ALGAL-HERBIVORE INTERACTIONS IN A HIGH CARBON WORLD: DIRECT AND INDIRECT EFFECTS THROUGH INDIVIDUALS, POPULATIONS, AND COMMUNITIES

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Zoology)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

November 2017

Abstract

Consumer-resource interactions play an important role in determining the structure and function of ecological communities. Thus, herbivores may buffer or magnify the impacts of environmental change. In this thesis, I examine the ways in which herbivory mediates the effects of one of the most important facets of environmental change in marine ecosystems: ocean acidification (OA). Responses to OA by invertebrate herbivores are wide ranging, typically negative, and depend on species traits (e.g. reliance on calcification), population dynamics, and shifts in interspecific interactions. My goal was to conduct research across levels of biological organization to better understand the main pathways by which OA and associated increases in carbon dioxide (CO_2) will drive ecological change in herbivore-dominated systems.

In Chapter 2, I examine the effect of CO_2 on herbivore growth and size-specific changes in feeding rate. I found that CO_2 had no impact on the size-specific feeding rates of the four-herbivore species I examined. However, changes in growth and body size in response to increased CO_2 may drive an overall reduction in the feeding rates of highly-calcified herbivores (e.g. urchins and gastropods), but not less calcified, crustacean herbivores. In Chapter 3, I used amphipod herbivores with short generation times to test the effects of CO_2 on *per capita* and abundance driven changes in herbivory. Again, I found no evidence for *per capita* changes in herbivory rate of this less calcified species, however increases in amphipod abundance lead to an increase in total herbivory. Finally, In Chapter 4, I manipulated both the abundance of gastropod herbivores and CO_2 in experimental tidepool communities *in situ*. I found that the indirect effects of CO_2 via the reduction of calcified herbivore pressure had a larger impact on tidepool community than CO_2 had directly.

These results show that changes in herbivore pressure in response to OA will be driven primarily through changes in individual body size and herbivore abundance. Further, these changes in herbivory pressure can be more important in determining community structure under conditions of high CO_2 than other species-specific responses.

Lay Summary

Increased carbon dioxide (CO_2) in the atmosphere is slowly entering the oceans and causing changes in ocean chemistry. This process, known as ocean acidification (OA), has large implications for how ecosystems look and function. Herbivores – animals that eat plants and algae – are important in maintaining healthy ecosystems. However, they are also affected by OA. I found that OA can reduce the growth rate and thus average body size of some herbivores, driving a decrease in how much they consume. Conversely, in other herbivores, an increase in abundance increased their ability to maintain a healthy ecosystem. My closer examination of vulnerable herbivores found that decreases in herbivore pressure in response to OA may have a more dramatic impact on ecosystem health than CO_2 addition on its own. This means that ecosystem vulnerability to OA, maybe directly determined by the degree to which herbivore species are impacted by OA.

Preface

The research presented in this thesis was primarily designed, executed, analyzed and written by me, Kathryn M. Anderson. Chapters 2-4 represent manuscripts in development, versions of which have or will be submitted to peer review. At this time, none of these manuscripts have been accepted for publication. Each manuscript has at least one co-author, including undergraduate research assistants who worked under my supervision (Chapter 2), collaborators in Australia (Chapter 3), and my research supervisor (all data chapters).

Chapter 2 will be submitted for publication shortly. I came up with the concept of comparing the feeding rates of multiple herbivores with different levels of calcification. I designed and led the manipulations for *Chlorostoma funebralis*, *Pugetia producta*, and *Idotea woesenskii*. J. Schultz and C. Ketcheson were instrumental in executing and collecting chemistry data related the *C. funebralis* manipulation, as were K. Flynn and C. Bullen in executing and collecting chemistry data related to the *I. woesenskii* manipulation. Data for *Strongylocentrotus franciscanus* were obtained from S. Nienhuis' MSc. Thesis; full citation:

> Nienhuis, S. B. (2009) Multiple impacts of ocean acidification on calcifying marine invertebrates (T). University of British Columbia, doi: http://dx.doi.org/10.14288/1.0067716.

Additional chemistry data on the *S. franciscanus* manipulation was collected by J. Schultz under my supervision. I collected the data relating to the *P. producta* manipulation. C. D. G. Harley was instrumental in discussions which lead to the development of the size specific feeding framework. I compiled and analyzed the data and wrote the resulting chapter.

A version of Chapter 3 has been submitted for review. The idea for the *MESO* manipulation was conceived by S. D. Connell and B. D. Russell. Presence of sharks in large outdoor mesocosms was approved by the University of Adelaide Animal Ethics Committee (permit: S-2013-095) according to the University's animal ethics guidelines. I designed the amphipod herbivory study with input from S. D. Connell, P. Munguia, and B. D. Russell. I led the herbivory experiments described herein and counted and identified the amphipods, with assistance from K.A. Heldt. I conducted all the chemical and statistical analyses (unless specifically noted in the methods) and wrote the resulting thesis chapter.

Chapter 4 is in preparation for publication. After co-developing the technique for manipulating carbon dioxide in tidepools with C.D.G Harley, I designed the experiment to explicitly look at the relative impacts of carbon addition and herbivore loss on community structure. I constructed, maintained, and monitored the tidepools in the field. I collected and analyzed the data and wrote the manuscript with important editorial feedback from and intellectual discourse with C. D. G. Harley.

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List of Abbreviations

Ω_A	Aragonite Saturation
Ω_C	Calcite Saturation
A_T	Total alkalinity
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate
DIC	Dissolved organic carbon
HCO_3^-	Bicarbonate
O_2	Oxygen
OA	Ocean acidification
pCO_2	Partial pressure of carbon dioxide, in this thesis this is always referring to a measurement

Acknowledgements

During the past seven years I have met and been helped by many incredible people and I wish I could describe each of them and what they have done for me during the process.

I am going to start by thanking my supervisor, Dr. Chris Harley. Chris is chill to a fault. He always celebrated my victories and brushed aside my many failures and shortcomings. In doing so, he taught me that even the small successes were meant to be enjoyed and that failures were unimportant so long as I kept working. I always left his office feeling more optimistic than I had when I walked in. Above all, Chris's superpower is to make others believe that they are smart, capable, and worthwhile and he does this without ever a care for his own ego or status. He has treated me like an equal from day one, and has done so without sacrificing my personal and professional growth. Chris, thank you so much for being both my scientific and personal mentor. I've grown so much over the past seven years and I will never forget how hard you have worked to get me here.

Next, I would like to thank my committee members: Dr. Colin Brauner, Mary O'Connor, Diane Srivastava, and Patrick Martone. My committee was larger than is standard, which happened because of a combination of maternity leave and me getting greedy. I have learned so much from each of you and I cannot believe how hard you all worked to support and push me along the way. I would also like to thank Sean Connell and Bayden Russell, who invited me to and mentored my while I was in Adelaide, SA. I have greatly benefited from having a high diversity of personalities, perspectives, and approaches on my team and I wouldn't have had it any other way.

In addition to my amazing team of mentors, I have been fortunate to have been a part of not one, but three incredible communities as my research has taken me from Vancouver, to Bamfield, to Adelaide and back again.

Living in Bamfield for eight-months a year, for three-years, was one of the most incredible, challenging, and simultaneously lovely experiences I have ever had. I would like to thank my *Bamily*, including, but not limited to: Karen Rickards, Catherine Thompson, JP Hastey, Susan Anthony, Travis Thai, Erin Reichsteiner, Alison McAfee, Sarah McLaughlin, Tyler Selvig, Jenny Aleman- Zometa, Gary Liu, Helen Yip, Amy McConnell, Jean Richardson, AJ Chapelsky, Christina Beulow, and the Bamfield Fall Program Classes of 2011, 2012, and 2013. Also, the countless other field assistants that gave their time to helping me pull off my multi-year field projects.

I will never forget how happy and at home I felt living in Adelaide. It was only seven-months, but I would have stayed for ever. In particular I want to send a shout out to my two Aussie besties Nicole Mertens and Kat Heldt; you guys made my world go around. I also want to thank an incredible community that took me in, made me feel welcomed, and helped me do some really cool science: Jon Mortimer, Jonathan Bush, Claudia Junge, Marc Jones, Laura Falkenberg, Skye Woodcock, and Jennie Pistevos.

Next is my Vancouver community. A quick shout out to my platonic life partner Stacey Hrushowy, this has been a crazy couple of years, thank you for being there to lean on. Also, my roommate, friend, and collaborator, Joey Bernhardt. I have learned so much about generosity and curiosity by being in your presence; I don't know anyone who does science or friendship quite like you. I am so grateful to the support and assistance of the Harley Lab (aka. Harley's Angels) in all its various permutations. I particularly want to thank Norah Brown, Alyssa Gehman, Katie

Marshall, Colin MacLeod, Thereasa Coyle, Amelia Hesketh, Crayon Snacks, Kelsey Flynn, Angela Stevenson, you guys have been my friends and my family! Incredible editorial help from within the Harley Lab came from wundergrads Graham Brownly, Emily Lim, Olivia Dyck, Carla Di Filippo, and Veronica Franzova. I would also like to thank Ruth Sharpe, Sara Miller, Andrew Cook, Katie MacIntosh, Andrew MacDonald, Sarah Richardson, Christie MacMillan, Stephanie Avery-Gomm, Matt Siegle, Matt Barbour, Mollie Chapman, Ross Whippo, Roya Eshragh, Virginia Noble, Natalie Stafl, and Angie Nicolás. I have been so well supported and I thank you for the long insightful discussions and wonderful memories.

Finally, I want to thank my family. You guys mean the world to me. I don't know who I would be had I not had you by my side. I want to thank my father and paternal-grandfather for making sure there was always a strange animal in the house. My dad, for telling me that I was describing a marine biologist, when at age-seven I explained to him what I wanted to do the rest of my life. I want to thank my brother, Chris, who I spent the first half of my life trying to become and the second half of my life trying to distinguish myself from. He always had my back and he always gave me something to strive for. My sister-in-law, Andrea, I love how family-oriented and feisty you are; we are a better team because you show up to play. My amazing little sister, Monkey, J-Bird, Jackie; you taught me to see the beauty in being flawed. Thank you for taking every one of my calls and for calling me out, when I needed it. My aunts, uncles, and cousins that seem continually confused by me, but love me anyways. My mother, who always put us kids before anything else, who I still call in the middle of the night when I need to talk things through. You are my rock. Thank you, family, for supporting me through this long crazy journey. I wouldn't be here without you.

For my mother and sister,
the two most wonderful women I known.

Thank you for teaching me how to be strong and how to love myself and others.

Chapter 1: General introduction

Understanding the mechanisms and community-level consequences of environmental change is a pressing issue in modern ecology. This thesis focuses explicitly on understanding how changes in the environment can alter producer-consumer interactions and how those changes can scale up to impact the whole community. In this general introduction, I outline the importance of herbivory – my focal consumer-resource interaction – for determining the structure and function of communities as well as provide background to how ocean acidification (OA) and the addition of carbon dioxide to marine environments may impact marine communities. I then discuss a traditional model that ecologists use to guide the way we understand and think about abiotic and biotic impacts on community structure and propose a few simple alterations to increase the functionality of the model. Finally, I use this improved model to help outline the ensuing research chapters and hypotheses.

1.1 Herbivory and ocean acidification

Plant-herbivore interactions are a fundamental component of most communities, and there are numerous examples of how herbivory can determine species composition and maintain diversity (Lubchenco 1978, Manier and Hobbs 2006, Hughes et al. 2007, Hillebrand et al. 2007, Burns et al. 2009). Plant-herbivore interactions are also key determinants of energy flow and function in ecosystems (Detling 1988, Ritchie et al. 1998, Bardgett and Wardle 2003, Duffy et al. 2003, Altieri et al. 2009). Like many important biological processes, the degree to which herbivores shape a community is often not fully appreciated until there is a breakdown in ecological function. For example, the loss of herbivores has

been shown to have large impacts on many different types of ecosystems, often with large scale consequences in terms of nutrient cycling and habitat maintenance (e.g. the loss of plant diversity in African and American grasslands after the removal of large herbivores, Burns et al. 2009, Dirzo et al. 2015; and the replacement of coral reefs by macroalgal assemblages following the over fishing of herbivorous fish and urchin die offs, reviewed in Mumby and Steneck (2008). Conversely, increases in herbivore abundance can also have just as drastic an impact on the ecology of ecosystems, including the decline of Mongolian grasslands in response to livestock grazing (Hilker et al. 2014), regime shifts driven by sea urchin overgrazing in kelp forests (Ling et al. 2014), and the loss of aspen seedlings and berry producing shrubs in North America associated with heightened elk grazing (Fortin et al. 2005, Ripple et al. 2015a). In this era of global change, shifts in the abundance and ecological role of herbivores may be more likely, and understanding the consequences of such changes is therefore ever more important.

Understanding the outcomes of anthropogenic environmental change is one of the most pressing issues facing biologists today. Not only is human-driven change associated with increases in extinction (Bellard et al. 2012) and changes in primary productivity (Boisvenue and Running 2006), but we are also seeing losses in ecosystem services beneficial to human welfare, such as decreased soil fertility and increased risk of forest fires (Schroter et al. 2005). In a recent survey of marine scientists, increased sea surface temperatures and ocean acidification were listed amongst the top four threats to the marine environment, along with over-harvesting and seawater contamination (Boonstra et al. 2015). Although these changes represent a threat to the stability of natural systems, they also

represent an opportunity for ecological understanding. As abiotic conditions change around the world, ecologists can use these new and projected environmental conditions to test theories of how communities are shaped. Further, the theories and models that ecologists have already developed may be fine-tuned with this understanding to help predict where projected abiotic changes may disproportionately influence community structure and function (Gaylord et al. 2015).

One of the most significant, ongoing environmental changes is the absorption of carbon dioxide (CO_2) into the world's oceans. As CO_2 levels in the atmosphere increase, the ocean acts as a chemical sink, absorbing a certain proportion of the excess CO_2 in order to maintain equilibrium with the surrounding atmosphere (Gattuso et al. 2014). This increase in dissolved CO_2 concentrations causes large shifts in the pH and carbonate chemistry of seawater, resulting in what has been termed OA (Gattuso et al. 2014). Efforts to understand the impacts of increased CO_2 on both individual species and communities have increased in the past 15 years, but to date, most of the general patterns that have emerged are limited to species level responses (Kroeker et al. 2010, 2013, Nagelkerken and Connell 2015). We are left wondering if the strongest impacts of OA will be from the additional CO_2 acting upon each species directly or if there will be ecological surprises arising from the complexities that are associated with species interactions.

Because herbivore pressure is known to be the primary determinant of algal biomass, diversity and community structure (Gruner et al. 2008, Altieri et al. 2009), they may be a particularly important leverage point through which a community's resilience to OA is affected. It is well documented that highly calcified invertebrate herbivores (like shelled

mollusks and urchins) do poorly in response to increased CO_2, while less calcified invertebrates (like crustaceans) tend not to be highly affected (Kroeker et al. 2010, 2013). In contrast, many species of marine macroalgae experience elevated growth rates in seawater that has been acidified by the addition of CO_2 (Kroeker et al. 2010, 2013, Connell et al. 2013). The degree to which this carbon addition affects an alga's productivity varies across taxa as each species responds based on its own physiological requirements (Wu et al. 2008, Falkenberg et al. 2013a, Connell et al. 2013). This uneven shift in productivity will likely result in substantial changes in interspecific competition (Connell et al. 2013), with poorly understood consequences for species diversity and ecosystem function. While there is some evidence that herbivores may help compensate for any changes in competitive hierarchies amongst seaweeds (Ghedini and Connell 2015, 2016, Ghedini et al. 2015), attempts to integrate the different scales and mechanism through which increased CO_2 may alter the role herbivores play in a community are lacking.

1.2 Adapting models of community assembly and maintenance

Community ecology as a field is concerned with explaining the abundance, distribution, and diversity of organisms through space and time. Given the complexity of ecological communities, several conceptual models have been developed to help guide our understanding (e.g., (MacArthur and Wilson 1967, Menge and Sutherland 1976, 1987, Keddy 1992, Hubbell 1997, Vellend 2016). One of the simplest models for understanding which organisms occur in any given space at a given time is the filter model of community assembly whereby the abiotic environment and then the biotic environment are envisioned as

sieves that prevent species from establishing themselves as members of the biological community (Figure 1.1A; Keddy, 1992; and summarized by Vellend, 2016). One notable weakness of this model is that it does not predict how and when abiotic and biotic filters will interact to alter the way in which the biological community assembles.

