0.698
CO_2 × Size	-0.014	0.085	0.028	1	0.867
Predator × Size	-0.227	0.085	6.697	1	0.009*

$N_{urchins}$ = 66 N_{tanks} = 22

Table 3-2. Mixed effects logistic regression testing the effect of cue type (seawater sham or predator), CO_2 treatment (control/high), and predator treatment to which the urchins were acclimated (absent/present) on red sea urchin (*Strongylocentrotus franciscanus*) alarm response. (*) indicates a significant effect.

Parameter	Est.	SE	Likelihood-ratio χ^2	*Df*	*p*	odds ratio
Cue	-3.21	0.925	35.846	1	<0.001*	0.040
CO_2	-0.536	0.788	1.030	1	0.310	0.585
Predator	-0.535	0.787	0.451	1	0.502	0.586
Cue × CO_2	0.299	0.984	0.091	1	0.762	1.348
Cue × Predator	0.893	0.986	0.826	1	0.363	2.442
CO_2 × Predator	-0.134	1.021	0.017	1	0.895	0.874

$N_{urchins}$ = 66 N_{tanks} = 22

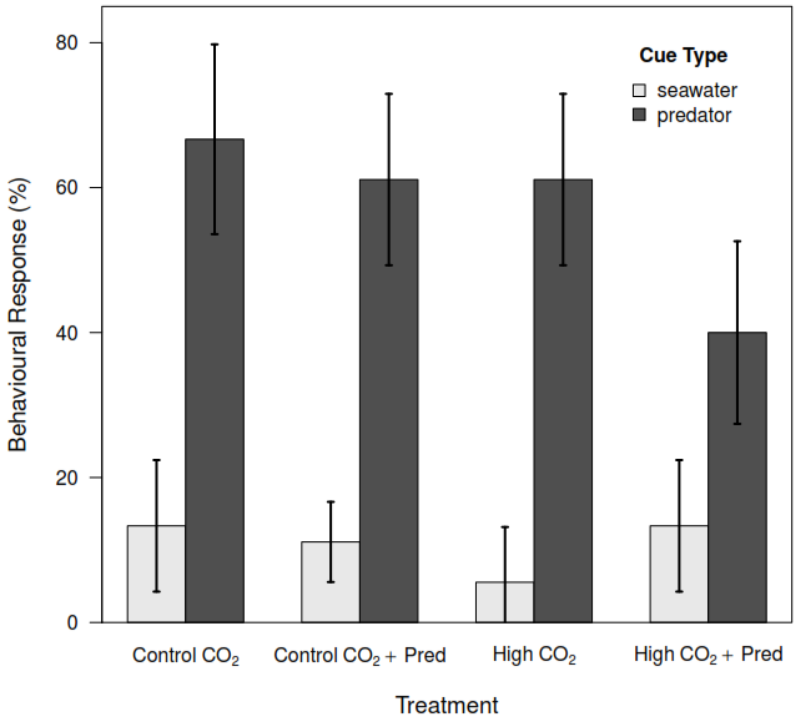

Figure 3-6. Percent avoidance response to seawater and predator chemical cues for red sea urchins (*Strongylocentrotus franciscanus;* N=66) exposed to ambient (pCO_2 ~ 500 µatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7) conditions for 22 weeks, with and without a caged predator (sunflower star, *Pycnopodia helianthoides*). Error bars represent 1 standard error of the proportion. There was no effect of either treatment (i.e., acclimation condition) on urchin behaviour, but the effect of cue type was statistically significant (see Table 3-2 for details).

3.4 Discussion

In this study, it was found that ocean acidification and predator presence influenced the performance of *Stronglyocentrotus franciscanus*. However, these two factors did not interact. Contrary to what has been observed in some other systems (e.g., littorinid snails, Bibby *et al.*, 2007), the effect of predator presence was not modified by high CO_2. Therefore, the hypothesis that OA would impair the development of induced defenses in *S. franciscanus* exposed to sea star cues was rejected. My results show predators and OA have an additive negative effect on urchin size-specific growth rate and calcified mass, but an additive positive effect on grazing rate.

Predator-induced effects on growth rate are well-studied in marine invertebrates (Brönmark and Hansson, 2000). Decreased prey growth rate in response to predation risk is often attributed to changes in behaviour (e.g., reduced foraging activity) or energy allocation (e.g., investment in defensive structures; Brönmark and Hansson, 2000). For example, Appleton and Palmer (1988) show exposure to chemical cues from a predatory crab (*Cancer productus*) induces shell thickening, but slower growth and feeding rates, in a marine gastropod (*Nucella lamellosa*). The precise mechanism by which predator presence affects growth in *S. franciscanus* remains unclear, but does not appear to be a result of reduced grazing or investment in skeletal structures. An alternative explanation could be that decreased growth is a consequence of elevated energetic demand associated with predator-induced physiological stress. Physiological responses to predation risk have been documented across a wide variety of invertebrate taxa, and may include increased cardiovascular activity, respiration, and metabolism (Hawlena and Schmitz, 2010).

The increase in kelp consumption observed in the presence of predators was unexpected as prey typically reduce foraging activity in response to predation risk (Brönmark and Hansson, 2000). Previous research shows predators limit red urchin grazing in nature by altering population spatial distribution (Watson, 1993). In addition, Matassa (2010) used laboratory experiments to show purple sea urchins (*S. purpuratus*) reduce grazing rates in the response to predator cues from spiny lobster (*Panulirus interruptus*). Increased grazing in the presence of predators may be a response to elevated energetic demand, although this does not appear to translate to higher growth rates. It is possible that predator-induced stress reduces the conversion efficiency of ingested food into biomass (Hawlena and Schmitz, 2010). Alternatively, Soto *et al.* (2005) show that nutritional condition affects the foraging strategy of intertidal whelks (*Acanthina monodona*) exposed to a sea star predator. They found that, in the presence of a predator, starved whelks fed faster than satiated whelks, but there was no difference when predators were absent.

Predator exposure resulted in a reduction in urchin calcified mass, which contrasts with expectations that urchins would upregulate calcification of defensive structures. Selden *et al.* (2009) show that *S. droebachiensis* develop thicker skeletons in response to crab cues. However, urchin physiological and behavioural responses are often predator-specific (Scheibling, 1995). Thicker skeletons may be more important for defense against crushing predators like crabs, but provide little protection from sea star predation. However, there was also no evidence of spine growth in response to predators. It is possible that increased investment in skeletal structures is not the most effective strategy for defending against *P. helianthoides*. Additionally, investment in defensive structures may be too energetically costly. *S. franciscanus* has been found to employ

a number of behavioural strategies to minimize predation risk, such as hiding in spatial refuges, aggregation, and nocturnal feeding (Tegner and Levin, 1983).

Decreased growth rates under high CO_2 may represent a physiological trade-off, where elevated metabolic demand associated with the increased cost of calcification and acid-base regulation reduces energy available for growth (Findlay *et al.*, 2011). Decreased urchin growth in the future may have consequences at both the population and community level. Slower-growing urchins spend more time in the vulnerable intermediate size class, which could translate to an increase in predation intensity and a decline in overall population growth (Scheibling, 1995). Reduced growth under acidification may also indirectly affect population size and recruitment by increasing time to reproductive maturity (Neinhuis, 2009).

Elevated CO_2 had no detectable effect on kelp consumption, although a positive trend was observed. This result is consistent with previous research, which suggests that OA does not directly impact mass-specific grazing rates (K. Anderson, personal communication). The positive trend may indicate that urchins are increasing consumption rates in an attempt to mediate higher maintenance costs under acidification. However, an increase in individual urchin grazing rate under high CO_2 may not necessarily translate to a change in *per capita* kelp consumption due to the concomitant decrease in urchin growth. OA may instead drive a decrease in kelp consumption because urchins spend more time smaller size classes (Neinhuis, 2009).