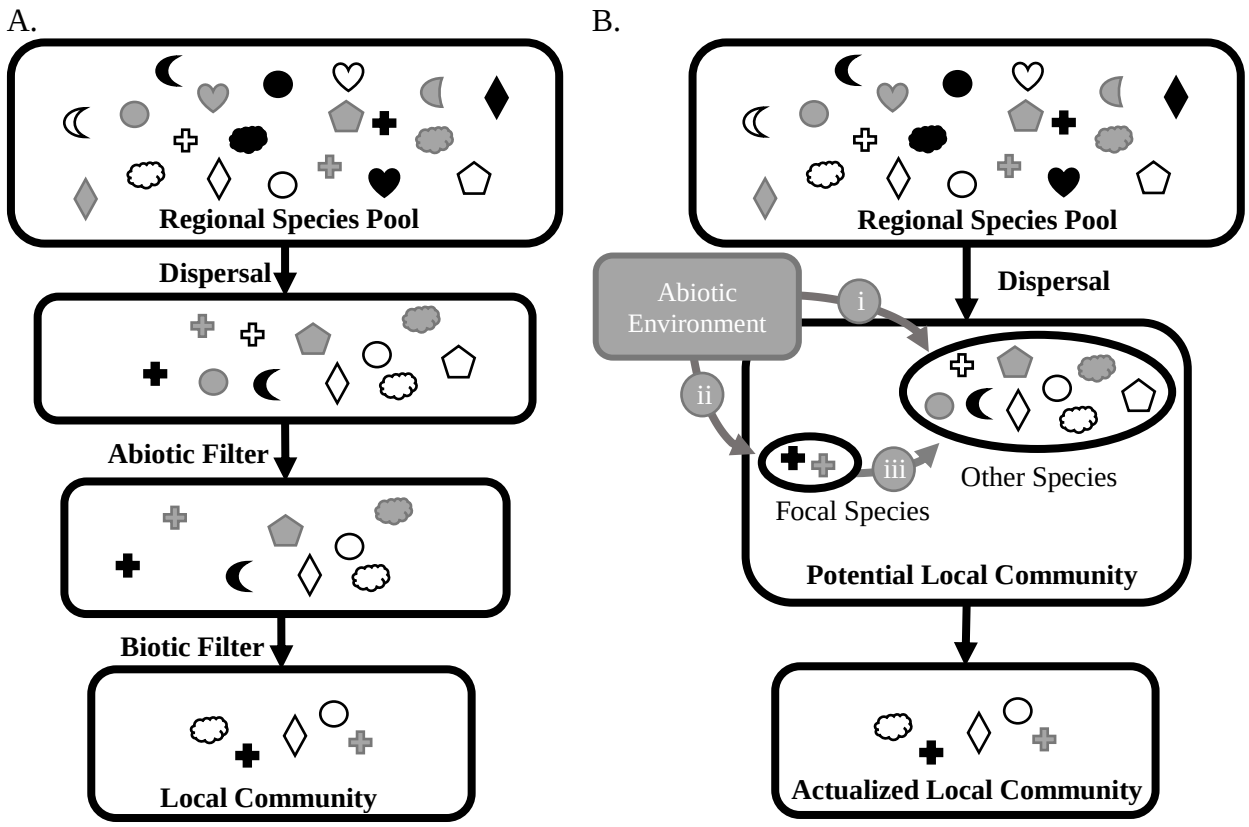


Figure 1.1. The filter model of community assembly.

In the traditional filter model (A; proposed by Keddy, 1992 and modified from Vellend, 2016), the local community is determined by a series of sequential filters which prevent species from moving from the regional species pool into the local community. In the updated model (B), the intermediate stages between the regional species pool and the actualized local community are combined to consider the abiotic and biotic environment simultaneously. This is because, while the abiotic environment can directly impact every species in the potential local community (i), by impacting an intermediate focal species or guild (in this case herbivores; ii), the abiotic environment may indirectly impact the rest of the species in the community via the focal species' impact on the rest of the community (iii). While neither model explicitly includes changes in abundance, changes in the abundance of each species are possible as indicated by each gray arrow in panel B.

In Figure 1.1B, I present an expanded view of how the abiotic environment may determine community structure. Instead of including three filtering steps I have included only one, dispersal, followed by a transition from a potential local community to the actualized community. The potential community contains more species than the actualized community, which is the community that is directly observed and quantified, in communities where membership is not dispersal limited. Whether or not species persist from the potential to the actualized community depends on the direct impacts of the environment on each species (Figure 1.1B, Line i) and the indirect effects of the (abiotic) environment on that species via (biotic) species interactions (Figure 1.1B, Lines ii & iii). In this way, the effects of both the local abiotic and biotic environment are considered simultaneously. What gives this approach more utility than its predecessor is that we can now consider the indirect effects that the abiotic environment can have on community structure via a focal species or guild (Figure 1.1B, Lines ii & iii). The abiotic environment does this by altering species interactions. This can happen in a multitude of ways. For example, the environment may directly favor one species over another, leading to competitive exclusion if there is no mechanism for maintaining diversity. Often a second abiotic factor can help stabilize these interactions by limiting the population growth of the dominant competitor (Chesson 2000). Trophic interactions can also be altered by the abiotic environment and can work as an equalizing or stabilizing mechanism between competing species, whereby the consumer preferentially consumes the dominant competitor, allowing the subordinate to remain present in the community (Chesson 2000).

1.3 Structure of this dissertation

This dissertation focuses on how producer-consumer relationships shift in response to increased atmospheric CO_2 (Figure 1.1B, Lines ii & iii), and ultimately weighs the relative importance of this change against the direct effects of CO_2 addition on community structure (composition and diversity; Figure 1.1B, Line i). This thesis is simultaneously reductionist and holistic in its approach. By breaking producer-consumer interactions down into their component parts, I aim to pinpoint potential mechanisms by which the abiotic environment can affect these interactions and identify the resulting consequences for an ecological community. By studying intact, interacting species assemblages, I aim to link these mechanistic drivers to resultant ecological change at more complex levels of biological organization.

Chapter 2 focuses on identifying how *per capita* consumption rates change in response to OA. As much of OA research has focused on how increased CO_2 can affect the growth rates and size of herbivores, I examine the importance of this potential change in body size in determining the consumption rates of herbivores as compared to any size-corrected changes in feeding rate. In this chapter, I investigate the relative importance of changes in size-specific feeding and overall herbivore growth and size in determining the effects of OA on rates of herbivory. In response to OA, it is likely that highly calcified herbivores would show both greater reductions in size and growth, and greater size-specific changes in feeding rates than less calcified crustacean herbivores (Kroeker et al. 2010, 2013). In particular, I address the following hypotheses:

1. Highly calcified herbivores, which are more vulnerable to OA, will show both decreased growth and size-specific feeding rates in response to CO_2 addition. Less calcified crustacean herbivores will not show such changes.
2. Reduced herbivore body-size – driven by reduced growth rates in highly calcified herbivores – will further exaggerate the already present size-specific reduction in feeding rates.

Chapter 3 builds upon this idea by taking a broader more integrative approach to herbivory. I also consider multiple stressors, adding increased temperature as a second environmental variable. In order to understand the relative role that a resource can play in altering resource-consumer interactions under climate change, I test the effects of increased temperature and CO_2 on both *per capita* feeding rates, when just the algae have been exposed to treatment conditions (a measure of palatability), when just the herbivores have been exposed to treatment conditions (a measure of herbivore condition), and finally when both have been exposed to treatment conditions. Finally, using multigenerational mesocosms, I examine the effects of long-term changes in consumer abundance on resource consumption. Specifically, I hypothesize that:

1. Both amphipod and algal responses to environmental change will alter *per capita* feeding rates of amphipods. I predicted that increased CO_2 and temperature will have a negative effect on algal palatability. However,

increased temperature alone will drive an overall increase in amphipod *per capita* feeding rates.

2. Additionally, changes in population abundance of amphipods in response to the environmental change will ultimately overshadow any effects on *per capita* level feeding rates.

In Chapter 4, I aim to understand the potential outcomes of these altered algal-herbivore interactions a long-term, *in situ*, artificial tidepool set up, I explicitly test the relative impacts of carbon addition on tidepool communities relative to the indirect effects of herbivore loss on species diversity and abundance. With this novel experiment, I attempt to demonstrate the potential importance of indirect effects of the abiotic environment on key ecological interactions and highlight that these may outweigh the direct effects of the environment on each species. In this chapter, I test the following hypotheses:

1. Increased CO_2 will have impacts on community both directly by impacting each species individually and indirectly through the reduction of highly calcified herbivores.
2. However, the indirect impacts of increased CO_2 via herbivore loss will be greater in magnitude than the addition of CO_2 on its own.

Finally, in Chapter 5, I summarize my results in an effort to help researchers refocus their approach to climate change biology in a way that uses climate change parameters as an

impetus to conduct targeted experiments that challenge our understanding of how ecological communities work. In this way, I hope to demonstrate that climate change biology is important not just for predicting potential impacts of future abiotic conditions, but also for helping to illuminate the rules and processes that govern species distribution, abundance, and diversity.

Chapter 2: Ocean acidification and herbivore pressure: growth-mediated consumption effects outweigh changes in size-specific consumption rates

2.1 Chapter summary

Changes in producer-consumer interactions may have dramatic impacts on primary producer biomass, community structure, and ecosystem function. Ocean acidification (OA) may alter marine algal diversity and abundance because algal species utilize additional CO_2 in acidified water based on their own physiology, potentially altering dominance hierarchies. Invertebrate herbivores, which are also vulnerable to OA, may buffer or exaggerate these direct effects of OA on algae. Here, I tested the effects of OA on rates of algal consumption by, and growth of, representative calcifying and non-calcifying herbivore species. I found no effect of herbivore exposure to acidified conditions on size-specific feeding rates of these herbivores. However, I did find evidence to suggest an alternative mechanism that may reduce top-down pressure: slower growth rates for calcifying organisms under OA lead to smaller herbivore body sizes at a given age. Changes in body size could drive an overall reduction in herbivore *per capita* feeding rates in response to OA. Furthermore, this trend may have previously been overlooked by short-term experiments, which focus on size-specific consumption without taking into account simultaneous changes in individual body size.

2.2 Introduction

By regulating the diversity and abundance of primary producers (Lubchenco 1980, Van Alstyne et al. 1999, Harley 2003, Gruner et al. 2008, Altieri et al. 2009), herbivores can

directly influence primary productivity (Paine 2002, Bracken and Stachowicz 2007). Further, the removal, overabundance, or behavior alteration of an herbivore can cause drastic shifts in ecosystem functions (Bertness 1984, Fortin et al. 2005, Hughes et al. 2007, Ripple et al. 2015a). Due to their ecological importance, herbivores serve as an important leverage point through which environmental change can indirectly impact communities and ecosystems by altering herbivore consumption.

In marine environments, one of the most wide-reaching environmental changes is ocean acidification (OA), the process by which excess CO_2 dissolves into the ocean, creating changes in the carbonate chemistry and increasing the acidity of the water. Under OA, growth rates of many algal species may increase, as they utilize the additional carbon as a resource (Kroeker et al. 2010, 2013). Algal species are differentially able to take advantage of this additional carbon source, potentially shifting competitive outcomes between algal species (Connell and Russell 2010, Falkenberg et al. 2013b). In the context of these direct effects of OA on algal production and diversity, indirect effects of OA on algae – mediated by changes in herbivory rates – may be particularly important for understanding the net effects of OA on algal dominated communities. In analogous systems where the addition of nutrients has caused shifts in the competitive hierarchy of primary producers, herbivores have been shown to be important mediators of competitive interactions (Russell and Connell 2005, Gruner et al. 2008). Two mechanisms by which OA may impact the grazing rates of herbivores are: (1) physiological changes that impact size-specific consumption rates; and (2) effects on growth that influence herbivore size.

OA can strongly influence the physiology of most invertebrate species (Kroeker et al. 2010, 2013, Wittmann and Pörtner 2013). Although these effects are highly variable among invertebrate taxa, a few generalities are beginning to emerge (Kroeker et al. 2010, 2013)). Highly calcified herbivores, such as urchins (echinoderms) and shelled gastropods, are particularly vulnerable to OA, showing decreased growth, calcification, and survivorship with acidification (Bibby et al. 2007, Dupont et al. 2010, Nienhuis et al. 2010, Kroeker et al. 2010, 2013). By contrast, non-calcifying crustacean herbivores have been shown to be generally resilient to OA owing to their internal pH regulation and minimal use of calcium carbonate in their exoskeletons (Wheatly & Henry 1992, summarized by Kroeker *et al.* 2010, 2013 and Wittmann & Pörtner 2013).

OA could also affect metabolic demand and lead to a change in size-specific feeding rates. However, despite strong evidence for negative physiological impacts of OA on herbivore fitness, meta-analysis has shown no consistent effect of OA on herbivore metabolic rate (Nagelkerken and Connell 2015). Furthermore, while some species do experience an increase in metabolic demand with OA, their size-specific feeding rates still may not change (Carey et al. 2016). This incongruence is because metabolic demand is not the same as feeding rate, particularly when there is no mechanism to alter feeding rate to keep up with a change in demand (e.g. increased digestive efficiency, decreased handling time, etc.; see Knutsen et al. 1999) for an empirical example).

Instead, OA could alter the *per capita* effects of herbivores through changes in herbivore body size, which could be more predictable than size-specific changes in feeding rate. As reduced growth rate is one of the most highly documented outcomes of OA on

invertebrates (Kroeker et al 2013), it is likely that OA will indirectly decrease the feeding rates of herbivores by making individuals within a population smaller. Just as the effect of OA on a species' growth rate depends on its level of calcification (summarized by Kroeker et al. 2010, 2013b), calcification level will likely be key in determining how *per capita* feeding rates will change in response to OA.

There is substantial evidence that OA affects size-specific feeding rates, yet less is known about how OA affects body size. For example, a recent meta-analysis showed an overall decrease in the feeding rates of consumers, driven primarily by mollusks and echinoderms, but not arthropods (Clements 2016). With no data on changes in body size or growth rate, it is not possible to distinguish between the hypothesis that OA is affecting *per capita* feeding rates via changes in size-specific consumption rates, or via changes in the body sizes of consumers. Thus, the mechanism for this pattern remains elusive.

I tested how OA affects size-specific feeding rates and somatic growth rates of four marine herbivores common to the northeast Pacific, including the red urchin (*Strongylocentrotus franciscanus*), the black turban snail (*Chlorostoma funebralis*), and two crustaceans: the kelp crab (*Pugettia producta*) and the rockweed isopod (*Idotea wosnesenskii*). Consistent with reported empirical patterns, I predicted reduced growth rates in highly calcified invertebrates (*S. franciscanus* and *C. funebralis*), but not in the crustaceans (*P. producta* and *I. wosnesenskii*). Similarly, as found in a meta-analysis (Clements et al. 2016), I predicted that exposure to acidified seawater would have a negative impact on size-specific feeding rates in the highly calcified invertebrates, but not in the crustaceans. Finally, because feeding rate should be positively correlated with size across all

taxa, the decrease in growth rates of *S. franciscanus* and *C. funebralis* should ultimately lead to lower *per capita* feeding rates under high CO_2 conditions, while the *per capita* feeding rates of *P. producta* and *I. wosnesenskii* should remain unchanged.

2.3 Methods

This study is a combination of four smaller studies, all designed with the shared goal of understanding how increased CO_2 affects *per capita* feeding rates and the degree to which changes in body size may explain this effect. Each species manipulation was designed separately from the others without the intention for direct comparison, and there are many differences between each manipulation, despite similar methods for manipulating CO_2 and quantifying feeding rates.

2.3.1 General design

Feeding rates of *S. franciscanus*, *C. funebralis*, *P. producta*, and *I. wosnesenskii* and growth rates of *S. franciscanus*, *C. funebralis*, and *I. wosnesenskii* – but not *P. producta* – were measured under ambient and elevated CO_2 conditions. I used dried or frozen wild-collected kelp in all feeding trials to isolate the impacts of reduced pH on the herbivore from potential impacts of future pH on the kelp. I defined ambient pH simply as the pH that was achieved when ambient air was bubbled through sea water. I achieved elevated CO_2 treatments by adding additional CO_2 to airlines until pH was significantly lower than that in the ambient treatment, but not so low that it would be out of the range expected by IPCC predictions for 2100 (*see results section*; Pachauri *et al.* 2015). I measured mesocosm pH

regularly (every 2-4 days) to the 0.01 unit using an Oakton Acorn pH 6 probe during both day and night to account for potential differences driven by any photosynthetic microbes. Photoperiods were not controlled during any of these manipulations. I collected water samples from the mesocosms for dissolved inorganic carbon (DIC) analysis and then processed them according to the methods of Dickson and colleagues (2007) using a Dissolved Inorganic Carbon Analyzer model AS-C3 (Apollo SciTech Inc., Bogart, GA, USA). DIC data was then combined with the associated pH, temperature, and salinity data and input into CO2Calc software (Robbins et al. 2010), along with equilibrium constants from Mehrbach et al. (1973) as adjusted by Dickson and Millero (1987), to determine the other carbonate parameters of the water: pCO_2, total alkalinity, carbonate and bicarbonate ion concentrations, as well as calcite and aragonite saturation states.