Ocean acidification had no effect on calcified tissue in the test, but urchin spines were significantly shorter in the high CO_2 treatment. Although OA has been shown to decrease calcification in other adult urchins (e.g., *Eucidaris tribuloides*, Ries *et al.*, 2009; *S. droebachiensis*, Holtmann *et al.*, 2013), this response appears to be highly species-specific (Dupont *et al.*, 2010a). The relatively low sensitivity of the test in *S. franciscanus* may be due to

differences in the protective external organic layer (Ries *et al.*, 2009). In addition, urchins have some ability to regulate the acid-base balance of extracellular fluid under acidification (Calosi *et al.*, 2013; Holtmann *et al.*, 2013). The mechanism by which acidification impacts calcification in red urchin spines remains unclear, but Holtmann *et al.* (2013) show acclimation to high CO_2 increases spine dissolution in *S. droebachiensis*. As spines are the primary structure used to defend against *P. helianthoides* in red urchins (Moitoza and Phillips, 1979), increased spine erosion could make urchins less resistant to predation in the future. A reduction in handling time due to weakened prey defense could potentially lead to an increase in *per capita* predation rate.

Chemical cues from *P. helianthoides* elicited a strong alarm response in *S. franciscanus*, but this was not significantly affected by high CO_2 or prior predator exposure. Although a slight trend was observed, where the percent avoidance response declined in urchins acclimated to predators or high CO_2, variation among individuals was high. OA has been shown to disrupt olfactory cue detection in a number of fish and invertebrate species (Leduc *et al.*, 2013), but this is one of the first studies to demonstrate a largely neutral effect. However, the mechanism by which OA may impact cue detection in sea urchins, if any, remains unclear. These results should be viewed with caution as it is uncertain if the cue concentrations used here are representative of natural settings.

It is important to note that the effects of OA on predators will also play a role in determining changes in predator-prey dynamics. Landes and Zimmer (2012) suggest that predator-prey interactions may not change if both predators and prey are negatively affected by acidification. However, differential effects of high CO_2 on predator-prey pairs could result in altered interaction strength and potentially scale up to changes in community structure (Keppel *et al.*, 2015). For example, the effects of OA on urchin populations could be magnified if the

performance of predators, such as sea stars, is improved (Dupont *et al.*, 2010b; Gooding *et al.*, 2009). Although these complex responses to acidification are largely unexplored in the literature, they may have major consequences for marine communities (Gaylord *et al.*, 2015). Understanding the effects of OA on predator-prey interactions may be particularly important for ecosystems with strong top-down control, such as kelp forests, where changes in the relative abundance of predators and herbivores can lead to dramatic phase shifts in kelp forest communities (Estes and Palmisano, 1974).

Chapter 4

Conclusion

4.1 Summary of Results

There is a growing consensus among researchers that disentangling the effects of ocean acidification on species interactions will be critical for predicting ecosystem-level changes in the future ocean (Gaylord *et al.*, 2015). This thesis sought to determine the effect of OA on a key predator-prey pair in benthic subtidal communities of the Pacific Northwest: the red urchin (*Strongylocentrotus franciscanus*) and the sunflower star (*Pynopodia helianthoides*). The data presented in Chapters 2 and 3 provide a number of important insights that help address considerable gaps in our understanding. In Chapter 2, I show that the growth and feeding rates of juvenile *P. helianthoides* increase with long-term exposure to elevated CO_2, but calcification is not affected. These data suggest *P. helianthoides* is highly tolerant of OA and *per capita* predation rate may increase in the future. In Chapter 3, the data suggest predators and OA have an additive negative effect on the growth and calcification of *S. franciscanus*, but a positive effect on grazing rate. The predator avoidance response, while pronounced, was not affected by either acidification or prior exposure to predator cue. These results suggest both predators and ocean acidification matter for individual urchin performance, but they do not appear to interact at the physiological level. Still, we have yet to explore how they may influence one another *via* changes in population sizes and *per capita* consumption rates.

4.2 Effects of OA on Predator-Prey Interactions

My results show that ocean acidification has direct effects on individual performance in both sunflower stars and red sea urchins, but predator-induced responses do not appear to be modified by high CO_2 (Figure 4-1). Altered pairwise dynamics in this predator-prey system could instead manifest through changes in interaction strength. Although the effect of CO_2 on interaction strength was not directly tested here, there are a few compelling examples from other systems that suggest OA may modify *per capita* consumption. Sanford *et al.* (2014) show that a reduction in the body size of Olympia oysters (*Ostrea lurida*) under elevated CO_2 increases the consumption rate of an OA-tolerant invasive snail. Increased oyster predation by muricid gastropods has also been attributed to weakened shell strength (Amaral *et al.*, 2012). Conversely, Keppel *et al.* (2015) found that the growth rate of the sea star *Asterias rubens* decreased with high CO_2 exposure, but the growth of its prey (blue mussels, *Mytilus edulis*) increased, resulting in a reduction in *per capita* predation rate. Furthermore, Munday *et al.* (2010) show acidification dramatically reduces the survival of larval reef fish due to impaired olfactory cue detection and altered predator avoidance behaviour.

The above examples suggest a number of mechanisms by which OA can modify predator-prey interactions (e.g., increased prey susceptibility, changes in predator and prey growth rates, decreased predator handling time and satiation, etc.). I propose that similar mechanisms may apply to the sea star – urchin relationship, where the differential effects of OA drive an increase in *per capita* interaction strength. Urchins that are smaller and more weakly defended may be more vulnerable to predation by *P. helianthoides*. Increases in consumption rate may also occur if predator energetic demands are elevated under high CO_2. As *P. helianthoides* is a key predator in benthic subtidal communities (Duggins, 1983), changes in *per*

capita consumption could have a considerable impact on community structure. It is well documented that increased predation pressure can reduce the abundance of herbivores and indirectly promote algal recruitment (Estes and Palmisano, 1974; Dayton, 1985). An important caveat is that *per capita* consumption is highly density-dependent (Holling, 1959) and the strength of trophic interaction may not increase if prey populations decline substantially in the future. Declines in prey abundance or energetic content may also drive shifts in predator diet (Gaylord *et al.*, 2015), causing further community-level change.

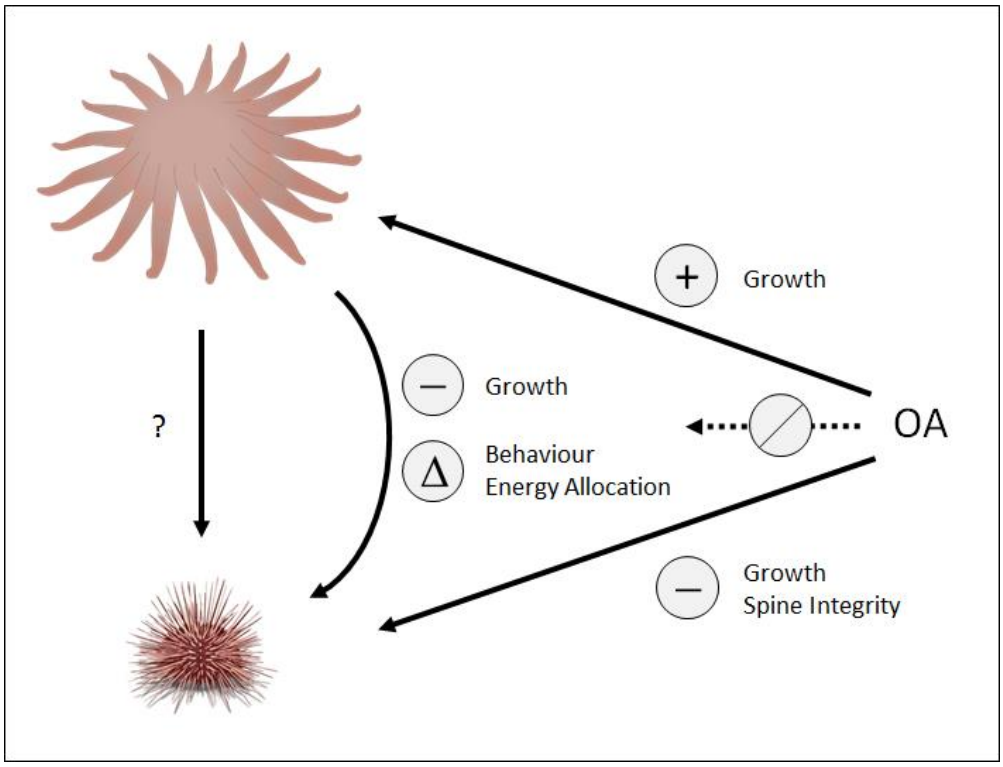

Figure 4-1. Conceptual diagram of the direct (solid lines) and indirect (dashed line) pathways through which ocean acidification (OA) may affect predator-prey interactions between sunflower stars (*Pycnopodia helianthoides*) and red sea urchins (*Strongylocentrotus franciscanus*). Nonconsumptive predator effects are indicated by the curved arrow. Circles indicate positive (+), negative (–), or neutral (/) effects. Image credit: Saxby (2005)