2.3.2 *Chlorostoma funebralis* – black turban snail

C. funebralis individuals were collected in the intertidal zone near Bamfield, British Columbia (48°50'04.7"N 125°08'04.8"W), and held for three months in a recirculating laboratory sea water system before manipulations began. As *C. funebralis* has slow feeding rates, I placed three individuals in each mesocosm to ensure a measurable change in kelp mass was achieved. I visually categorized *C. funebralis* individuals into one of three size classes: small; medium; or large (1.32 ± 0.04, 1.69 ± 0.03, and 2.27 ± 0.10 grams submerged wet weight ± SE; respectively). One individual from each size class was randomly assigned to each of twelve replicates for both CO_2 treatment (N = 72 snails). Each set of three *C. funebralis* individuals were housed in 14x14x9 cm mesh containers, submerged in ~250L

recirculating tanks with natural seawater (see Gooding et al. 2009 for details on the design of these mesocosms). I held the *C. funebralis* in these treatment conditions for twelve weeks and supplied dried bull kelp, *Nereocystis luetkeana*, *ad libitum*, replacing food once every 3-7 days (depending on the level of algal degradation); the dry mass of all kelp was measured before and after each feeding to quantify consumption. I also paired each feeding assay with no-herbivore controls, which were achieved by submerging additional kelp in a second mesh container within the larger recirculating tank. I measured *C. funebralis* shell mass to the nearest 0.001 gram using the submerged mass technique (described in Palmer 1982) before and after the 12 week manipulation. As gastropod body size is limited by shell growth, submerged shell mass is a reasonable proxy for overall gastropod size. I collected 10 mL water samples for DIC analysis every two weeks during the *C. funebralis* manipulation (N = 220 samples).

I conducted an additional short-term experiment to quantify the effect of individual size directly on feeding rates, in which I fed dried *N. luetkeana* to twenty *C. funebralis* individuals of various sizes (17.5 ± 1.3 mm ± SE) for 5 days. I measured dried *N. luetkeana* mass at the start and end of these experiments. This short-term feeding trial was run under ambient conditions only, as I simply wanted to establish a relationship between size and feeding rate for *C. funebralis*, which the original experimental design would not permit.

2.3.3 *Idotea wosnesenskii* – rockweed isopod

I. wosnesenskii individuals were collected from the intertidal zone near the University of British Columbia's Point Grey campus in Vancouver, British Columbia (49°16'24.6"N

123°15'25.1"W), and were kept in a communal recirculating seawater system for two weeks before beginning the manipulation. During the manipulation, I housed twenty-three *I. wosnesenskii* individuals in 1L flow-through mesocosms made from 1L plastic bottles, which also experienced a flow rate of 1L recirculating filtered seawater per hour. *I. wosnesenskii* were fed dried *N. luetkeana ad libitum*, which was replaced every 3-7 days (depending on the level of algal degradation). The *I. wosnesenskii* feeding trial lasted one week and started two weeks after the *I. wosnesenskii* individuals had been exposed to treatment conditions. Dried *N. luetkeana* was weighed before and after this feeding trial but not throughout the manipulation. Independent herbivore-free controls for each CO_2 treatment were run simultaneously in identical mesocosms. To measure *I. wosnesenskii* growth rates, blotted dry mass of *I. wosnesenskii* individuals was measured to the nearest 0.0001 grams at the start of the manipulation and after six weeks of exposure to the treatments to ensure measurable growth. I collected water samples at the beginning and end of the *I. wosnesenskii* manipulation for DIC processing (n = 40, per CO_2 treatment).

2.3.4 *Pugettia producta* – kelp crab

P. producta individuals were collected from the subtidal zone near Bamfield, British Columbia. *P. producta* were kept in a communal tank with flowing seawater for 48 hours before beginning the manipulation. During the manipulation, I housed twenty individual *P. producta* (57.2 ± 4.6 grams ± SE) in flow-through mesocosms made from 2L glass jars, which experienced a flow rate of 1L fresh, filtered seawater per hour. *P. producta* individuals were acclimated to conditions for a week prior to a single 24 hour feeding trial, using dried

N. luetkeana. Independent herbivore-free controls for each CO_2 treatment were run simultaneously in identical mesocosms. Dried *N. luetkeana* mass was measured before and after the feeding trial. To estimate the relationship between size and feeding rate, I measured the blotted dry mass of each *P. producta* prior to the start of the manipulation to the nearest 0.001 grams. I collected water samples for DIC processing once during the *P. producta* manipulation from a subset of the mesocosms (n = 7 and n = 8 for the ambient and elevated CO_2 treatments, respectively).

2.3.5 *Strongylocentrotus franciscanus* – red urchin

S. franciscanus growth and feeding data are from Nienhuis (2009). In brief, individuals were collected in the subtidal zone near Bamfield, British Columbia, and kept in artificial seawater aquaria for four months prior to being exposed to treatment conditions. Two *S. franciscanus* individuals were housed in each of ten 20 L aquaria, and partial (1/3 volume) water changes were conducted every 2-3 days to keep the water clean and prevent extensive alteration of pH (n = 5 tanks per CO_2 level, N = 20 urchins total). This artificial seawater was mixed from de-chlorinated water and Instant Ocean^{TM} aquarium mix. Seawater pH, temperature, and salinity were measured every 2-3 days, and DIC samples were taken after the conclusion of the experiment from tanks of fresh mixed seawater under the same treatment conditions (N = 10). *S. franciscanus* were fed frozen blades of giant kelp (*Macrocystis pyrifera*). To quantify consumption, the blotted wet mass of the alga was measured before and after each feeding trial. To control for kelp degradation, kelp mass loss in herbivore-free mesh containers submerged in each replicate aquarium was measured four

times over the course of the 12-week manipulation. Before and after the experimental manipulation, *S. franciscanus* were blotted dry to remove the effects of excess water on mass estimates and then weighed individually.

2.3.6 Statistical analysis

I first fit linear models to test whether CO_2 addition created a significant decrease in pH. For pH data from the *C. funebralis*, *I. wosnesenskii*, and *S. franciscanus* trials, I used a mixed-effects linear model with mesocosm and date as random variables, nesting date within mesocosm, which controlled for variation between tanks and sampling dates. I used a single-factor linear model to examine the effects of CO_2 addition on pH in the *P. producta* experiment and the *S. franciscanus* (absent of *S. franciscanus*) experiments, as these pH measurements were only taken at one point in time.

I ran separate statistical analyses for each species to quantify effects of OA on both feeding and growth rates. The effect of CO_2 treatment on growth rate for *C. funebralis*, *I. wosnesenskii*, and *S. franciscanus* was examined using separate mixed-effect models with CO_2 treatment (current or elevated) as a fixed effect, initial mass as a covariate, and total mass change (for all individuals in each replicate) as the dependent factor. Because there was more than one individual per mesocosm, mesocosm was nested within CO_2 level when analyzing the *C. funebralis* and *S. franciscanus* data. When the p-value for this nested term was greater than 0.9, as it was for the *C. funebralis* analysis, I dropped the term from the analysis as it had no significant effect on growth.

For all herbivore species, I used a two factor, linear model to determine if feeding rate (total algae consumed over the whole experiment) was affected by pH. I used herbivore

presence as a second fixed effect, so that our herbivore-free controls could be compared directly to the experimental treatments, i.e. an effect of CO_2 on feeding rate would be represented by a significant interaction between CO_2 and herbivore presence. Because herbivory rates were measured multiple times in the *C. funebralis* assay, I used mesocosm as a random variable to account for multiple measurements.

I used a final series of linear models to look at the effect of body size on consumption rates of the herbivores. I used mixed-effect models to look at the effects of body size and CO_2 on feeding rates simultaneously for *P. producta, I. wosnesenskii,* and *S. franciscanus* trials. As size-specific feeding rates for *C. funebralis* were only examined under ambient conditions, I used a single factor linear regression to look at the effect of body size on their consumption rates.

I verified null results with *post hoc* power analyses. I conducted all analyses in R using the *nlme* and *base* statistic packages, and functions lm() for linear models without random effects and lme() for linear models with random effects (R Development Core Team 2004).

2.4 Results

2.4.1 Seawater chemistry

The addition of CO_2 into experimental mesocosms resulted in a significant reduction in pH within the 0.05- 0.4 unit range predicted to occur by 2100 (Gattuso et al. 2014, Pachauri et al. 2015; Tables 2.1). This reduction resulted in a subsequent change in all relevant carbonate chemistry parameters, while leaving temperature and salinity stable (Table

2.2). Although carbonate chemistry for the *S. franciscanus* manipulation was measured separately from the experiment, I note that the magnitude of pH decrease during the *S. franciscanus* manipulation was conservative compared to changes observed during the period of carbonate chemistry measurement (7.75 pH ± 0.01 to 7.68 pH ± 0.01, Table 2.1).

Table 2.1. Analysis of mesocosm pH for *P. producta* and *S. franciscanus.*

ANOVA table showing the effect of CO_2 addition on pH in mesocosms of (A-B) *S. franciscanus*, (C) *C. funebralis*, (D) *I. wosnesenskii*, and (E) *P. producta*. *S. franciscanus* data were taken both when organisms were present (A) and absent (B) from mesocosms. Date analyzed with a univariate linear model for *P. producta* (E) and *S. franciscanus* when urchins were absent (B), because data was not collected over multiple sampling periods. All other data we analyzed with a mixed effect linear model where mesocosm and date random effects.

Source	**Df**	**Sum Sq**	**F-value**	**p-value**
A) *S. franciscanus* – Present				
Date [Mesocosm] – Random	1, 110		459892	**<0.0001**
Treatment	1, 8		15	**0.004**
B) *S. franciscanus* – Absent				
Treatment	1, 1	0.30	548	**<0.0001**
Residuals	1,8	0.00		
C) *C. funebralis*				
Date [Mesocosm] – Random	1, 203		374395	**<0.0001**
Treatment	1, 15		61	**<0.0001**
D) *I. wosnesenskii*				
Date [Mesocosm] – Random	1, 400		790354	**<0.0001**
Treatment	1, 38		542	**<0.0001**
E) *P. producta*				
Treatment	1, 1	0.85	531	**<0.0001**
Residuals	1, 38	0.06		

Table 2.2. Summary of seawater carbonate chemistry of four manipulations.

Data categorized according to herbivore species. The asterisk (*) indicates that the data is based upon pH and DIC measurements taken while the organisms where not in their tanks. *S. franciscanus* (n=480, each for salinity and temperature, however n = 10 for all other seawater chemistry parameters). *P. producta* (n=20, each for salinity, temperature and pH, however n = 7 for control and n = 8 for high CO_2 for all other seawater chemistry parameters). *I. wosnesenskii* (n=220 for salinity, temperature and pH, however n = 40 for all other seawater chemistry parameters).

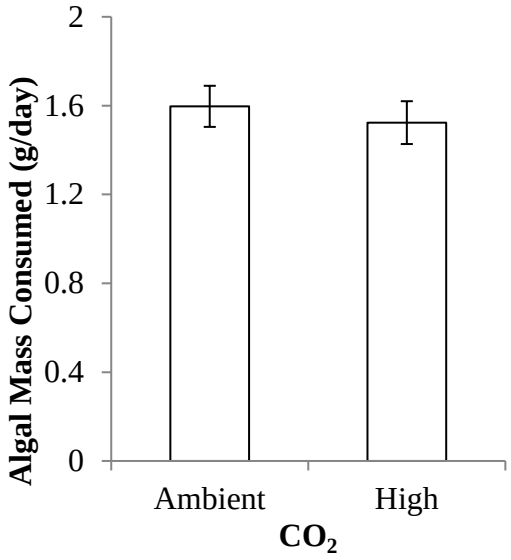
Herbivore	Treatment	Salinity (ppt)	Temp. (°C)	pH	DIC	A_T (µmol/kgSW)	HCO_3^- (µmol/kgSW)	CO_3^{2-} (µmol/kgSW)	pCO_2 (ppm)	Ω_C	Ω_A
A) *S. franciscanus* *	Control	32.6 (0.25)	12.70 (0.03)	8.44 (0. 01)	2150 (4)	2507 (7)	1883 (6)	257 (4)	209 (4)	6.23 (0.09)	3.96 (0.06)
	High CO_2	32.4 (0.25)	12.64 (0.04)	8.10 (0. 01)	2226 (9)	2390 (4)	2077 (11)	127 (3)	509 (19)	3.10 (0.08)	1.97 (0.05)
B) *P. producta*	Control	36.0 (<0.01)	10.60 (0.01)	8.07 (< 0. 01)	2021 (13)	2177 (14)	1888 (12)	113 (1)	460 (7)	2.69 (0.02)	1.72 (0.02)
	High CO_2	36.0 (<0.01)	10.61 (0.01)	7.78 (0. 01)	2077 (24)	2124 (23)	1977 (23)	57(1)	997 (25)	1.36 (0.02)	0.87 (0.02)
C) *C. funebralis*	Control	33.7 (0.08)	12.6 (0.03)	8.08 (0. 01)	1859 (4)	2066 (6)	1699 (4)	146 (2)	13.3 (0.3)	333 (7)	3.51 (0.05)
	High CO_2	33.9 (0.07)	12.7 (0.03)	7.87 (0. 01)	1936 (6)	2060 (6)	1815 (6)	98 (2)	23.6 (0.7)	591 (16)	2.35 (0.05)
D) *I. wosnesenskii*	Control	36.9 (0.07)	12.58 (0.05)	7.89 (0. 01)	1174 (14)	1267 (15)	1104 (13)	55 (1)	375 (15)	1.30 (0.04)	0.83 (0.03)
	High CO_2	36.5 (0.07)	12.55 (0.02)	7.49 (0. 01)	1206 (10)	1220 (14)	1141 (9)	27 (2)	950 (63)	0.64 (0.05)	0.41 (0.03)

2.4.2 Herbivores and feeding assays

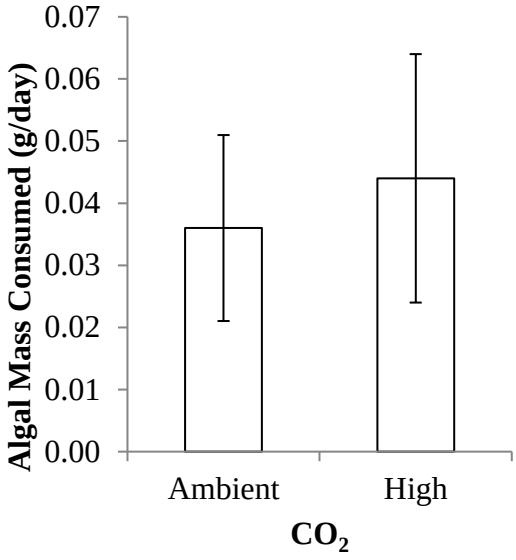
In all cases, herbivore presence significantly increased the amount of algal tissue that was lost (interpreted as consumed) compared to the no-herbivore controls (Figure 2.1, Table 2.3). However, there was no detectable effect of herbivore exposure to CO_2 on the amount of algal tissue lost during our feeding trials, nor was there an interactive effect of CO_2 and herbivore presence. This indicates that CO_2 does not affect either the rate of algal consumption or decomposition (Figure 2.1, Table 2.3).

Unlike feeding rate, increased CO_2 did negatively affect the growth rates of *C. funebralis* and *S. franciscanus* (Figure 2.3 A&C, Table 2.4C). The was no effect of CO_2 on the growth rates of *I. wosnesenskii*, however (Figure 2.3B, Table 2.4B). The herbivores, *C. funebralis*, *I. wosnesenskii*, and *S. franciscanus*, all increased feeding rate with body size (Figure 2.2A-C, Table 2.5A-C). For two species, *S. franciscanus* and *I. wosnesenskii*, I examined the effect of body size on feeding rates in combination with CO_2, and found that there was no effect of CO_2 on how feeding rates varied with size (Figure 2A-B, Table 2.5A-B). In contrast, the crab *P. producta* showed no change in feeding rates as size increased (Figure 2.2D, Table 2.5D). As with the other species, there was no effect of CO_2 on the feeding rates of *P. producta* (Table 2.5D).

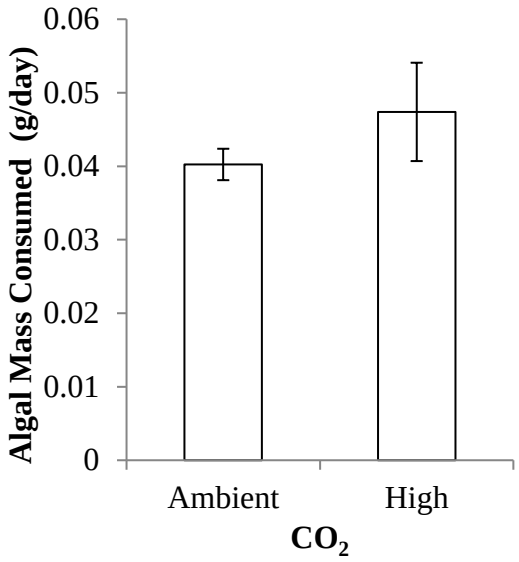
A. *S. franciscanus* (highly calcified)



B. *I. wosnesenskii* (less calcified)



C. *C. funebralis* (highly calcified)



D. *P. producta* (less calcified)

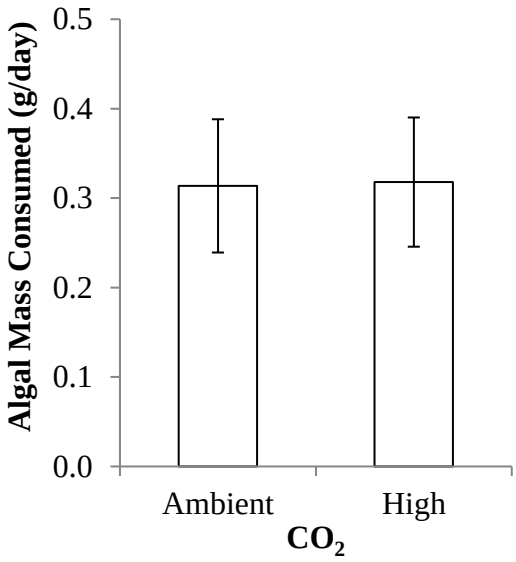


Figure 2.1. Algal mass consumed.

Algal mass consumed in the presence of four different species of herbivores under projected high and current ambient CO_2 conditions. Data shown relative to no-herbivore controls. Heavily calcified herbivores (A) *S. franciscanus* and (C) *C. funebralis* are shown on the left, while the less calcified crustacean herbivores (B) *I. wosnesenskii* and (D) *P. producta* are shown on the right. The zero line represents the mean mass lost in each

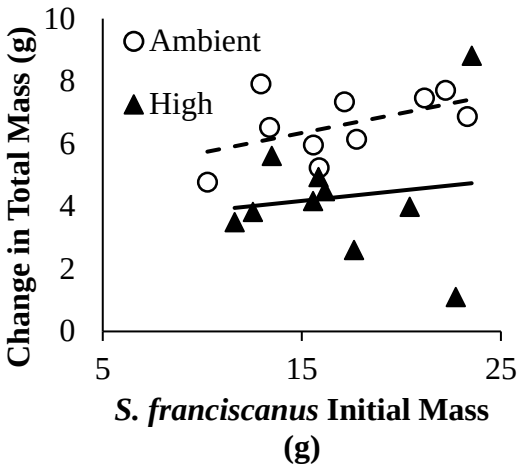
treatment in the no herbivore controls. Error bars indicate the standard error (SE) of the mean. As *S. franciscanus* and *C. funebralis* had paired no herbivore controls, SEs were calculated using the difference between the algal mass lost in the presence of the herbivore and the algal mass lost in the paired no-herbivore control (A; C). As the no-herbivore controls for *I. wosnesenskii* and *P. producta* were not with herbivore treatments, means and SEs are bootstrapped estimates (B; D).

Table 2.3. Analysis effect of CO_2 on the amount of algal mass consumed.

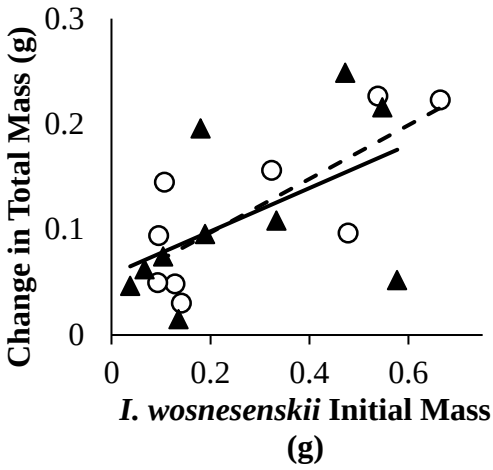
The effect of CO_2 condition on the amount of algal mass lost in the presence of herbivores. Information shown for (A) *S. franciscanus* (B) *C. funebralis*(C) *I. wosnesenskii* and (D) *P. producta*. Algal mass loss was higher in the presence of herbivores than in no-herbivore controls. There was no effect of CO_2 nor interaction of CO_2 and herbivore presence on algal mass loss.

Source	DF	Sum Sq	F-value	p-value
A) *S. franciscanus*				
CO_2	1,16	0.006	0.18	0.67
Herbivore Presence	1,16	12.2	371.7	**<0.0001**
CO_2* Herbivore Presence	1,16	0.007	0.20	0.66
B) *C. funebralis*				
Mesocosm- Random	1,15		200044	**<0.0001**
CO_2	1,15		0.12	0.74
Herbivore Presence	1,15		210	**<0.0001**
CO_2* Herbivore Presence	1,15		0.037	0.85
C) *I. wosnesenskii*				
CO_2	1,35	0.002	1.14	0.29
Herbivore Presence	1,35	0.01	6.82	**0.01**
CO_2* Herbivore Presence	1,35	<0.001	0.16	0.69
D) *P. producta*				
CO_2	1,36	0.02	0.45	0.50
Herbivore Presence	1,36	0.97	25.8	**<0.0001**
CO_2* Herbivore Presence	1,36	<0.001	0.004	0.95

A. *S. franciscanus* (highly calcified)



B. *I. wosnesenskii* (less calcified)



C. *C. funebralis* (highly calcified)

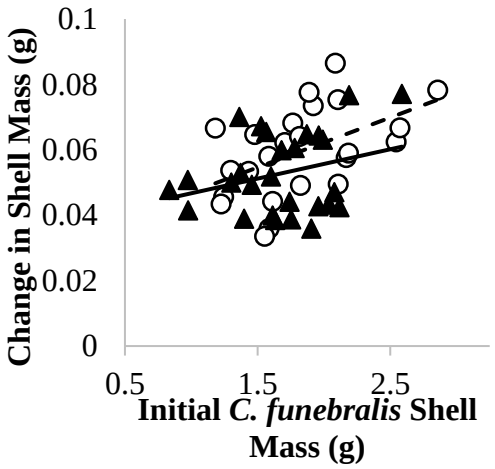


Figure 2.2. Herbivore growth.

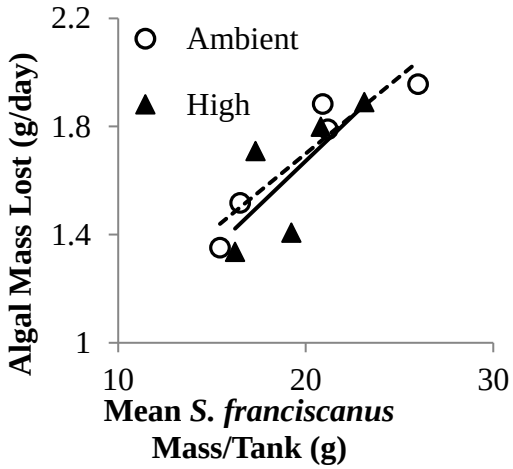
Growth of three different species of herbivores under high and ambient CO_2 conditions. For easy comparison, heavily calcified herbivores (A) *S. franciscanus* and (C) *C. funebralis* are shown on the left, while the less calcified crustacean herbivore (B) *I. wosnesenskii* is shown on the right. There was a significant effect of initial mass on the growth of all three species of herbivores. Increased CO_2 had a negative effect on the growth of both *S. franciscanus* and *C. funebralis*. However, there was no effect of increased CO_2 on the growth of *I. wosnesenskii*. The scale of the x- and y-axes vary between each of the three plots to best show the spread of the data.

Table 2.4. Analysis of herbivore growth rates

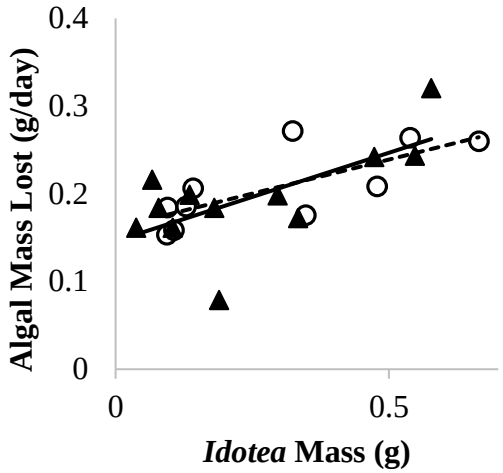
ANOVA table on the effect of CO_2 on the growth rates of (A) *S. franciscanus* (B-C) *C. funebralis* and (D) *I. wosnesenskii*. Initial size was used as a covariate in all cases. Mesocosm was nested inside the CO_2 treatment for *S. franciscanus* (A) and *C. funebralis* as (C) each tank contained two to three individuals. However, the Mesocosm factor was dropped for *C. funebralis* (C) because $p > 0.90$.

Source	DF	Sm Sq	F-value	p-value
A) *S. franciscanus*				
CO_2	1, 9	26.22	11.94	**0.007**
Initial Size	1, 9	2.98	1.36	0.27
Mesocosm [CO_2]	8, 9	24.04	1.37	0.32
B) *C. funebralis*				
CO_2	1, 48	<0.001	3.92	**0.05**
Initial Size	1, 48	0.001	7.69	**0.008**
C) *I. wosnesenskii*				
CO_2	1, 16	<0.001	0.07	0.80
Initial size	1, 16	0.04	10.84	**0.005**

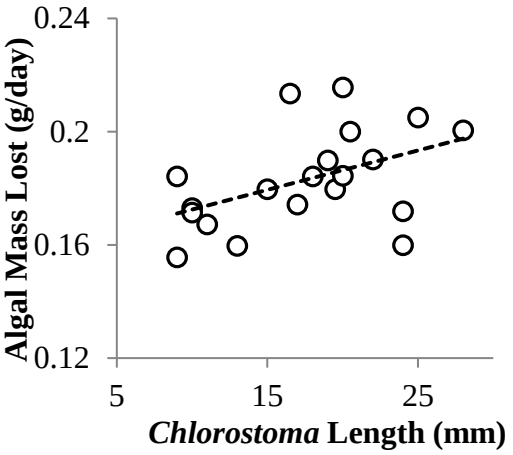
A. *S. franciscanus* (highly calcified)



B. *I. wosnesenskii* (less calcified)



C. *C. funebralis* (highly calcified)



D. *P. producta* (less calcified)

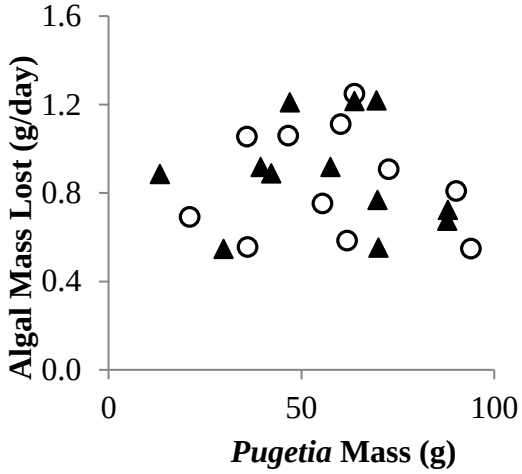


Figure 2.3. Algal mass lost according to herbivore size.

Size shown in mass or length of the individual. Heavily calcified herbivores (A) *S. franciscanus* and (C) *C. funebralis* are shown on the left, while the less calcified crustacean herbivores (B) *I. wosnesenskii* and (D) *P. producta* are shown on the right. Results are shown under both current ambient (open circles and dashed lines) and future projected high (black triangles and solid lines) CO_2 conditions for all the herbivores except for *C. funebralis* (C). Algal mass loss increases linearly with increased herbivore size for both species of highly calcified herbivore (A & C), but only in one of the two species of crustacean herbivore, the *I. wosnesenskii* (B).

Algal mass loss was consistent across body sizes of *P. producta* (D). In all cases where the effect of CO_2 level was tested simultaneously with herbivore size, there was no effect of CO_2 level nor an interaction between herbivore size and CO_2 on the amount of algal mass lost (A, B, & C). The scale of the y-axis varies between each of the four plots as each herbivore consumes at different rates. The x- and y- axes for the *S. franciscanus* data begin at 10 and 1, respectively; this was done to better show the spread of the data range. The x-axes for the *S. franciscanus* data represent the mean mass of the two urchins in each tank.

Table 2.5. Analysis of size specific feeding rates.

The effect of size on CO_2 condition on the size specific feeding rates of (A) *S. franciscanus* (C) *I. wosnesenskii* and (D) *P. producta*. As there were two *S. franciscanus* in each tank, I used mean herbivore mass instead of individual mass in this analysis. I also show the effect of body size on the feeding rates of *C. funebralis* (B). I did not include CO_2 in this last analysis, as this experiment was conducted separately from CO_2 and *C. funebralis* manipulation.

Source	DF	Sm Sq	F-value	p-value
A) *S. franciscanus*				
CO_2	1,6	0.01	0.55	0.48
Mean Herbivore Size	1,6	0.36	15.73	**0.007**
CO_2* Mean Herbivore Size	1,6	0.001	0.08	0.78
B) *C. funebralis*				
Herbivore Size	1,18	0.03	4.86	**0.04**
C) *I. wosnesenskii*				
CO_2	1,18	0.001	0.30	0.59
Herbivore Size	1,18	0.02	12.0	**0.003**
CO_2* Herbivore Size	1,18	0.001	0.28	0.60
D) *P. producta*				
CO_2	1,19	0.004	0.07	0.79
Herbivore Size	1,19	0.008	0.13	0.72
CO_2* Herbivore Size	1,19	<0.001	0.002	0.97

2.5 Discussion

Top-down pressure is important for the structuring of both marine and terrestrial communities (Burns et al. 2009, Ling et al. 2014, Ripple et al. 2015a, 2015b). For example, changes in top-down pressure can dramatically change ecosystem function, particularly when foundation species are affected (Paine 1966; Hughes et al. 2007; Ling et al. 2014). Past attempts to find a predictable mechanism for how OA may affect top-down pressure have so far been fruitless: while OA seems to cause an overall decrease in herbivory rates under high CO_2 conditions, this appears to be an idiosyncratic phenomenon caused by multiple species-specific responses (Clements *in review*). Here, I looked at the effect of OA on *per capita* herbivory pressure to demonstrate that lower growth rates may explain this pattern of reduced feeding rates.

As predicted, I found no effect of experimental acidification on the size-specific feeding rates of any of the four herbivore species. This is likely because even when increased CO_2 drives an increase in the metabolic demand of a consumer, there does not seem to be a mechanism by which increased CO_2 can predictably increase ingestion efficiency to compensate for the change in demand (Carey et al. 2016). However, I did find that there was a significant effect of body size on the feeding rates of *S. franciscanus*, *C. funebralis*, and *I. wosnesenskii*, and that experimental acidification reduced the growth rates of both highly calcified herbivores: *S. franciscanus* and *C. funebralis*. As predicted, there was no change in the growth rates of *I. wosnesenskii*, which, as a crustacean, is not highly calcified. All else being equal, slower growth rates of highly calcified herbivores under acidified conditions should lead to an overall reduction in body size in individuals of the same age class, and therefore an overall decrease in the *per capita* feeding rate.

Changes in feeding rates – driven by changes in body size – may be a general phenomenon for highly calcified herbivores such as *S. franciscanus* and *C. funebralis*, where the impacts of acidification on growth are pronounced, but not for crustaceans such as *P. producta* and *I. wosnesenskii*. While I did not test the effect of increased CO_2 on the growth rates of *P. producta*, the data suggest that there would be little to no change in feeding rate driven by any changes in growth rate, as *P. producta* impacts on algal biomass were size independent, at least across the size range and at the time scale of this experiment. It is important to note that this lack of size dependence may be due to the destructive means by which *P. producta* interact with their food, tearing and picking it apart. In this way, *P. producta* may have a much larger impact on its algal food source than through simple ingestion. Thus, species like *P. producta* demonstrate an important exception to using changes in average body size to predict changes in the top-town impacts of herbivores.

Our study adds to a growing body of literature that shows an overall decrease in the feeding rates of highly calcified herbivores under acidified conditions. For example, a recent meta-analysis revealed that consumption rates of highly calcified consumers show an overall decline under conditions of high CO_2, whereas the consumption rates of less calcified consumers such as arthropods were unaffected (Clements 2016). It would be interesting to know if the majority of studies included in this meta-analysis took into account changes in body size. If they did not, the significant negative effects of increased CO_2 on consumption rates could have been driven by changes in body size as opposed to physiological, size-specific effects. Further, it appears that although highly calcified herbivores are functioning at sub-optimal levels under high CO_2, they may be unable to increase their feeding rates to compensate for the increased stress. A recent paper showed that while invertebrates may

indeed increase their metabolism (by about 20%) to compensate for the stress of a high CO_2 environment, they did so without increasing their feeding rates (Carey et al. 2016). In such instances, it is likely that ingestion and digestion rates are already operating at maximum efficiency and are thus unable to meet an increase in metabolic demand. Regardless of the exact mechanisms, it is becoming apparent that many species experience no change in size-specific feeding rates in response to increased CO_2.

Although the examples in this study show either a reduction or no change in body size with CO_2 addition, it is important to mention that there may be species that show the opposite pattern. Gooding et al (2009) observed an overall increase in individual body size of *Pisaster ochraceous* with increased CO_2, with no significant change in size-specific feeding rates, leading to an overall increase in *P. ochraceous* consumption rates. This is, however, likely a notable exception, as very few consumers overall have been shown to exhibit increased growth with increased CO_2 (Kroeker et al. 2010, 2014).

Ultimately, in order to fully understand and predict how *per capita* consumption rates will change with increased CO_2, ecologists need to break these rates down into their component parts: (1) the effect of CO_2 on size-specific consumption; (2) the effect of CO_2 on individual body size; and (3) the effect of body size on consumption (Figure 2.4). In cases where size-specific feeding rates change in response to a stressor (Figure 2.4, Line A), one would expect to see a subsequent change in *per capita* feeding rates as well. However, when body size is affected by CO_2 and also has a direct effect on feeding rate (Figure 2.4, Line B), whether or not CO_2 will influence *per capita* feeding rates would depend on the relative direction and magnitude with which CO_2 affects both size and size-specific feeding rates.