4.3 Study Limitations

Variation in seawater pCO_2 and pH was relatively high over the course of the experiment, which was a result of fluctuation in ambient CO_2 levels. On week 8 of the experiment, pCO_2 in the acidified treatment increased to almost double target levels (~ 2000 μatm). The implications of this spike in pCO_2 are unclear, but given that my results suggest the effects of OA on calcification are relatively minor, it appears both red urchins and sunflower stars are able to tolerate extreme conditions to some degree. It is possible that experimental variation in seawater chemistry parameters is more representative of natural conditions in coastal ecosystems. For example, a short-term increase in acidity could occur in nature during a seasonal upwelling event (Hoffman *et al.*, 2011). Variation in biological activity and tidal mixing also create heterogeneity in the pH of near-shore habitats (Hoffman *et al.*, 2011). This variability may influence organism physiological responses, resilience, and potential for adaptation.

Longer time scale consideration of adaptation and acclimatization are also important. The 22 week acclimation in this study does not capture the long-term and trans-life-cycle effects of OA. Long-term responses to OA may be very different from those observed over a single generation or life-stage (Dupont *et al.*, 2013). These effects are discussed in more detail in the following section.

4.4 Recommendations for Future Research

A number of questions remain as to how OA will impact interactions between *S. franciscanus* and *P. helianthoides*. A key avenue of investigation will be to experimentally test the effect of OA on *per capita* predation rate, which has considerable influence on community structure *via*

indirect effects on habitat-forming kelp species (Dayton, 1985). Scaling up to multi-species food webs would also provide valuable insight into the complex effects of OA on species interactions.

Population viability and persistence will depend not only on the sensitivity of adults to OA, but also on variation in sensitivity across life-history stages. Early developmental stages are generally considered most vulnerable to changes in seawater acidity (Byrne, 2011; Kroeker *et al.*, 2013a). Studies show acidification can cause delayed larval development and reduced fertilization success in both sea stars and urchins (Kurihara, 2008; Havenhand *et al.*, 2008; Parker *et al.*, 2009; Gonzalez-Bernat *et al.*, 2013, Reuter *et al.*, 2011). In addition, carry-over effects across multiple life-history stages and generations could have serious implications for long term population sustainability (Dupont *et al.*, 2013).

Determining the degree of genetic variation and phenotypic plasticity among populations will also be essential for assessing the potential for adaptation and acclimatization to OA. Due to the naturally variable pH environment found throughout the Pacific Northwest (Feely *et al.*, 2008), *P. helianthoides* and *S. franciscanus* may have a greater capacity to acclimatize and adapt to changing conditions. There is some evidence to suggest green urchins (*S. droebachiensis*) can acclimate to elevated CO_2 over relatively short time spans (Dupont *et al.*, 2013). In addition, Sunday *et al.* (2011) suggest evolutionary responses to rising CO_2 in *S. franciscanus* may be relatively fast due to high levels of both genetic and phenotypic variation, although their study only considered one trait (early larval growth). Understanding long-term adaptation across a suite of genetically-determined traits will require a great deal of additional research.

The simultaneous increase in ocean temperature predicted under current climate change scenarios (IPCC, 2013) also has the potential to affect species interactions. In general, temperature appears to enhance sensitivity to OA, although this effect varies depending on the

species or response being tested (Kroeker *et al.*, 2013a). For example, increased temperature stimulates faster arm regeneration in the brittlestar *Ophiocten sericeum,* but this effect is counteracted by acidification (Wood *et al.*, 2011). Understanding the impact of rising ocean CO_2 on predator-prey dynamics will require further research into the interactive effects with warming.

Given the various gaps in our understanding, disentangling the effects of ocean acidification on species interactions presents a formidable challenge. The strong context-dependent nature of responses to OA makes it difficult to form general predictions about future ecological change. However, we may begin to tackle these complex questions through empirical tests of conceptual models. Few studies have taken such an approach to understanding the indirect effects of OA on predator-prey relationships. My thesis demonstrates that the role of biotic stressors should not be overlooked when considering organismal responses to high CO_2.

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Appendices

A. Seawater Chemistry

Table A-1. Seawater chemistry parameters (mean ± SE) measured or calculated for control and acidified treatments. Temperature, salinity, pH, and dissolved inorganic carbon (DIC) were measured. Parameters identified with an asterisk (*) – total alkalinity (TA), CO_2 partial pressure (pCO_2), CO_2 fugacity (fCO_2), bicarbonate and carbonate ions concentration ([HCO_3^-] and [CO_3^{2-}]), calcite and aragonite saturation (Ω_{calc} and Ω_{arag}) – were calculated using CO2SYS (Pierrot *et al.*, 2006).

Parameter	Control	Acidified
Temperature (°C)	12.80 ± 0.05	12.74 ± 0.04
Salinity (ppt)	32.8 ± 0.1	32.6 ± 0.1
pH	7.96 ± 0.01	7.68 ± 0.01
DIC (µmol kg^{-1})	1569.28 ± 14.25	1652.91 ± 11.35
TA (µmol kg^{-1}) *	1664.59 ± 14.87	1676.08 ± 11.44
pCO_2 (µatm) *	511.50 ± 10.88	1052.81 ± 26.18
fCO_2 (µatm) *	510.30 ± 10.77	1048.89 ± 26.08
CO_3^{2-} (µmol kg^{-1}) *	69.36 ± 1.33	39.56 ± 0.91
HCO_3^- (µmol kg^{-1}) *	1479.17 ± 13.45	1570.60 ± 10.77
Ω_{calc} *	1.67 ± 0.03	0.95 ± 0.02
Ω_{arag} *	1.06 ± 0.02	0.61 ± 0.01