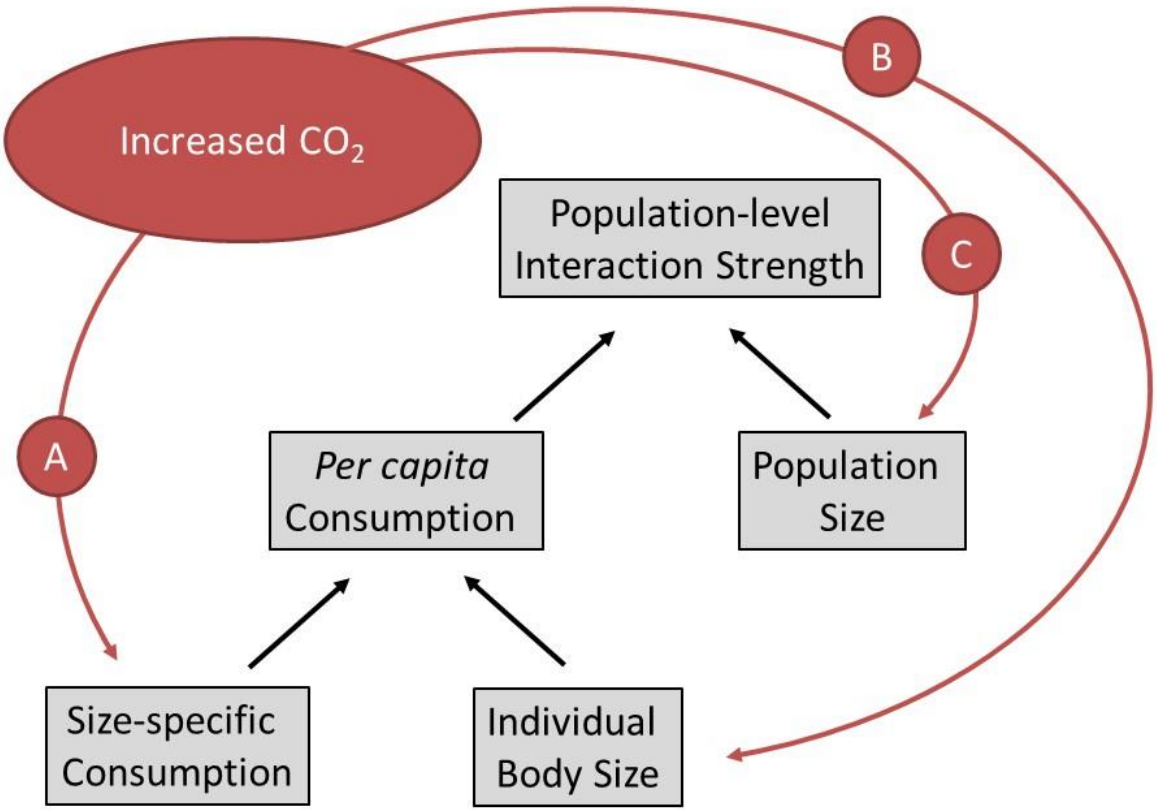


Figure 2.4. Pathways through which increased CO_2 can impact herbivory rates.

The overall impact of a consumer population on its resource, represented here as the population-level interaction strength, depends on both the number of consumers and the *per capita* effects of those consumers. *Per capita* interaction strength, in turn, depends on the size of the consumers and on their size-specific consumption rates. Increases in dissolved CO_2 can influence population level interaction strength through several pathways: (A) via changes in organismal physiology or behavior and thereby feeding rates; (B) via changes in growth rates (e.g., due to reduced calcification) and thereby the distribution of body sizes within a population; or (C) via changes in population size.

None of the herbivores I observed in this study altered their size-specific feeding rates with increased CO_2 (Figure 2.4, Line A). Instead, when there has been no observed change in size-specific feeding rates in response to a stressor, one would only expect to see changes in *per capita* consumption rates when CO_2 alters the growth of the individual (Figure 2.4, Line B) and size is linked to feeding rates. This is what I observed in *S. franciscanus* and *C. funebralis*. However, if there is no relationship between CO_2 and body size, as with *I. wosnesenskii*, one would not expect to see any change in *I. wosnesenskii per capita* consumption rates. As I saw no effect of body size or CO_2 on the feeding rates of *P. producta*, one also would not expect a change in the consumption rates of this herbivore, even if CO_2 did alter their growth rate (Figure 2.4, Line B).

Extending beyond this study, I note that changes in population size (Figure 2.4, Line C) will combine with changes in *per capita* feeding rates to determine population level changes in top-down pressure. Research and meta-analyses consistently show echinoderms and mollusks to be particularly vulnerable to changes in pH during early development (Kroeker et al. 2010, 2014). Such vulnerability during early development may ultimately lead to reduced survivorship and smaller population sizes of these herbivores, as has already been observed at natural CO_2 vents (Hall-Spencer et al. 2008, Kroeker et al. 2011). Reductions in body size may amplify reductions in population size when larger females produce disproportionally more offspring than their smaller counterparts. In contrast, recent research has shown an increase in the population size of amphipod herbivores, another crustacean (Heldt et al. 2016), which may directly result in increased feeding rates at the population level (see chapter 3 of this thesis). Whether or not there will be some type of compensatory mechanism by which crustacean herbivores are able to increase their population size to

maintain guild-level herbivory pressure is unknown. Such a possibility may also be true for calcified herbivores (they could be smaller and more numerous), however I suspect this is unlikely given the other ways in which calcified herbivores are negatively impacted by increased CO_2 (see above discussion of larval development and survivorship).

This framework (Figure 2.4), while developed to help understand the different ways in which CO_2 addition can impact herbivory rates, is widely applicable to understanding changes in the feeding rate of almost any consumer in almost any varying environment. For example, we know that below a species' thermal optimum, increased temperature can increase the size-specific ingestion rate of many invertebrates (Figure 2.4, Line A; Kordas et al. 2011). Further, according to the temperature-size rule, body size is inversely related to the temperature of the environment (Figure 2.4 Line B; Atkinson 1994; Kingsolver & Huey 2008). Thus, assuming the species in question has a positive relationship between body size and consumption rates (Brown 2004), the actual change in *per capita* consumption would be driven by the magnitude by which temperature affects size and feeding rate (Osmond et al. 2017). In this way, the framework enables researchers to consider both size and size-specific consumption rates when making predictions about how consumers will impact communities.

Understanding changes in top-down pressure in response to changing abiotic conditions is critical to understanding and predicting changes in community structure and diversity; both urchins and gastropods have been found to be important shapers of marine communities (Lubchenco 1978, Ling et al. 2014). While a reduction in urchin grazing may not have drastic impacts on a healthy temperate algal dominated community, reductions in urchin grazing in tropical systems have been linked to substantial declines in coral cover due to overgrowth by macroalgae (Hughes et al. 2007). Likewise, changes in gastropod herbivory

have led to significant changes in intertidal and fresh water benthic community structure (Lubchenco 1978, Sheldon 1987). This is a particularly important issue as OA has already been shown to alter the competitive hierarchies in algal dominated communities (Connell et al. 2013), making it important to understand which, if any, herbivores may be able to buffer these changes.

2.6 Conclusions

Increased scientific effort has been put towards understanding how changes in oceanic CO_2 and the process of OA may affect trophic interactions. While the initial data show a multitude of directions in which size-specific consumption rate can change (Clements 2016), ecologists have overlooked an important part of the puzzle: the role individual body size plays in determining the feeding rate of consumers, a universal mode by which many environmental changes may impact rates of consumption. As increased CO_2 has a negative effect on the growth rates and body size of highly calcified species, it will likely also cause an overall decrease in the *per capita* consumption rates of consumers, as these rates are tightly linked to body size. This effect of OA may be overlooked in the literature, as many experiments that measure consumption under conditions of high CO_2 control for body size and/or do not allow enough time for the effects of reduced growth to manifest as changes in body size. For this reason, when considering what the impact of OA will be on consumption rates, it is important to consider body size (Figure 2.4, Line B) in addition to per gram consumption rates (Figure 2.4, Line A). From this research, it is apparent that changes in the environment – in this case, the addition of CO_2 – can have large impacts on *per capita* feeding rates without altering size-specific consumption.

Chapter 3: Future CO_2 and temperature alter an algal-herbivore interaction via changes in herbivore abundance.

3.1 Chapter summary

Top-down pressure by herbivores is known to control primary productivity and diversity. The relative intensity of this process is determined by *per capita* feeding rates and herbivore abundance, and mediated by the palatability of the producers. Although all of these facets of plant-herbivore interactions can be influenced by environmental conditions, they are seldom considered simultaneously in realistic climate change scenarios. I manipulated temperature and carbon dioxide concentration to examine the effects of these two climate variables on benthic algal-herbivore interactions. I used a mesocosm experiment spanning several consumer generations in conjunction with targeted short-term manipulations to investigate amphipod grazing pressure at both the individual and population level. At the population level, a strong interactive effect of elevated CO_2 and temperature resulted in an increase in herbivore abundance and, consequently, a near doubling in overall herbivory on turf algae. When algae and herbivores were manipulated separately, elevated temperature negatively impacted *per capita* herbivore feeding rates, and elevated CO_2 negatively impacted algal palatability, but when both algae and herbivores were simultaneously exposed to projected future levels of both variables, there was no overall effect of temperature, CO_2, or their combination on *per capita* feeding rates. Therefore, in this plant-herbivore system the impacts of climate change on abundance were much greater than minimal changes in *per capita* level interaction strength. This chapter highlights the importance of conducting longer-term experiments that allow for the development of population-level responses in

addition to *per capita* level effects. Further, changes in abundance may be reasonable predictors of population level impacts and thus need to be examined when considering consumer-producer interactions under novel environmental conditions.

3.2 Introduction

Changes in plant-herbivore interactions are becoming increasingly likely as anthropogenic climate change accelerates. In marine systems, two of the most important environmental changes are increased sea surface temperature and ocean acidification (OA). In general, small changes in temperature are thought to intensify top-down pressure by disproportionately increasing metabolic rates relative to any changes in primary production (O'Connor 2009, Gilbert et al. 2014). While there may be increased metabolic costs associated with OA, the strength of this metabolic response is variable among taxa (Nagelkerken and Connell 2015). A few key studies have found an increase in the *per capita* feeding rates of herbivores in response to OA (Falkenberg et al. 2014, Ghedini et al. 2015). However, a recent meta-analysis found an overall reduction in *per capita* feeding rates in response to OA across taxa and trophic levels (Clements 2016). Additionally, changes in *per capita* feeding rates are difficult to predict based solely on the response of the herbivore, as decreases in primary producer palatability by either OA or increased temperature may alter any changes in *per capita* feeding rates driven by the herbivores alone (Dury et al. 1998, Gaylord et al. 2015).

The intensity of herbivore pressure is not just determined by *per capita* grazing rates, but by a combination of *per capita* grazing rates and herbivore abundance (Atkins et al. 2015), with many of the largest shifts caused by changes in herbivore abundance (Hughes et

al. 2007, Ling et al. 2014, Ripple et al. 2015b). OA and increased temperatures have the potential to affect physiological and demographic rates, thus affecting individual performance as well as population growth rates, size, and persistence (Harley et al. 2006, Gilbert et al. 2014). However, individual and population level effects are often difficult to reconcile because population level responses occur over multiple generations and thus may be out of sync with the *per capita* physiological responses, which tend to occur days or even minutes after being exposed to a novel environment. This difference in temporal scale makes population level effects more difficult to study experimentally and thus attempts to reconcile these two levels are few.

Theory predicts that the same increases in metabolic rates associated with small increases in temperature should also drive increases in herbivore population growth rates (Savage et al. 2004). Conversely, OA has been shown to have adverse effects on many marine invertebrates, which may ultimately lead to reduced population sizes amongst calcified herbivores (Kroeker et al. 2010, Gaylord et al. 2015). However, no manipulative experiments have yet documented this over multiple generations. There is also evidence that future conditions may increase herbivore resources (Falkenberg et al. 2013a, 2014, Connell et al. 2017), decrease predation pressure (Pistevos et al. 2015), and increase fecundity of amphipod herbivores (Heldt et al. 2016), all of which may lead to larger herbivore populations.

To explore the ecologically realistic effects of multiple stressors on a plant-herbivore interaction, I manipulated temperature and CO_2 in a temperate reef amphipod-algal system over time scales short enough to capture fine-scale changes in *per capita* feeding rates and long enough to capture population-level responses. Specifically, I manipulated temperature

and CO_2, alone and in combination, and in long-term outdoor mesocosms as well as in short-term laboratory microcosms. I predicted that increases in both OA and temperature would decrease the palatability – and thus the consumption – of turf algae. Further, I expected that both elevated temperature and increased CO_2 would increase the grazing rates of amphipods by increasing their physiological demands, similar to previous observations on other herbivores (Ghedini et al. 2015). Additionally, given the previous evidence that OA can increase amphipod population size (Heldt et al. 2016), I predicted that changes in population size in response to future conditions would have a larger effect on total algal consumption than changes in *per capita* consumption rates. In other words, while *per capita* feeding rates would be highest in response to combined increased temperature and CO_2, I expected populations to consume more algae under elevated CO_2 conditions because there would simply be a greater number of amphipods feeding.

3.3 Methods

3.3.1 Study system

Temperate kelp forests in South Australia are dominated by *Ecklonia radiata* which compete with a mixed assemblage of filamentous turf algae (mainly *Feldmannia spp*.). Under ambient atmospheric and temperature conditions, *Ecklonia* easily outcompetes turf algae and inhibits their growth and recruitment by light reduction and mechanical abrasion (Connell 2003). However, increases in temperature and CO_2 have been shown to alter this interaction such that the turf algae begin to overgrow kelp recruits, preventing replenishment of lost individuals (Connell and Russell 2010). Here I combine herbivory assays done in large scale multigenerational outdoor mesocosm communities with short-term laboratory studies to tease

apart the relative impacts of CO_2 and temperature on both primary producers and their associated amphipods.

Despite their small body size, amphipods have been shown to be important grazers in marine systems (Brawley and Adey 1981, Jernakoff and Nielsen 1997, Duffy and Hay 2000, McSkimming et al. 2015). Furthermore, their small size, fast generation time, and relative robustness in mesocosm systems makes them a tractable organism for studying the effects of abiotic change on population and *per capita* level processes. Non-calcified herbivores, such as amphipods, may become even more important under conditions of OA since other dominant temperate marine herbivores such as urchins and gastropods are highly calcified and thus more vulnerable to the negative impacts of OA (Kroeker et al. 2010, 2014).

3.3.2 Multigenerational mesocosms

3.3.2.1 Experimental site and set-up

Experimental mesocosms were located within the Marine Experiments for a Sustainable Outcome facility at SARDI (South Australian Research and Development Institute) Aquatic Sciences, West Beach, South Australia (34.9453 ºS, 138.5038 ºE). These 2400 L flow-through experimental mesocosms (polyethylene, TeamPoly, Australia) were fitted with transparent covers (high density polyethylene with woven scrim, SolarPro, PolyFab, Australia). I filled the mesocosms with natural filtered seawater so that initial seawater chemistry (i.e. before experimental manipulation) was characteristic of the local coastal waters. I pumped ocean water directly into the mesocosms at a flow rate of 4 L min^{-1}, or 2.5 total volume turnovers per day to maintain water quality. I used a total of 12 mesocosms, consisting of three replicates for each of four CO_2-temperature combinations.

This fully factorial design featured two CO_2 treatments (ambient, at a pH of 8.16 ± 0.01, and future elevated, at a pH of 8.00 ± 0.01; n = 72) and two temperature treatments (also ambient, at 15.4 ± 0.1 °C, and future elevated, at 18.0 ± 0.3 °C; n=72; see Falkenberg et al. 2016 for more detail).

I maintained CO_2 concentrations by aerating the tanks at a rate of 15 L min^{-1} with either ambient atmospheric air, or air enriched with CO_2. CO_2 enrichment was achieved by using a gas mixer (Pegas 4000 MF, Columbus Instruments, Columbus Ohio USA). I measured temperature, pH and salinity daily, and total alkalinity (AT) weekly, using a potentiometric titrator (888 Titrando, Metrohom, USA). Then, I calculated concentrations of CO_2, carbonate (CO_3^{2-}), and bicarbonate (HCO_3^-) from measured AT, pH, salinity, and temperature using the CO2SYS program for Excel (Pierrot et al. 2006) with constants from Mehrbach et al. (1973) as adjusted by Dickson and Millero (1987). I used individual heater/chiller units (TC-60 Aquarium Chillers, TECO Refrigeration Technologies, Ravenna, Italy) on each mesocosm to maintain consistent temperature differences between control and elevated temperature treatments and allowed mesocosms to track natural variations in temperature, CO_2, and light conditions.

The mesocosms were stocked with three trophic levels: primary producers, five individual *E. radiata* and recruitment tiles that had been seeded with a self-recruiting turf algal assemblage that quickly covered much of the sides and bottom of the mesocosms; herbivores, six urchins (*Heliocidaris erythrogramma),* 15 snails (*Turbo undulatus*), and an amphipod assemblage dominated by *Cymadusa pemptos*; carnivores, one crab (*Ozius truncatus*), one spiny rock lobster (*Jasus edwardsii*), and three juvenile Port Jackson sharks *(Heterodontus portusjacksoni*). Urchins, snails, and kelp were replenished regularly during

the thirteen-week period of the experiment, in order to keep the biomass consistent between mesocosms. In order to achieve a natural seed population, both in terms of initial density and diversity, amphipod assemblages were collected along with *E. radiata,* by enclosing each kelp entirely in a plastic bag in the field. Amphipod diversity and abundances were homogenized between fronds, by keeping kelp in a communal holding tank prior to placement in experimental mesocosms. An estimated 100 amphipods were placed into each mesocosm. Amphipods were not restocked during the duration of the experiment and thus their population densities were allowed to change on their own over approximately 2-3 generations.

3.3.2.2 Population measurements

In order to estimate the number of amphipods present in each population, I removed all the kelp from each mesocosm at the end of the thirteen-week period and rinsed them in salt water and then in fresh water. The resulting mix of invertebrates and detritus was strained using a 0.5 mm mesh filter and stored in 100% ethanol. I removed and counted all *C. pemptos* individuals that were visible with a dissecting microscope set to 5x magnification (N=12 populations; for more detail see Heldt et al. 2016). While there were a few other amphipod morpho-species present in the mesocosms, they were not quantified for this study due to their extremely low abundances.

3.3.2.3 Mesocosm feeding assays

To quantify population level consumption rates in the large experimental communities, I periodically introduced six small jars (160 mL) into each mesocosm and

seeded each jar with kelp (0.557 ± 0.004 g blotted dry mass, BDM) and turf (0.547 ± 0.005 g BDM) sourced from its host mesocosm. I took kelp clippings from healthy lateral blades, while I used turf removed from the side of mesocosm tanks using forceps. Half of the jars contained amphipods while the other half were used as no herbivore controls. I varied the number of individual amphipods in each jar according to the relative population size found in its corresponding experimental tank (min = 1 amphipod, max = 20 amphipods per jar). Due to limitations on estimating amphipod population size non-destructively and the large differences in populations sizes between the mesocosms, the number of amphipods used in the feeding trials had a saturating relationship with population size by which amphipod density was systematically underestimated in the high density mesocosms (Figure 1). Relative population size was approximated based on 20 minutes of search time. All amphipods caught in 20 minutes of search time per mesocosm were divided into three jars to be used in that round of feeding assays. The searches were discontinued when 20 minutes was up or when 60 amphipods had been found maxing out the number of amphipods per replicate. Because searches were ended earlier than 20 minutes when amphipods were highly abundant, amphipod density in jars is a conservative representation of amphipod abundance in mesocosms when population sizes are high. Each mesocosm contained three control and three grazer jars and amphipods were allowed to graze for three days. I measured the BDM of both kelp and turf algae before and after the grazing trials. The experiment was repeated at 3 time intervals during December 2013 after amphipod populations had been exposed to experimental treatments for 11-12 weeks.

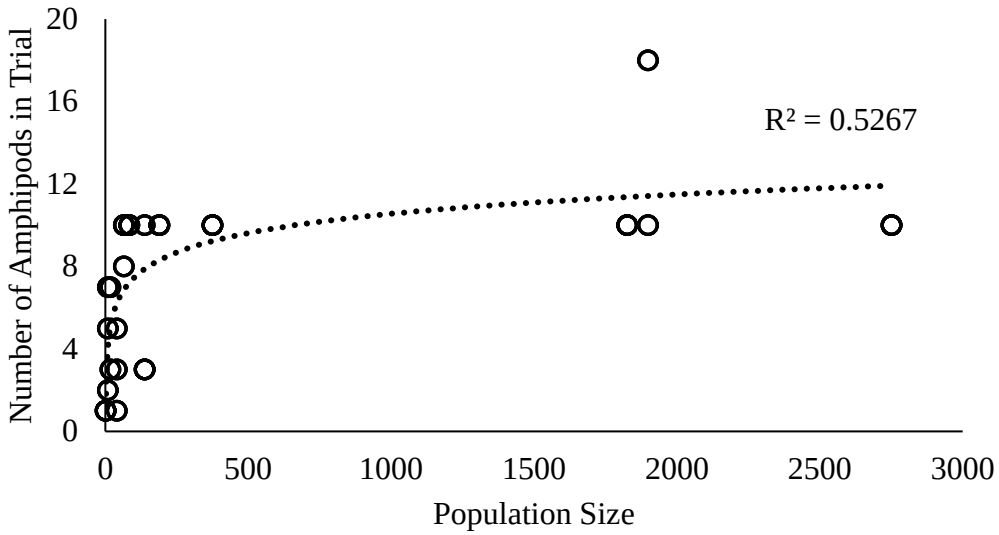


Figure 3.1. Amphipod density was systematically underestimated in the high density mesocosms.

The relationship between the populations size in the multigenerational mesocosms and the number of individual amphipods used in the population level herbivory assay. Dashed line is a logarithmic fit to the data showing that the number of amphipods used in the trial increased with population size more quickly at low population sizes than at high population sizes. This saturating relationship makes the estimates of population level herbivory more conservative for the larger populations than the smaller populations.

I measured amphipod, kelp and turf biomass at the beginning and end of each trial. Consumption of primary producers was calculated using the correction of the controls (Equation 3.1; Sotka et al. 2002, Long et al. 2007). I found that amphipods did not affect the biomass of kelp ($F_{1,201} = 1.45$, $p = 0.23$), but significantly reduced the biomass of turf ($F_{1,201} = 42.36$, $p < 0.0001$), thus I focused exclusively on the interaction between amphipods and turf algae.

Equation 3.1. Calculating consumption rates using no-herbivore controls.

Consumption of primary producers was defined as the corrected mass lost where *H* and *C* are algae BDM (either kelp or turf) in the herbivore and control jars at the initial, *i*, and final, *f*, time point of each grazing trial. I calculated the term C_f/C_i, which represents the average expected proportional mass change of the algae in the tank in the absence herbivores, separately for each large mesocosm at each trial period

$$\text{Consumption} = \mathrm{H_i}\left(\frac{\mathrm{C_f}}{\mathrm{C_i}}\right) - \mathrm{H_f}$$

3.3.3 Laboratory feeding trials

I experimentally manipulated conditions of the amphipods and algae independently and simultaneously in separate experimental replicates to test whether it was changes in algal or amphipod physiology, or some combination of the two, that drove changes in herbivore-plant interactions. I conducted the experiment in three parts (Figure 2). First, I tested for the effect of temperature and CO_2 on algal palatability by feeding algae from all four treatment conditions to amphipods from ambient conditions. Second, I tested the effect of amphipod condition on feeding rates. I fed algae grown only under control conditions to amphipods raised at all four experimental conditions. Finally, to test whether *per capita* interactions change when both algae and amphipods are exposed to projected conditions simultaneously, I fed amphipods acclimated to each of the four treatment conditions algae grown under those same treatments. I designed, manipulated, and monitored the experimental conditions of these laboratory microcosms to directly match the conditions of the large outdoor mesocosms.

AMPHIPOD ↓ \ ALGAE →	Control	↑Temp	↑ CO2	↑ Temp & CO2
Control	X	X	X	X
↑ Temp	X	X		
↑ CO2	X		X	
↑ Temp & CO2	X			X

Figure 3.2. Experimental combinations used in the laboratory feeding trials.

Columns show algal conditions and rows show amphipod conditions as indicated by their respective headers. The far-left column shows control amphipod conditions, the environment which amphipods were acclimated to prior to feeding trials. "X" indicates a treatment combination used in the experiment. The top row of "X's" shows the portion of the experiment targeting the effects of CO_2 and temperature on algal palatability. The left column of "X's" shows the portion of the experiment targeting the effects of CO_2 and temperature on amphipod feeding rates independent of changes in algal palatability. The diagonal of "X's" shows the portion of the experiment looking at how CO_2 and temperature impact algal-herbivore interactions when both algae and amphipods are exposed to treatment conditions. This diagonal is most similar to changes being examined by the large MESO manipulation.

To expose the amphipods to treatment conditions independent of algae, I dried turf algae that I had grown in the outdoor mesocosms. I ground this algae into a homogenized powder and made it into agar plates using 0.2 g dried turf algae to 0.25 g agar powder and 40 ml milliQ water. After setting, the agar plates were sliced into cubes (approximately 1 cm^3)

and placed into small replicate jars. Each jar contained three amphipods that had been held in the laboratory under conditions reflecting the original experimental mesocosms for 8-10 days. Each replicate was paired with a no-herbivore control from a cube cut adjacent to the experimental cube to control for variation between agar batches. I ran these feeding trials for 72 hours.

During the agar setting process, the ground algae settled to the bottom of the agar plate, creating a 2-dimensional feeding area. Agar cubes were placed floating in experimental jars at the start of the experiment allowing easy access to the algae rich side. I used photo analysis of this 2-dimensional feeding area on the control and experimental cubes to determine how much area had been consumed by the amphipod grazers.

3.3.3.1 Chemical analysis of turf algae

In order to determine a mechanism behind the changes in the palatability of the turf algae, I analysed both phlorotannin content and carbon-nitrogen ratios (C:N) of the turf algae. For C:N analysis, small samples of dried turf algae (≤ 0.1 g) from each of the 12 experimental mesocosms were analyzed via flash combustion by the Sprigg Geobiology Centre at the University of Adelaide's School of Earth and Environmental Science (Mawson Laboratories, Adelaide, South Australia). I then calculated C:N ratios from the raw mass of carbon and nitrogen found in each sample.

I conducted phlorotannin analysis on small samples of dried turf algae (≤ 0.1 g; N=12) using a Folin-Ciocalteu assay based on Van Alstyne's Folin-Ciocalteu assay for compounds dissolved in 80% methanol (Van Alstyne 1995). I extracted these small dried samples in 78% methanol for 24 hours. After extraction, I took 0.1 mL of each sample and

added it to vials containing 0.4 mL Folin-Ciocalteu reagent, which had been diluted with 1.5 mL deionized water. Finally, I added 1 mL of a saturated $NaCO_3$ solution to catalyze the reaction. After allowing the reaction two hours to complete, each sample was analyzed for pigment intensity using a spectrophotometer at 765 nm. I used a standardized curve to back calculate phlorotannin content into percent dried algal tissue.

3.3.4 Data analysis

To assess whether the amphipods were consuming algae in the feeding trials, I used a linear model to test the null hypothesis that consumption was not different between our herbivore trials and our no herbivore controls, using herbivore presence and absence as the only fixed factor. Kelp and turf algae were treated independently, and as there was no evidence that kelp was being consumed, no further analysis was conducted on the kelp.

I used linear models to test the effect of CO_2 and temperature on population level consumption rates with CO_2 and temperature as fixed factors. Population level consumption was transformed using the formula logit(x + 0.08) to ensure residual normality. This was the only data that needed to be transformed in order to meet the assumptions for the statistical analyses.

Three separate mixed-effects linear models were run on the laboratory feeding assays: one to look at the effect of treatment conditions on amphipod feeding rates in isolation from changes in the algae; one to look at changes in algal palatability; and one to look at the effect of treatment when algae and amphipods were manipulated simultaneously. The control data is the same in all of these analyses and it comes from amphipods under ambient conditions that where fed algae grown under ambient conditions. In all three linear models temperature

and CO_2 were used as fixed effects. The algae used in this manipulation came from one of three tanks from each of the four treatment conditions. Thus, algal source was used as a random effect in these three linear models.

I conducted all analyses in R using the *nlme* and *base* statistic packages, functions lm() for linear models without random effects and lme() for linear models with random effects (R Development Core Team 2004).

3.4 Results

3.4.1 Water chemistry

The water chemistry of the long-term outdoor mesocosms and indoor laboratory microcosms reflected temperature and carbonate chemistry of both current ambient conditions and conservative projected local ocean conditions for the year 2100 (Table 3.1; Falkenberg et al. 2016).

Table 3.1. Carbonate chemistry of short-term laboratory microcosms.

Mean values (± SE) for multiple water chemistry parameters important to the carbonate chemistry of our short-term laboratory microcosms. For the long-term mesocosm manipulation, each of the 21 microcosms were measured for all A_T, pH, temperature, and salinity at three occasions. All other chemical parameters were calculated from these data: pCO_2; bicarbonate (HCO_3^-); carbonate (CO_3^{2-}); and saturation states for calcite and aragonite (Ω_C and Ω_A, respectively).

CO_2 Treatment	**Temperature Treatment**	**Salinity (ppt)**	**Temp. (°C)**	**pH**	**A_T (µmol/kgSW)**	**pCO_2 (ppm)**	**HCO_3^- (µmol/kgSW)**	**CO_3^{2-} (µmol/kgSW)**	**Ω_C**	**Ω_A**
Ambient CO_2	Ambient	41 (<1)	18.04 (0.1)	8.04 (0.01)	2534 (10)	627 (13)	2128 (15)	165 (3)	4 (<1)	2 (<1)
	Elevated	43 (<1)	21.1 (0.1)	8.04 (0.01)	2599 (27)	633 (23)	2128 (21)	191 (8)	4 (<1)	3 (<1)
Elevated CO_2	Ambient	42 (<1)	18.1 (0.1)	7.83 (0.01)	2616 (16)	1142 (36)	2346 (19)	110 (3)	3(<1)	2 (<1)
	Elevated	43 (<1)	20.7 (0.1)	7.82 (0.01)	2643 (50)	1212 (59)	2342 (50)	123 (4)	3 (<1)	2 (<1)

3.4.2 Consumption rates

At the population level, I observed an interactive effect of CO_2 and temperature on algal consumption (Figure 3.3, Table 3.2). CO_2 and temperature, when manipulated independently of each other, had no detectible effect on consumption rates. However, when I increased CO_2 and temperature simultaneously, I observed a 1.8x increase of consumption relative to current conditions (Figure 3.3, Table 3.2).

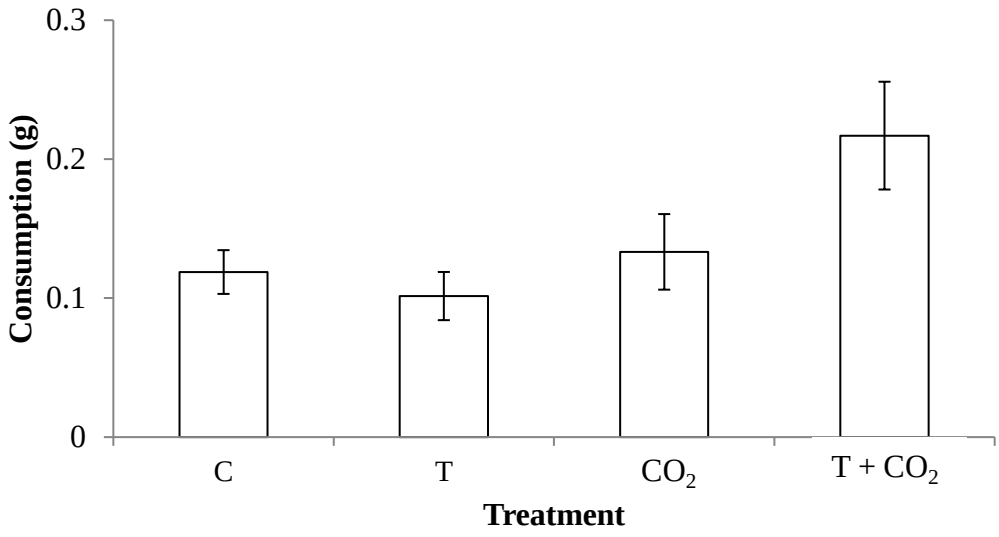


Figure 3.3. Consumption of turf algae by amphipod when abundance was allowed to vary.

The effect of increased temperature (T) and CO_2 on population level amphipod consumption of turf algae (g) in long term mesocosms in terms of change in mass corrected for algal growth. There is no significant effect of either temperature or CO_2 on turf consumption. However, there is an interactive effect of temperature and CO_2 where under combined elevated conditions, there is an increase in turf consumption by amphipods. "C" indicates control conditions.

Table 3.2. Analysis of the effects of CO_2 and temperature on consumption of turf by amphipods.

Statistical details for the linear model of the effects of CO_2 and temperature on consumption of turf algae by amphipods. Data were transformed using the formula f(x)=logit (x + 0.08). Treatment levels of CO_2 and temperature included current or elevated levels in each case (DF= 1, 95).