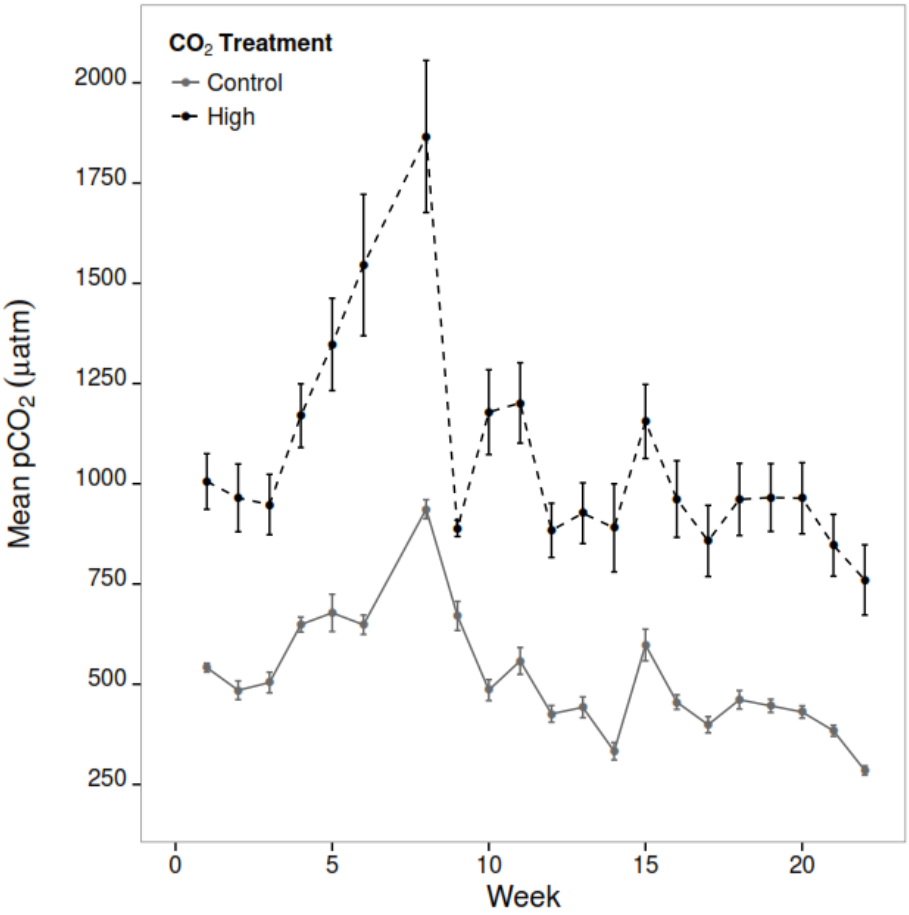

Figure A-1. Mean pCO_2 (µatm) measured weekly in laboratory mesocosms airated with ambient (~500 µatm) and elevated (~1000 µatm) levels of CO_2. Partial seawater changes were carried out at weeks 8 and 15. The increase in pCO_2 from weeks 5-8 was a result of high ambient CO_2 levels in the laboratory. The effect of ambient air on seawater pCO_2 was mitgated by installing CO_2 scrubbers on week 9. Error bars represent standard error of the mean.

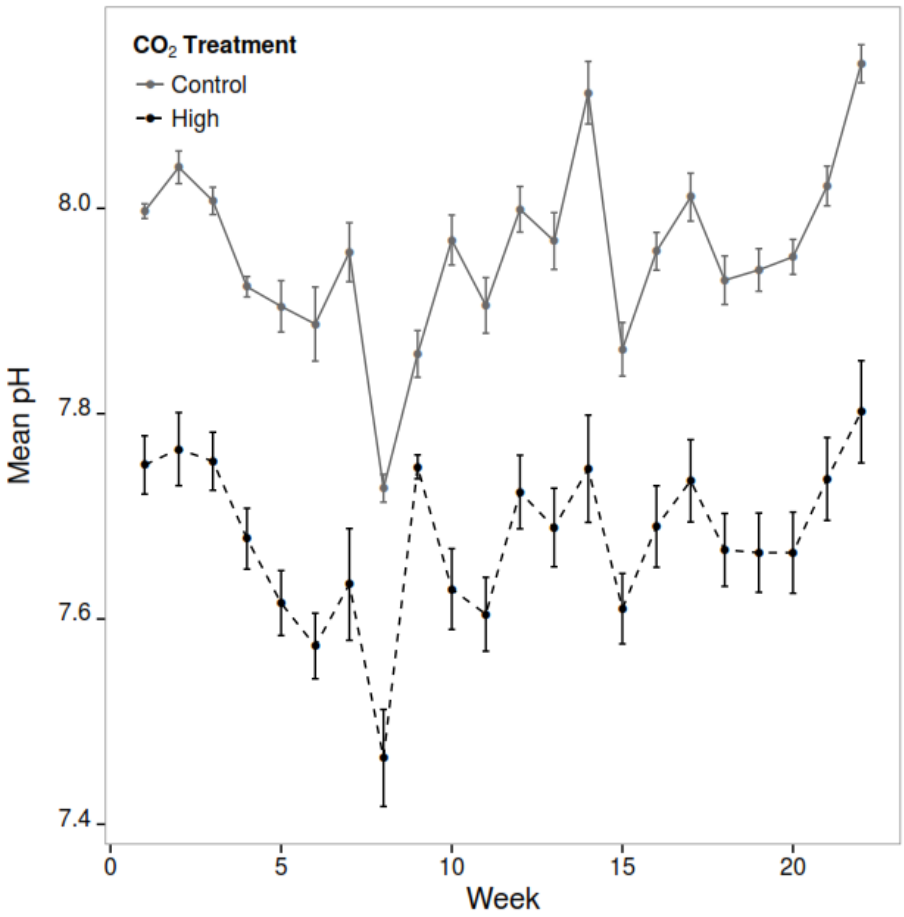

Figure A-2. Mean pH measured weekly in laboratory mesocosms airated with ambient (~500 µatm) and elevated (~1000 µatm) levels of CO_2. Partial seawater changes were carried out at weeks 8 and 15. The decrease in pH from weeks 5-8 was a result of high ambient CO_2 levels in the laboratory. The effect of ambient air on seawater pH was mitgated by installing CO_2 scrubbers on week 9. Error bars represent standard error of the mean.

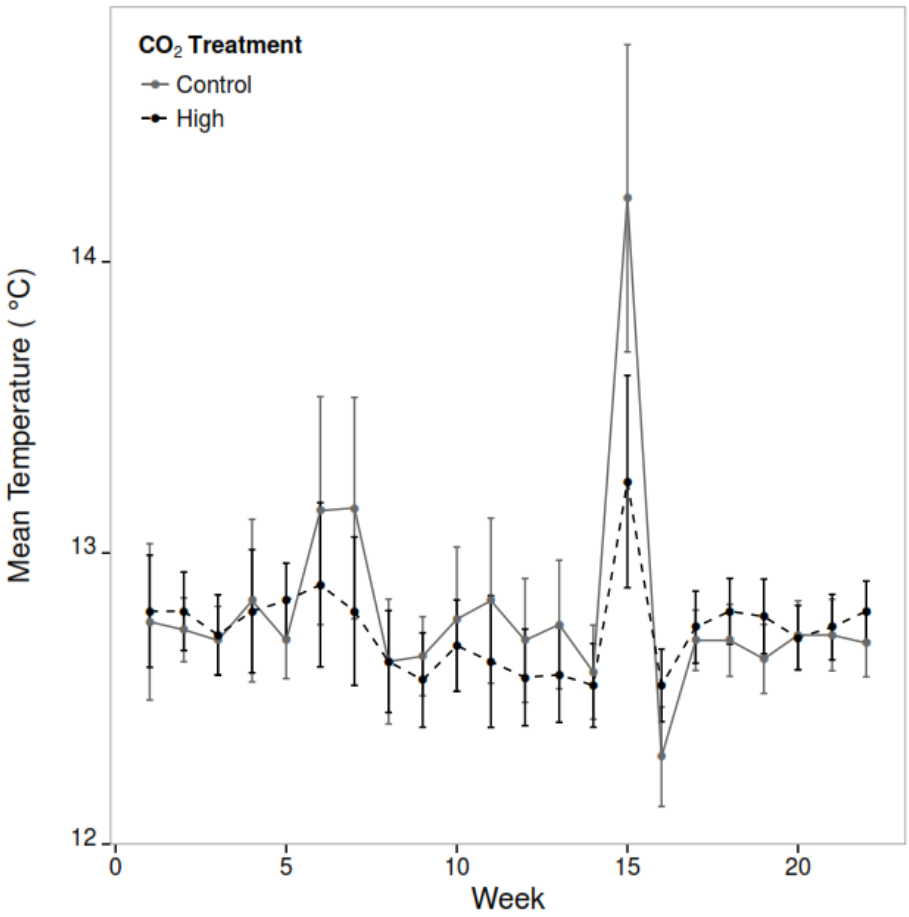

Figure A-3. Mean temperature (°C) measured weekly in laboratory mesocosms airated with ambient (~500 µatm) and elevated (~1000 µatm) levels of CO_2. The spike in temperature at week 15 occurred due to a mechanical failure in the air conditioning system, but had no observable effect on the experimental animals. Error bars represent standard error of the mean.