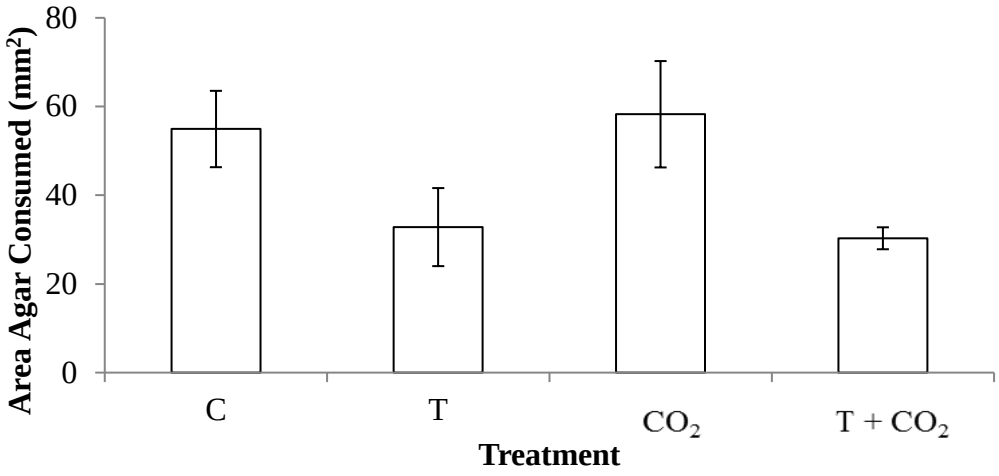
Source	F-value	p-value
CO_2	2.91	0.09
Temperature	0.98	0.32
CO_2*Temperature	4.17	**0.04**

Amphipod population density did not change with increased temperature. However, there was a significant and substantial increase in population size with CO_2 addition under both ambient and elevated temperatures. Mean population size was highest when both temperature and CO_2 were increased simultaneously with up to a 25x increase in amphipod abundance (Heldt et al 2016).

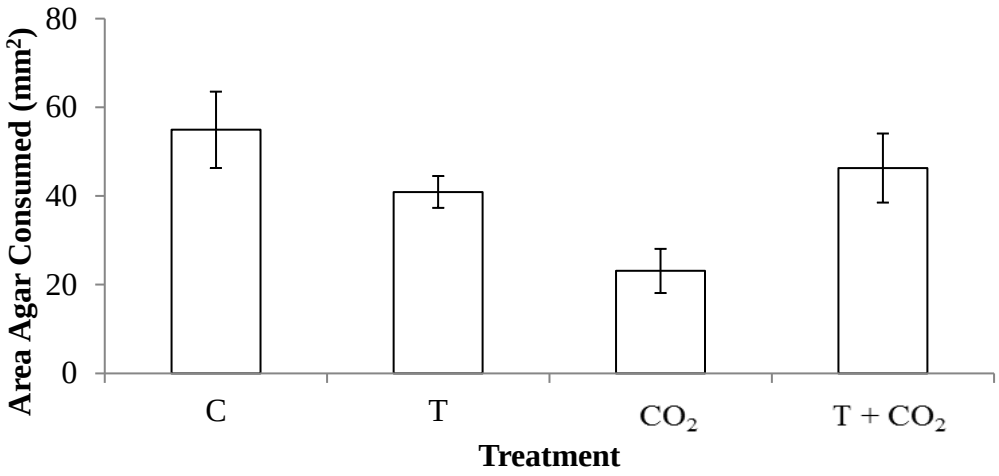
In our laboratory *per capita* feeding rate manipulation, when only the amphipods were exposed to treatment conditions, temperature had a significant and negative effect on amphipod consumption of turf algae, (Figure 3.4A, Table 3.3A), resulting in a 40% decrease in consumption rate at higher temperatures regardless of CO_2 condition. There was no effect of CO_2 on the amphipods *per capita* feeding rates. When I fed control amphipods algae that had been grown under treatment conditions, I found an interactive effect of CO_2 and temperature (Figure 3.4B, Table 3.3B). In this case, CO_2 negatively impacted the palatability of the algae with over a 50% reduction in consumption compared to current conditions. This effect of CO_2 on palatability was only seen at ambient temperatures. Palatability of algae under elevated temperatures (regardless of CO_2 treatment) did not differ significantly from that of control algae. Finally, when both algae and amphipods were exposed to the same

treatment conditions, there was no observed effect of treatment conditions, and consumption at treatment conditions did not vary significantly from current control conditions (Figure 3.4C, Table 3.3C).

A) Amphipods Only



B) Algae Only



C) Both Amphipods and Algae

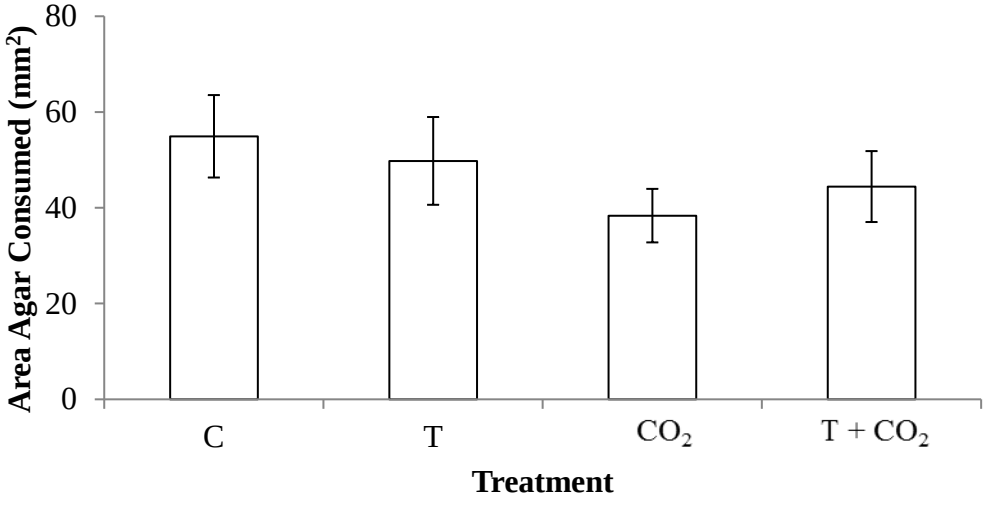


Figure 3.4. Amphipod *per capita* consumption rates.

Consumption of agar-suspended algae (in area grazed) when only amphipods (A), only algae (B), or both amphipods and algae (C) were exposed to treatment conditions. Note the Control ("C") bar is the same in all three figures. Consumption by amphipods is negatively affected by temperature (A), while CO_2 decreases the palatability of turf algae, but only at ambient temperature (B). When both amphipods and algae are exposed to the same experimental condition, there is no effect of temperature, CO_2, or their interaction on feeding rates (C).

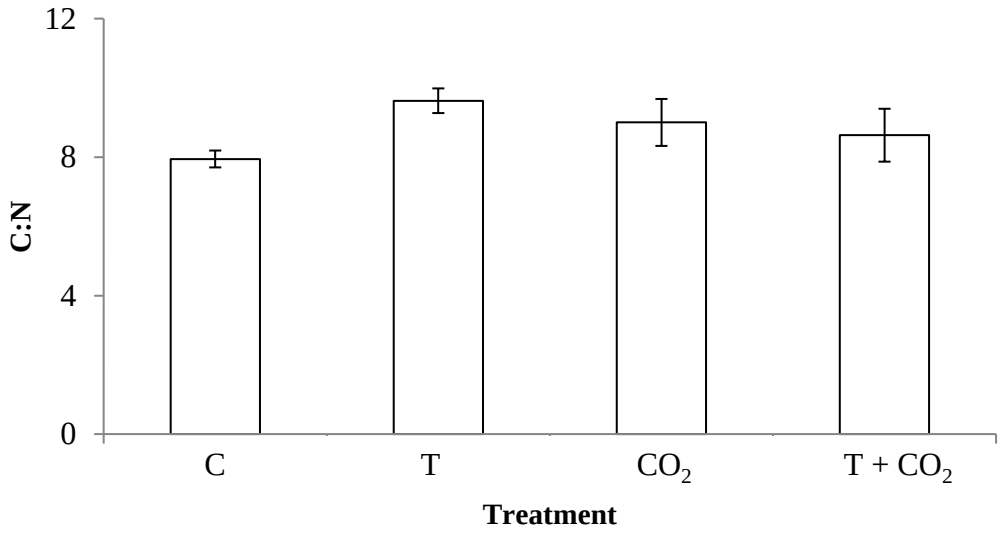
Table 3.3. Analysis of amphipod *per capita* consumption rates.

Statistical details for mixed effects linear model of the effects of CO_2 and temperature on (A) consumption of control turf algae by amphipods subjected to treatment condition, (B) consumption of algae subjected to treatment condition by control amphipods, and (C) consumption of algae by amphipods when both were subjected to the same treatment conditions. Treatment levels of CO_2 and temperature included current or elevated levels in each case. Turf algal origin was a random effect due to the way the agar feeding cubes were made (DF = 1, 20).

Source	F-value	p-value
A) Amphipods at Treatment		
CO_2	<0.01	0.97
Temperature	7.04	**0.02**
CO_2*Temperature	0.10	0.76
B) Algae at Treatment		
CO_2	5.54	**0.03**
Temperature	1.75	0.20
CO_2*Temperature	7.04	**0.02**
C) Both at Treatment		
CO_2	1.90	0.18
Temperature	<0.01	0.95
CO_2*Temperature	0.51	0.48

There was no change in phlorotannin concentration or C:N ratio under different CO_2 or temperature combinations (Figure 3.5, Table 3.4).

A)



B)

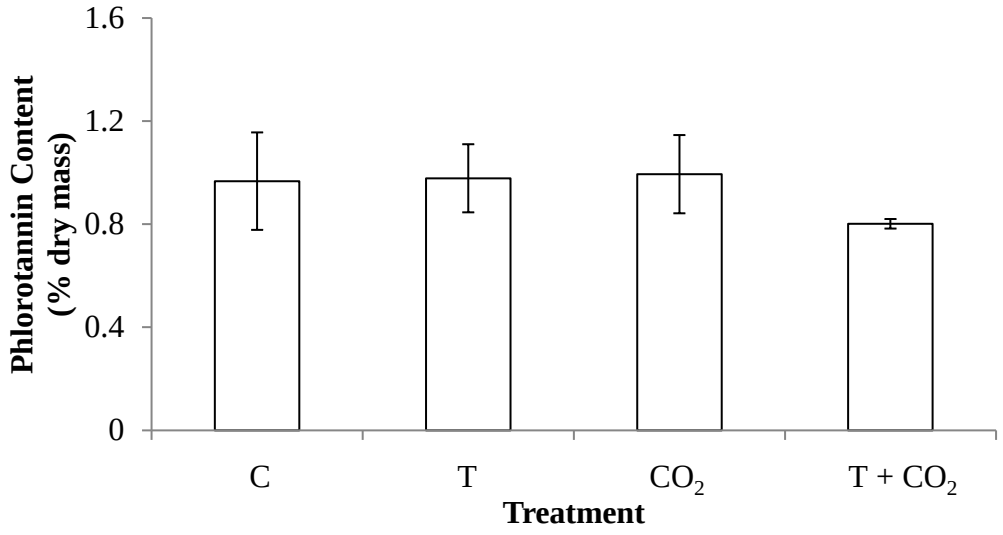


Figure 3.5. Chemical analysis of turf algae.

Effect of increased temperature (T) and CO_2 on turf algal (A) carbon-nitrogen ratios (C:N) and (B) phlorotannin content (mean ± SE). There was no significant effect of temperature, CO_2 or their combination on C:N ratios nor phlorotannin content. "C" indicates control conditions.

Table 3.4. Analysis of the effects of CO_2 and temperature on chemistry of turf algae.

Statistical details for the linear model of the effects of CO_2 and temperature on turf algae (A) carbon-nitrogen ratios (DF = 1, 8) and (B) phlorotannin content expressed in percent per dry mass (DF = 1, 1). Treatment levels of CO_2 and temperature included current or elevated levels in each case.

Source	F-value	p-value
A) Carbon-Nitrogen Ratios		
CO_2	<0.001	0.66
Temperature	1.40	0.27
CO_2*Temperature	3.42	0.10
B) Phlorotannin Contentent		
CO_2	0.02	0.60
Temperature	0.02	0.53
CO_2*Temperature	0.03	0.48

3.5 Discussion

Herbivory is a process that is important for determining ecosystem structure and function but can be highly affected by anthropogenic change. Changes in herbivore pressure can be driven by both changes in individual *per capita* feeding rates or by changes in herbivore abundance. Further, *per capita* feeding rates are influenced both by the condition of the resource and the condition of the consumer. Where a stressor does not show parallel effects on consumer abundance, resource condition, and consumer condition, there is the potential for ecological surprises. This potential for discordant effects may be even higher under multiple stressor scenarios. In this experiment, I used a multigenerational mesocosm experiment as well as targeted microcosm experiments to examine how OA and temperature can affect amphipod herbivory both at the population and *per capita* level.

Metabolic theory predicts a non-linear relationship between temperature and most biological responses (feeding rate, growth rate, population growth, etc.), where there is a single optimum temperature at an intermediate value (Savage et al. 2004, Kordas et al. 2011). As temperate species tend to live slightly below their temperature optimum, many studies observe an increase in the feeding rates of mesograzers with the small increases in temperature associated with climate change (Deutsch et al. 2008, O'Connor 2009, O'Connor et al. 2009). However, once warming pushes herbivores beyond this optimal temperature, consumption rates decrease steadily with any further increase in temperature (Lemoine and Burkepile 2012, Mertens et al. 2015). Here, I observed a negative effect of temperature on the *per capita* consumption rates of the amphipods in response to increased temperature. This suggests that amphipods in our system are already operating near their optimal temperature and that further warming may be detrimental to their role as consumers (Kordas et al. 2011).

Multiple studies have focused on the effect of CO_2 addition on the foraging rates of mesograzers, showing that CO_2 indirectly affects *per capita* consumption rates by altering the palatability of algal tissue (Poore et al. 2013, 2016, Falkenberg et al. 2013b). These studies have generally found an increase in algal palatability in response to CO_2. While certain studies were able to link this increased palatability to decreases in the C:N (Falkenberg et al. 2013b)other studies demonstrated changes in phlorotannin content (Swanson and Fox 2007) or fatty acid composition (Rossoll et al. 2012), which would likely also affect algal palatability. Here, I found a decrease in algal palatability with increased CO_2, but only at ambient temperatures. Furthermore, the exact mechanism for this decrease in palatability is unknown and could not be explained by carbon-nitrogen ratios or phlorotannin concentrations. I suspect this decrease in palatability was caused by a change in

the chemical composition of the algae that was not measured in this experiment (e.g. fatty acids or other upregulated chemical defense; Rossoll et al. 2012), as grinding the up the algae prior to the feeding assay would have eliminated any changes in palatability due to algal structure. It is unclear why there was no effect of CO_2 on algal palatability under high temperature conditions. Likely, this lack of effect is related to temperature altering the physiology of the algae in a way that minimizes the CO_2 effect, but further research needs to be done in this area to decipher an exact mechanism.

This laboratory manipulation provides important insight into looking at multiple species under multiple stressors simultaneously (Harley et al. 2006, Gilbert et al. 2014). I found no effect of temperature or CO_2 on the consumption of turf algae by amphipods when both species were exposed to treatment conditions in our laboratory feeding trials. This lack of treatment effect was not predictable from the manipulations looking at each species in isolation, which found a significant effect of temperature on amphipod feeding rates and a significant interactive effect of temperature and CO_2 on algal palatability. This outcome is important as it changes the way one interprets the algal and amphipod responses. For example, the decrease in palatability of turf algae in response to increased CO_2 might indicate that amphipod herbivores would consume less algae under high CO_2 conditions. Combining this information with known increases in growth rates and competitive dominance of turf algae over habitat forming kelp *E. radiata* (Connell and Russell 2010) paints a bleak picture for the future of South Australian kelp forests. However, the negative effect of algal palatability is not a factor if amphipods have also been exposed to enriched CO_2, indicating that herbivory could still be an important mechanism for regulating dominance hierarchies. Similarly, the negative effect of increased temperature on amphipod

feeding rates disappeared when the algae had been grown under similar conditions. These responses were not predictable based on my single species manipulations, and the mechanism behind them is yet unknown. However, this result clearly demonstrates the need for assessing interacting species simultaneously when attempting to understand the ecological implications of environmental change.

While there is no doubt that *per capita* level grazing rates may have large implications for communities, it is also quite apparent that herbivore abundance can drive large changes in community structure (Fortin et al. 2005, Ling et al. 2014, Atkins et al. 2015, Ripple et al. 2015a). For this reason, one cannot make assumptions about how changes in herbivory will affect a local ecosystem without first understanding how local herbivore abundance changes in response to future conditions. Here, I found an interactive and synergistic effect of temperature and CO_2 level on turf algal consumption by an experimental population of amphipods, despite not finding this effect in our *per capita* level manipulations. This increase in population level consumption rates was likely largely driven by increases in population density with increased CO_2 enrichment (Heldt et al. 2016). Heldt and colleagues (2016) did not find an effect of temperature on amphipod abundance. However, I found an interactive effect of temperature and CO_2 on population herbivory. I suspect that this disconnect between Heldt's (2016) findings and the ones in this study relates to the large variability in amphipod population size under high CO_2 and ambient temperature conditions. This increased variability would make amphipod abundances patchier under high CO_2 at ambient temperatures, making an effect of herbivory more difficult to detect. Finally, while I observed a 1.8x increase in population level consumption rates under conditions of elevated temperature and CO_2, I suspect that this result is conservative: while amphipod

density in the experimental units were varied according to population density at the mesocosm scale, the experimental densities were proportionately smaller when mesocosm densities were highest. In spite of this conservative design, there was still strong effect of CO_2 and temperature on population level herbivory.