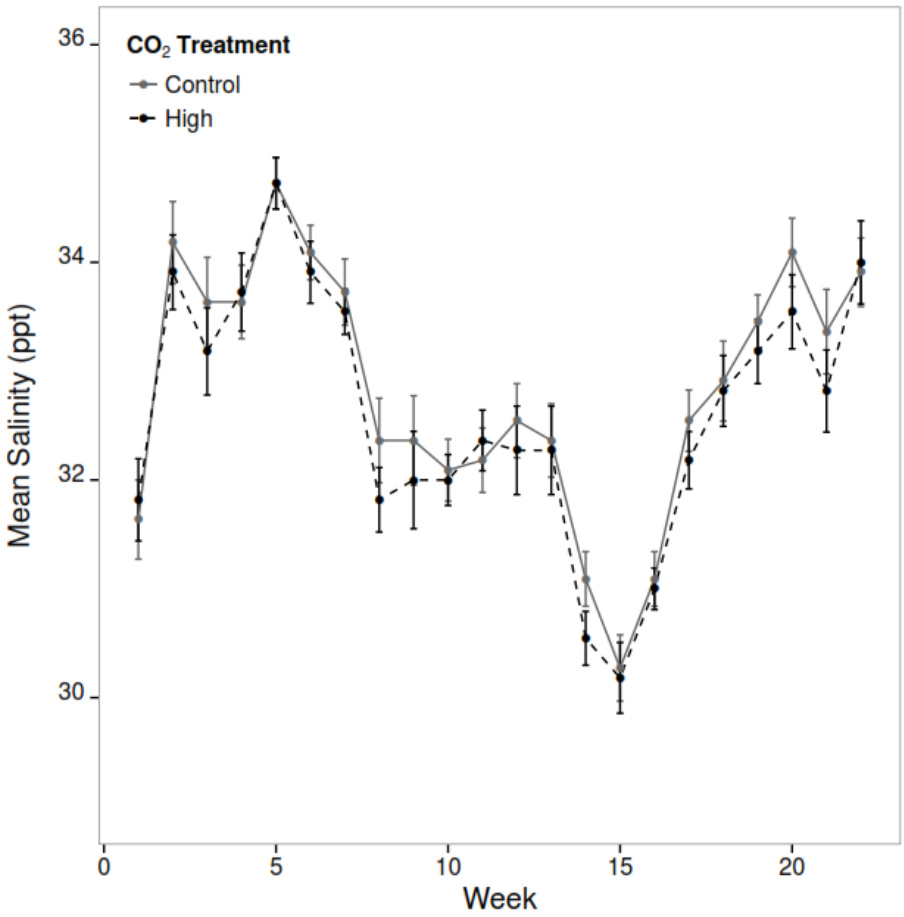

Figure A-4. Mean salinity (ppm) measured weekly in laboratory mesocosms airated with control (~500 µatm) and high (~1000 µatm) levels of CO_2. Partial seawater changes were carried out at weeks 8 and 15. Error bars represent standard error of the mean.

B. Chapter 2 Supplementary Figures

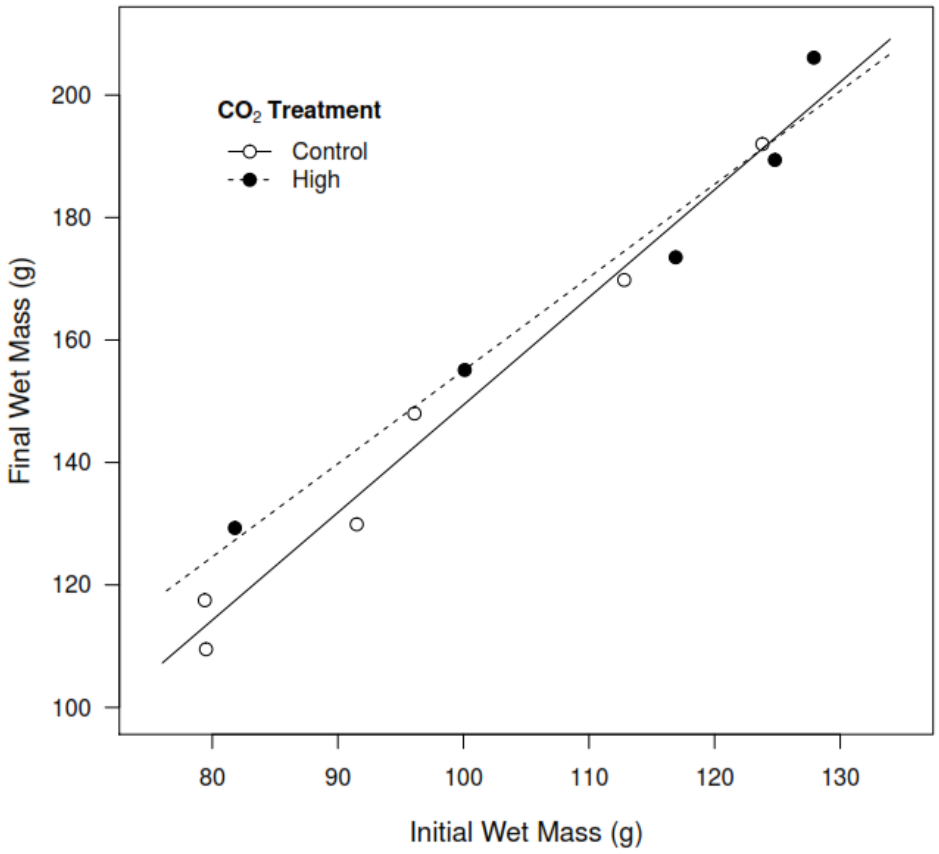

Figure B-1. Scatterplot of final wet mass (g) by initial wet mass (g) of *Pycnopodia helianthoides* (n=11) exposed to ambient (pCO_2 ~ 500 µatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7). Lines represent linear regressions. There was no significant difference between CO_2 treatments (ANCOVA; CO_2, $F_{1,7}$ = 1.55, p = 0.25, initial wet mass, $F_{1,7}$ = 253.64, p < 0.0001, interaction, $F_{1,7}$ = 1.30, p = 0.29).

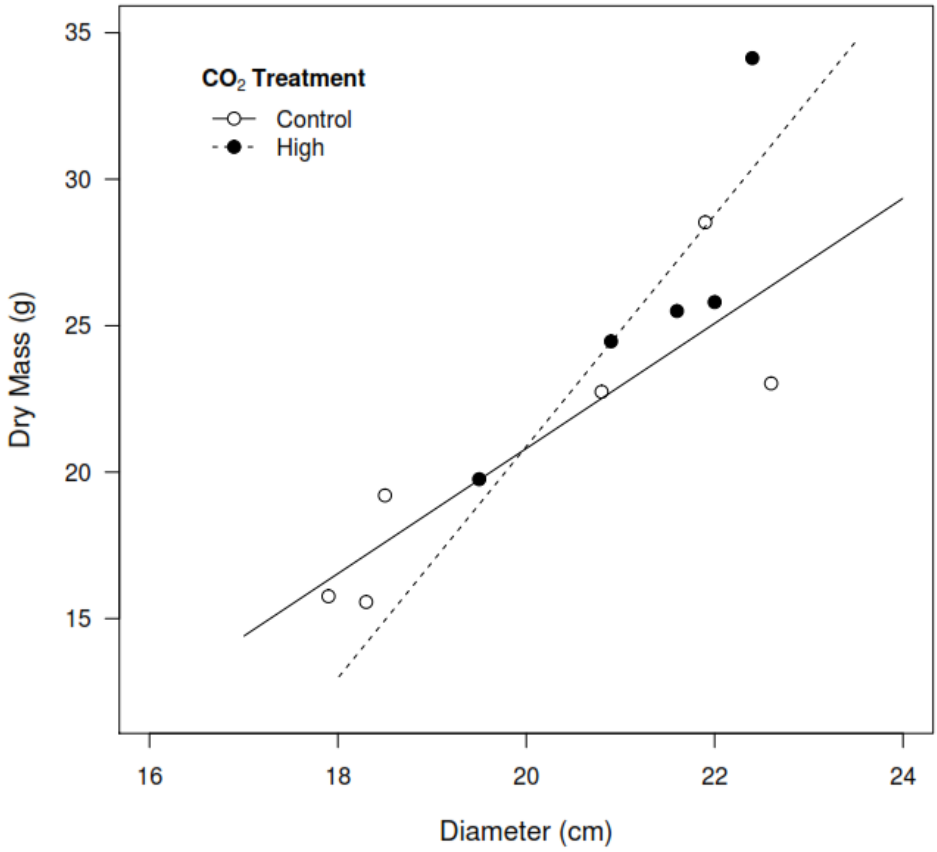

Figure B-2. Scatterplot of final diameter (cm) by dry mass (g) of *Pycnopodia helianthoides* (n=11) exposed to ambient (pCO_2 ~ 500 µatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7) conditions for 22 weeks. Lines represent linear regressions. There was no significant difference between CO_2 treatments (ANCOVA; CO_2, $F_{1,7}$ = 1.06, p = 0.34, diameter, $F_{1,7}$ = 19.87, p = 0.003, interaction, $F_{1,7}$ = 1.69, p = 0.24).

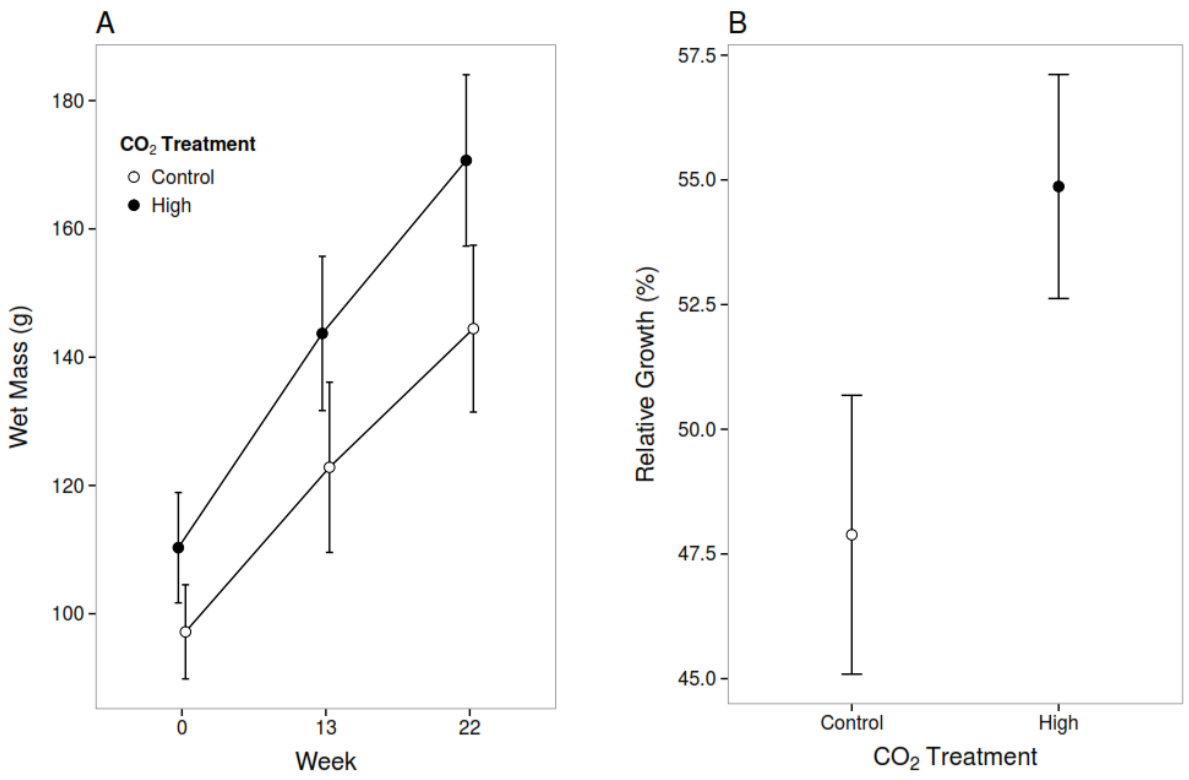

Figure B-3. Effect of CO_2 manipulation on A) mean wet mass (g) and B) mean relative growth (percent change from initial wet mass) of *Pycnopodia helianthoides* (n=11). Sea stars were exposed to ambient (pCO_2 ~ 500 µatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7) conditions for 22 weeks. Error bars represent 1 standard error (SE) of the mean. The effect of initial size, but not CO_2, on final sea star wet mass was statistically significant (ANCOVA; CO_2, $F_{1,7} = 1.56$, $p = 0.25$, initial wet mass, $F_{1,7} = 253.64$, $p < 0.0001$, interaction, $F_{1,7} = 1.3$, $p = 0.29$).

C. Chapter 3 Supplementary Figures

Table C-1. Linear mixed effects model testing the effects of CO_2 treatment (control/high) and predator treatment (absent/present) on log final wet mass (g) of red sea urchins (*Stronglyocentrotus franciscanus*). Urchin size (test diameter) is included as a covariate. (*) indicates a significant effect.

Parameter	**Est.**	**SE**	**Likelihood-ratio χ^2**	***df***	***p***
CO_2	-0.272	0.138	3.583	1	0.058
Predator	-0.454	0.138	5.352	1	0.021*
log(Size)	0.661	0.034	190.18	1	<0.001*
CO_2 × Predator	0.029	0.062	0.216	1	0.642
CO_2 × log(Size)	0.056	0.039	2.142	1	0.143
Predator × log(Size)	0.109	0.039	7.055	1	0.008*

$N_{urchins}$ = 66 N_{tanks} = 22

Table C-2. Linear mixed effects model testing the effects of CO_2 treatment (control/high) and predator treatment (absent/present) on log test calcified mass of red sea urchins (*Stronglyocentrotus franciscanus*). Urchin size (test diameter) is included as a covariate. (*) indicates a significant effect.

Parameter	**Est.**	**SE**	**Likelihood-ratio χ^2**	***df***	***p***
CO_2	0.136	0.161	0.260	1	0.610
Predator	-0.380	0.162	3.240	1	0.072
Size	-0.050	0.003	179.94	1	<0.001*
CO_2 × Predator	-0.024	0.061	0.156	1	0.693
CO_2 × Size	0.002	0.003	0.484	1	0.487
Predator × Size	0.006	0.003	4.450	1	0.035

$N_{urchins}$ = 66 N_{tanks} = 22

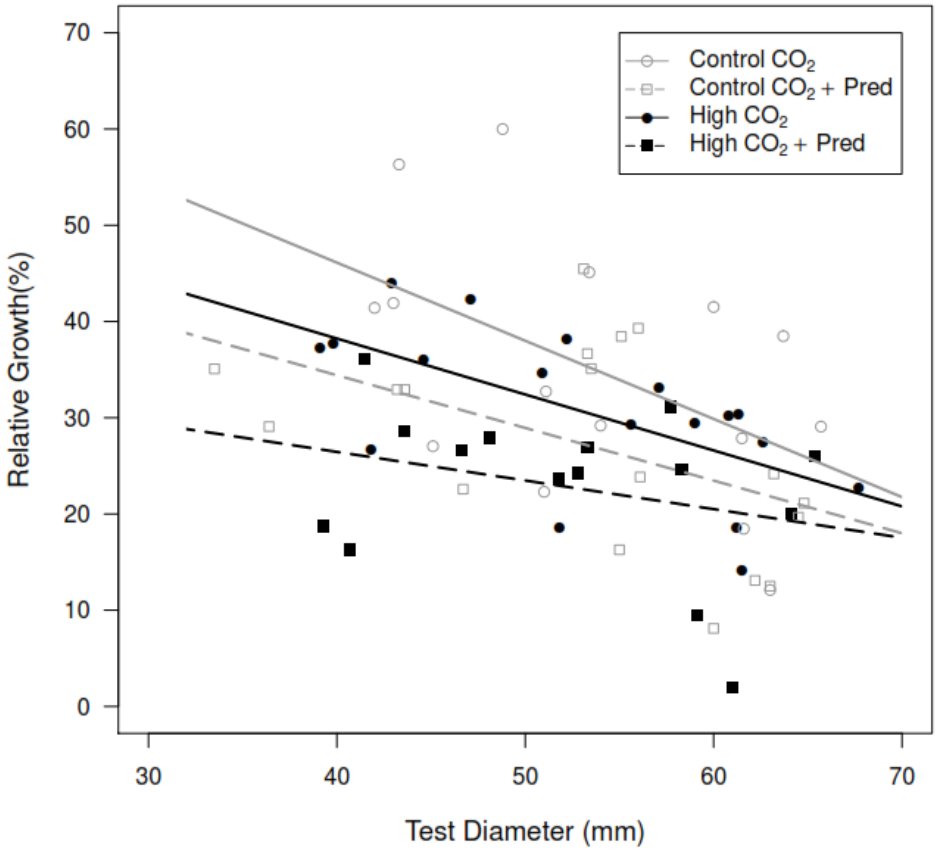

Figure C-1. The relationship between test diameter (mm) and relative growth (percent change from initial diameter) for red sea urchins (*Strongylocentrotus franciscanus;* N=66) exposed to ambient (pCO_2 ~ 500 µatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7) conditions for 22 weeks, with and without a caged predator (sunflower star, *Pycnopodia helianthoides*). The effect was statistically significant for both treatments (see Table 3-1 for details).

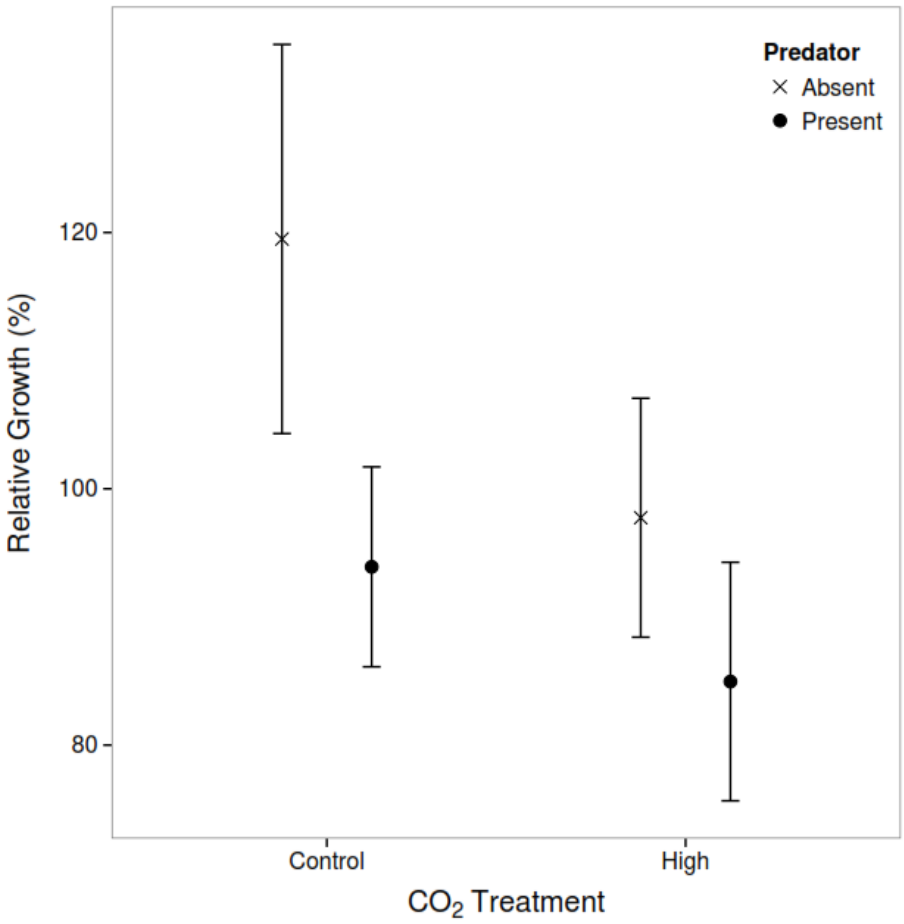

Figure C-2. Mean relative growth (percent change from initial wet mass) for red sea urchins (*Strongylocentrotus franciscanus;* N=66) exposed ambient (pCO_2 ~ 500 µatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7) conditions for 22 weeks, with and without a caged predator (sunflower star, *Pycnopodia helianthoides*). Error bars represent 1 standard error of the mean. There was a statistically significant effect of predator presence, but the effect of CO_2 was marginally non-significant (see Table C-1 for details).

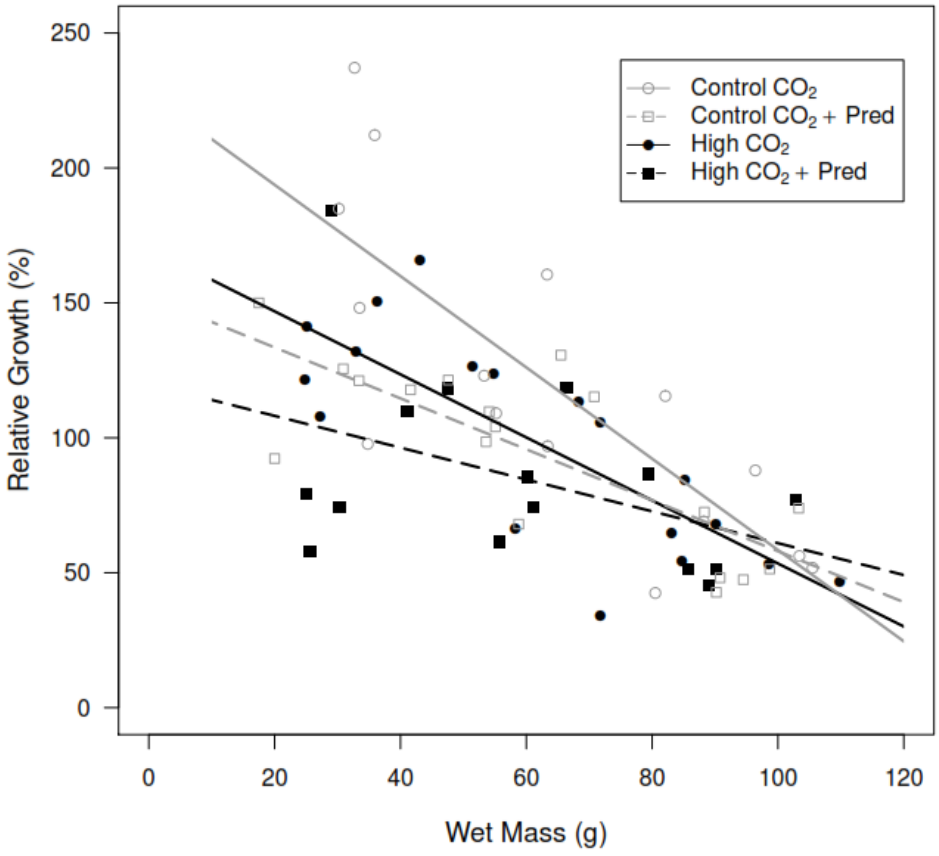

Figure C-3. The relationship between wet mass (g) and relative growth (percent change from initial wet mass) for red sea urchins (*Strongylocentrotus franciscanus;* N=66) exposed to ambient (pCO_2 ~ 500 µatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7) conditions for 22 weeks, with and without a caged predator (sunflower star, *Pycnopodia helianthoides*). There was a statistically significant effect of predator presence, but the effect of CO_2 was marginally non-significant (see Table C-1 for details).

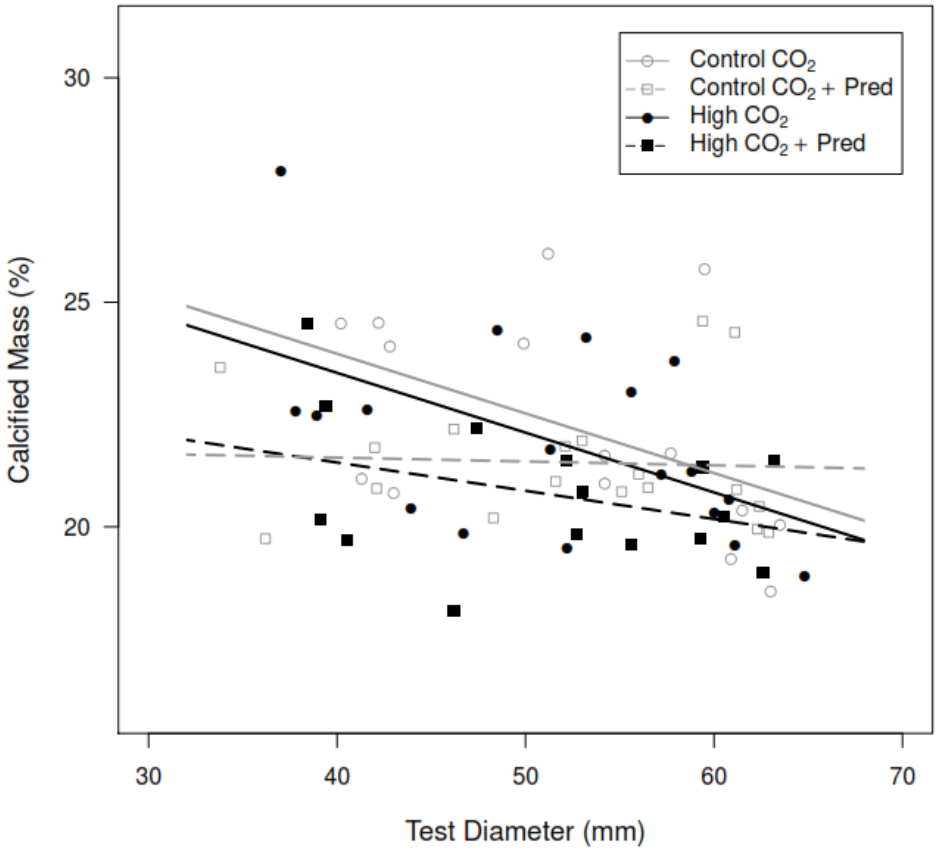

Figure C-4. The relationship between test diameter (mm) and calcified mass (as a percentage of total wet mass) for red sea urchins (*Strongylocentrotus franciscanus;* N=66) exposed to ambient (pCO_2 ~ 500 µatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7) conditions for 22 weeks, with and without a caged predator (sunflower star, *Pycnopodia helianthoides*). The effect of predators, but not CO_2, was statistically significant. There was also a significant interaction between size and predator presence (see Table 3-1 for details).

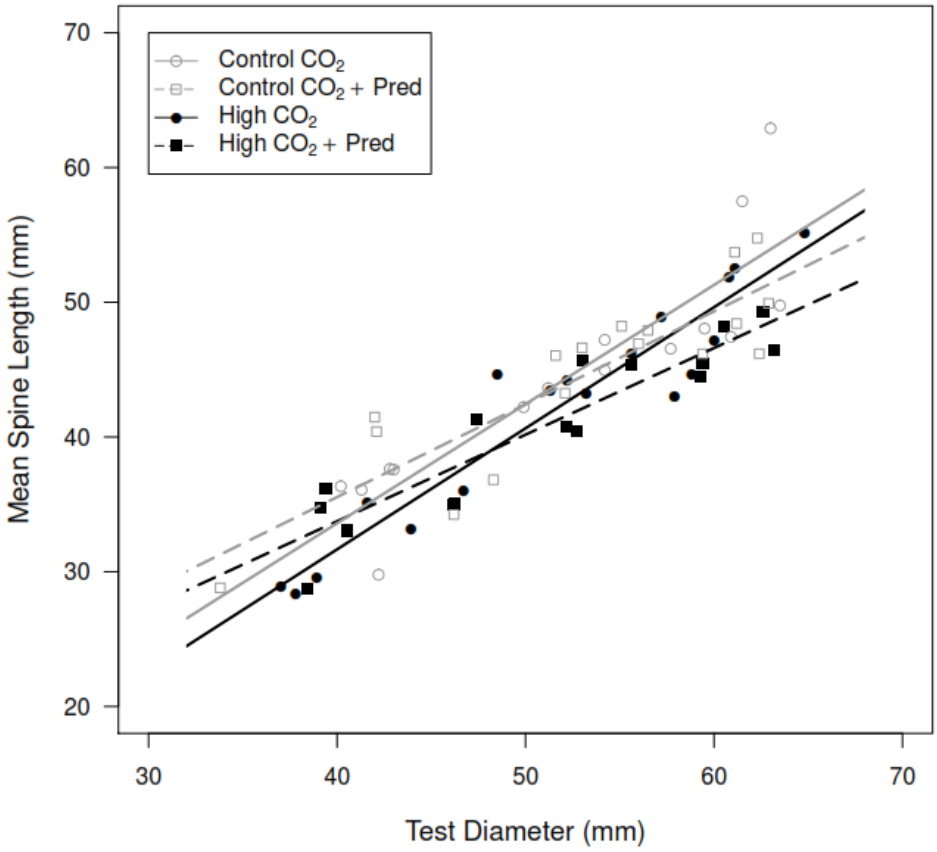

Figure C-5. The relationship between test diameter (mm) and mean length (mm) of the longest spine (n=10 spines per urchin) for red sea urchins (*Strongylocentrotus franciscanus;* N=66) exposed to ambient (pCO_2 ~ 500 µatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7) conditions for 22 weeks, with and without a caged predator (sunflower star, *Pycnopodia helianthoides*). The effect of CO_2, but not predator presence, was statistically significant (see Table 3-1 for details).

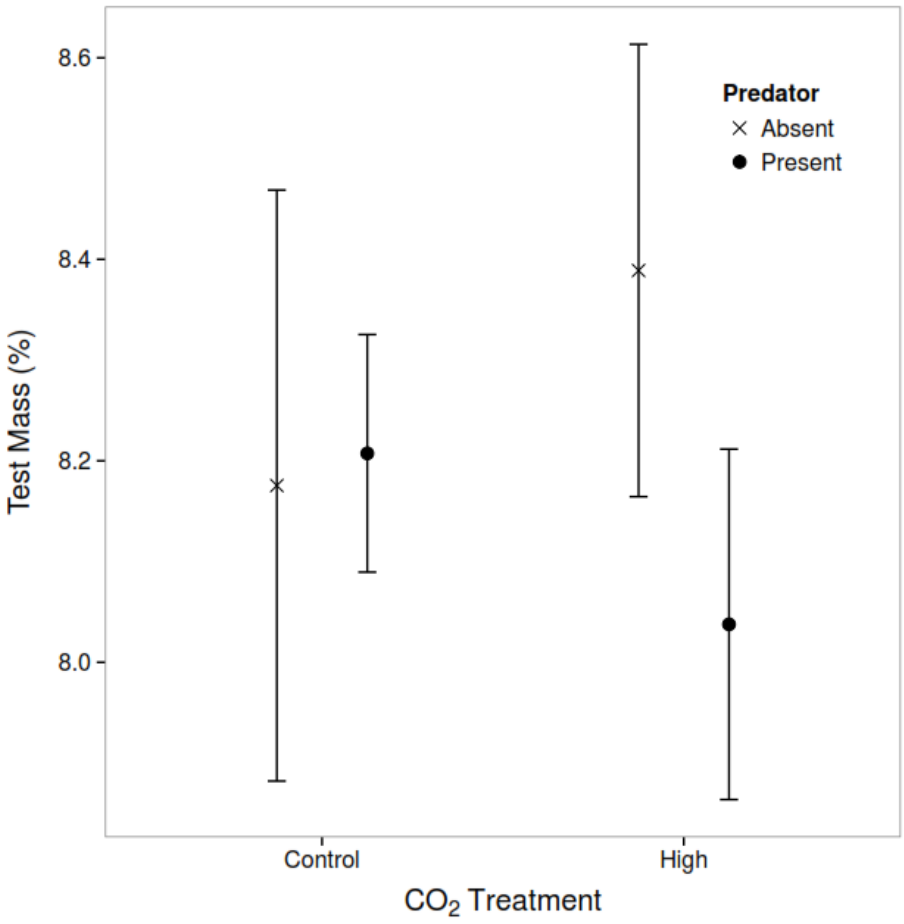

Figure C-6. Test calcified mass (as a percentage of total wet mass) for red sea urchins (*Strongylocentrotus franciscanus;* N=66) exposed to ambient (pCO_2 ~ 500 µatm, pH ~ 8.0) or acidified (pCO_2 ~ 1000 µatm, pH ~ 7.7) conditions for 22 weeks, with and without a caged predator (sunflower star, *Pycnopodia helianthoides*). Error bars represent 1 standard error of the mean. There was no significant effect of either treatment (see Table C-2 for details).