In many ecosystems, herbivores can cause large scale reductions in primary producer biomass, changing the environmental and community assembly of that environment (Lubchenco 1978, Hughes et al. 2007, Ling et al. 2014, Ripple et al. 2015a, 2015b). This study suggests that herbivory on turf algae will increase under future oceanic conditions off the western coast of Australia. This may have implications for the Australian *E. radiata* kelp forest. The turf algae that I utilize in this study are historically competitively inferior to the habitat-forming *E. radiata*. However, increased CO_2, temperature, and nutrients have all been shown to shift the competitive hierarchy in favor of the turf algae (Russell and Connell 2005, Connell and Russell 2010). Increased herbivory on turf algae is expected to equalize the competitive interaction between *E. radiata* and turf algae under projected future conditions, as turf algae is more palatable than its habitat-forming competitor (Falkenberg et al. 2012, 2014, Ghedini et al. 2015).

Similar suites of conditions are likely applicable in other communities where habitat structure is created by non-palatable, less competitive species (e.g. coral reefs Harley et al. 2012; Gaylord et al. 2015). However, large increases in herbivore populations may not always be beneficial for the maintenance of community structure (Ripple et al. 2015a). Increases in herbivore populations may be quite detrimental to communities where the habitat-structuring species is also a highly palatable primary producer (e.g. *Macrocystis* dominated kelp forests; Ling et al. 2014). For this reason, the implications of having

increased herbivore pressure by small arthropods may have differing effects on ecosystem health depending on the palatability of foundation species. Furthermore, it is important to note that not all herbivores are likely to experience increases in population size in response to OA. In fact, many important marine herbivore species such as urchins and gastropods will likely experience reduced population sizes or reduced population biomass due to reduced larval viability, fertilization success, and adult growth as a result of OA (Kroeker et al. 2011, 2014). In such situations, the presence of a species that is more resilient to the effects of increased CO_2 may be especially important in the maintenance of community structure and diversity.

3.6 Conclusions

The results of this study draw attention to the fact that the outcomes of multi-species, multi-stressor experiments are often not predictable based on the outcomes of their individual species components. I also showed that the emergent properties of a population may be quite different than what would be predicted from the behavior of individuals alone; while there was no change in the amphipods *per capita* consumption under future projected conditions, increased population density did cause an overall increase in herbivory rates. These impacts are particularly important to understand when the outcomes have a bearing on foundation species and their key competitors and consumers, as impacts on these taxa have the potential to scale up to impacts on whole communities. Thus, in order to understand how consumers shape a community, it is important to understand the drivers of *per capita* and population level consumption as well as how *per capita* interaction strength changes when both species are exposed to treatment conditions. As research on climate change biology progresses,

scientists are finding it more and more important to look not only at multiple interacting species, but multiple climate change variables at the same time (Rudd 2014). This study demonstrates the need to conduct ecological studies on environmental change over longer time scales that encompass not only processes at the individual level but also at the population and community level.

Chapter 4: Calcified herbivore loss may disproportionately drive responses to ocean acidification in natural communities.

4.1 Chapter summary

Ocean acidification (OA) is expected to be one of the largest challenges facing marine biodiversity. While we continue to catalogue the effects of OA on a growing number of species at the individual level, there is increased recognition that we must understand the effects of OA in a community context and, whenever possible, in the field. Although many communities will likely be reshaped by each species responding individually to the immediate, direct effects of OA, other communities will be affected by longer-term indirect effects driven by the reduction of a single important species or guild. For example, a decline in OA-sensitive calcified grazers over multiple generations may have secondary effects on benthic community structure, as described by diversity and composition, in addition to any direct effects of increased CO_2 on other species. Here, I simultaneously measured the direct and indirect effects of OA by factorially manipulating CO_2 and molluscan grazer abundance in field-based artificial tidepools over a full annual cycle. I manipulated gastropod herbivore density (control and reduced density) and CO_2 aeration (high and ambient CO_2 aeration, along with a no aeration control) in the field. Tidepool community structure responded significantly to herbivore abundance over the entire 15-month time series. This community response included an increase in mussel and diatom cover coupled with a decrease in average species richness, Shannon diversity, and barnacle cover. By contrast, the only taxa that showed a response to increased CO_2 were diatoms. While I did observe an effect of CO_2

aeration treatment on community structure, this effect was driven by differences between aeration and no aeration, not by a difference in CO_2 concentration in aerated treatments. This was likely caused by the large fluctuations in oxygen saturation in the non-aerated tidepools, which the aerated tidepools did not experience. My results suggest that predicted long-term, indirect consequences of OA, such as the reduction of top-down control by calcified species, may have impacts larger than the direct effects of OA on abundance and diversity.

4.2 Introduction

Ocean acidification (OA), which is caused by increased carbon dioxide dissolved in the oceans, can impact organisms directly (e.g. reductions in survival) or indirectly through the alteration of ecologically important interactions. OA ecology is a relatively new field, and much of the initial work on OA has focused on establishing the direct effects of CO_2 on individual species (Kroeker et al. 2010, 2013) and documenting differences in community structure along naturally occurring CO_2 gradients (Hall-Spencer et al. 2008, Kroeker et al. 2011, 2012, Johnson et al. 2013). More recently, ecologists have begun to explore the ways in which species interactions may mediate the role of OA in determining how ecosystems and communities will respond to increasing CO_2 (Falkenberg et al. 2013a, 2014, Connell et al. 2013, Kroeker et al. 2014, Gaylord et al. 2015, Ghedini et al. 2015). Despite this increased interest in the role of species interactions, few studies consider more than two species at a time and those that do tend to infer the role of species interactions rather than explicitly testing it (Kroeker et al. 2012, Brown et al. 2016, Goldenberg et al. 2017). Without these explicit tests, one is left to speculate about the role that a particular species plays in producing observed patterns or how measured pairwise interactions scale up to shape the whole community.

Herbivory is a particularly important ecological interaction, which may undergo substantial changes with OA. The relevance of herbivory is twofold; first, there is growing evidence that a healthy herbivore population can help combat some of the negative outcomes of climate change (and OA specifically) on community structure by the removal of a competitively dominant algal species if that species also benefits disproportionately from the change in the abiotic environment (Harley et al. 2012, Falkenberg et al. 2013b, 2014, Ghedini and Connell 2015, Ghedini et al. 2015). However, there is ample evidence that increased CO_2 can reduce herbivore biomass and their net consumption if many of the important herbivores are highly calcified invertebrates (e.g. urchins and gastropods). Such invertebrates tend to be highly sensitive to the decrease in pH associated with increased CO_2. This prediction is supported by numerous organismal-level studies on calcified herbivores, which have shown significant negative effects of OA on fertilization, embryonic and larval development, and recruitment, along with survival, calcification and growth of adults (Ellis et al. 2009; Kroeker et al. 2010, 2013). Negative impacts of OA on calcified grazers are also evident in the loss or reduction of gastropod and urchin herbivore biomass at naturally occurring CO_2 vent sites (Hall-Spencer et al. 2008; Kroeker et al. 2011; but see Connell et al. 2017). Despite this recurrent and predictable observation, no study has teased apart the direct effect of OA on community structure from the indirect effect of OA via reduced herbivore abundance.

Tidepools provide a useful model system for understanding the direct and indirect effects of OA on community structure. One may hypothesize that the organisms living in tidepools will be relatively robust to changes in ocean chemistry as adults, since tidepools are strongly characterized by the large shifts in carbonate chemistry that occur over a single tidal

cycle (Morris and Taylor 1983, Vargas et al. 2017). During high tide, when tidepools are submerged, tidepools experience the same carbonate conditions as the nearshore water. However, at low tide, when tidepools are isolated, tidepool carbonate chemistry is largely determined by biological activity within the pool. When low tide occurs during daylight hours, autotrophs can rapidly deplete CO_2 in the pool via photosynthesis, causing an increase in pH. Conversely, if there is no light (i.e. a nighttime low tide) or few autotrophs, respiration can increase the CO_2 concentration in the tidepool, causing a decrease in pH that can exceed changes predicted with OA in the next 100 years by at least an order of magnitude (Morris and Taylor 1983, IPCC 2007). Because the pH environment is highly variable, organisms found living in tidepools may be particularly well adapted to rapid decreases in pH (Vargas et al. 2017). However, if tidepool organisms are living closer to their pH tolerance limit, any further change in pH could have disproportionately severe consequences – similar to what has been observed with animals living in extreme thermal environments (Stillman and Somero 2000). Finally, it is worth noting that an increase in CO_2 may serve as a resource for photo-autotrophs and thus alter their relative competitive abilities as has been seen in other systems (Connell et al. 2013). This effect may be more profound in tidepools than in other benthic ecosystems as the isolated nature of the tidepools can cause them to become carbon-limited during daytime low tides (Williamson et al. 2014).

Like many other benthic communities, tidepool community structure is known to be significantly altered by the presence and abundance of herbivores, many of which are highly calcified (particularly gastropods; Lubchenco 1978; Bracken & Nielsen 2004), taxa that have already been shown to exhibit strong negative responses to OA (Kroeker et al. 2010, 2013). Thus, herbivory may be a particularly important leverage point when it comes to

understanding the indirect effects of OA on community structure. Analogous herbivore species at natural CO_2 vent sites do not seem to have evolved a tolerance to high CO_2, but rather experience dwarfing in response to chronic exposure to a low pH environment (Garilli et al. 2015) or, more commonly, simply show a reduction in abundance –or even absence – in areas of extreme low pH and high CO_2 (Hall-Spencer et al. 2008, Kroeker et al. 2011). Herbivore populations may be further limited by demographic effects of OA at the larval stage, which tends to be particularly susceptible to low seawater pH (Kroeker et al. 2010, 2014). Therefore, one might expect to see large reductions in herbivore abundance based upon changes in recruitment and post-recruitment mortality.

Here, I experimentally disentangle the direct and indirect effects of CO_2 by independently manipulating gastropod abundance and dissolved CO_2 in artificial tidepools. I use tidepool communities because they are (1) self-contained and therefore amenable to manipulations (Bracken and Nielsen 2004, Bracken et al. 2008), and (2) known to experience changes in community structure associated with changes in herbivore density (Lubchenco 1978). While I do not know exactly what future herbivore densities will be, I can reasonably predict that they will be reduced to some degree in systems dominated by large, calcified herbivores. Here, I reduced herbivore abundance considerably to simulate the more extreme herbivore response to OA observed in some vent systems (Hall-Spencer et al. 2008, Kroeker et al. 2011). I predict that tidepool communities, which may contain a number of taxa that are robust in their response to abiotic change (in this case OA), but may in fact be vulnerable indirectly through the loss of a key species interaction.

4.3 Methods

4.3.1 General design and construction

To understand the impacts of increased CO_2 and reduced gastropod herbivore abundance on community structure, I constructed 60 artificial tidepools in the high rocky intertidal in Bamfield Inlet, Bamfield, British Columbia (48°50'04.7"N 125°08'04.8"W). I constructed the tidepools out of vertical sections of large bore PVC pipe (diameter – 20cm, length – 15cm). These PVC pipes were then cemented using fast-setting concrete (Quickrete™) into the rocky intertidal so that the pipe was below the top of the *Fucus* zone (2.04± 0.01 meters ± SE, lower low water, large tide). This height was chosen so that each tidepool would be submerged at least once a day, to allow for the dispersal and recruitment of new individuals, but were above the waterline for an average of 13 h/day. During this period, the tidepools would be isolated from the rest of the ocean so that treatment conditions would be more easily maintained. I used epoxy (Z-Spar™ splash zone epoxy) to increase the strength and seal of the tidepool to the rock, but only did this on the outside of the tidepools to allow for ample recruitment space on the natural rock inside.

I used a fully factorial design where I manipulated herbivore abundance and CO_2 via tidepool aeration. The aeration treatment had 2 levels (ambient and CO_2 enriched aeration) as well as a no aeration as a procedural control. This procedural control was added specifically so that I could consider the effects of CO_2 concentration separately from the physical effects of bubbling associated with aeration, which does not naturally occur in tidepools. Gastropod herbivore reductions were carried out by painting large rings of copper paint around the outside of the tidepools; copper is mildly toxic to gastropods so they avoid coming into contact with it (Range et al. 2008). In addition, any gastropod herbivores (limpets, turban

snails, and periwinkles) that were found inside the tidepools during diversity surveys (see below) were removed from the community at that time. Because gastropod herbivores re-entered the pools in low numbers, despite the copper paint, gastropod herbivore populations were reduced but not completely eliminated, simulating a substantial, but not complete, reduction in grazing pressure.

4.3.2 Biodiversity surveys

The communities in each tidepool were allowed to re-establish naturally over a period of 15 months beginning in early July 2012. Over the course of the experiment, tidepools were surveyed every 1-2 months. In each tidepool, the percent cover of sessile species was visually approximated (Dethier et al. 1993), as the three-dimensional nature of the tidepools did not allow for two-dimensional point counts. During the first month of the manipulation, microscope slides were attached to the side of each tidepool. Slides were collected after 4 weeks and viewed under a microscope to verify that the developing biofilm was dominated by diatoms. Mobile species (e.g. hermit crabs and limpets) were counted individually. I identified each species to the lowest possible taxonomic unit, which varied greatly across taxonomic groups. This variation was driven by an inability to remove sessile organisms from the tidepools for more detailed identification without interfering with the natural succession of the community.

4.3.3 Seawater chemistry

While pH and CO_2 of the tidepools were deliberately manipulated, they were not tightly controlled and were influenced by biotic feedback loops within the developing

communities (e.g. photosynthesis and respiration). Aeration was achieved by piping freshly compressed air down to the intertidal from a storage shed near the field location. Compressed air was piped down to a splitter box in one of two long tubes: one with just compressed air, the other with CO_2 enriched compressed air. The splitter box contained multiple gang valves in order to divide the flow from each of the source tubes into individual lines that lead to each of the tidepools receiving manipulation. Each of these smaller lines ended in a tidepool with a sealed end that had been perforated with thin needle to imitate an air stone.

I calibrated the aeration treatments by altering air flow rates before the start of the experiment so that the ambient aeration treatment had a pH similar to that of non-aerated controls during the day. I then adjusted the CO_2 concentration of the high CO_2 aeration treatment so that it consistently altered tidepool pH to be 0.52 ± 0.07 pH units below that of the tidepools ambient aeration pre-recruitment, which is slightly more extreme than the largest change predicted by 2100 (Pachauri et al. 2015). Aeration was then held constant for the duration of the experiment and seawater chemistry was allowed to fluctuate from this starting point.

Seawater chemistry parameters were measured regularly throughout the entire experiment. Temperature and pH were usually measured twice each week between May and September and at least once per month October - April (time between measurements: 6.7 ± 0.8 days, mean ± SE) in each tidepool, as well as a single sample from the adjacent inlet. Consequently, each tidepool was measured an average of 68 times ± 1.5, mean ± SE, during the experiment (487 days, plus 14 days before the official start of the experiment). Slow water leaks caused some of the artificial to drain at low tide, and occurred randomly across all treatment groups, and leaky tidepools were only kept in the experiment if they could be

fixed before visible damage occurred to the existing community. Tidepools that experienced irreparable leaks were excluded from the experiment from that point forward. Early on I found very little variation in salinity between tidepools (SE < 0.04 ppt, on any given day). Thus, I reduced measurements of salinity, whereby for 2/3 of the sampling days I would measure salinity in each tidepool and for the remainder measured the salinity in a single haphazardly selected tidepool. Because carbonate chemistry is strongly affected by changes in the rates of photosynthesis and respiration, tidepools were sampled both during the day and the night. The nighttime samples were interspersed between the daytime sampling to the best extent possible given the constraints of the fortnightly and seasonal tidal cycles; in total, 25% of all samples were taken at least an hour after sundown

To understand tidepool seawater chemistry and my CO_2 treatments at a finer scale, there were two instances where tidepools were sampled repeatedly within a single low tide or over a complete tidal cycle. On September 23, 2012, I measured pH, temperature, and salinity in each pool every half hour for 2.5 hours starting when each tidepool was first isolated from the surrounding ocean. I did this to verify that each tidepool exhibited treatment variation (according to pH) within the first half hour of being isolated. Additionally, I took repeated samples (pH temperature, salinity, and O_2) from six tidepools, one from each of the 6 treatment groups, every hour for 72 hours while they were isolated from the ocean between daytime low tide on October 29, 2013 and daytime high tide on November 1, 2013 (days 483-486). During this intensive sampling period, the same parameters were measured once every 2 hours in Bamfield inlet for a detailed diurnal characterization of background carbonate chemistry. Finally, additional oxygen measurements were taken during daytime on October 10, 2012, during the night on January

12, 2013, and during the day and night on November 2, 2013 (days 109, 193, and 487, respectively).

To fully characterize carbonate chemistry in experimental tidepools, water samples were fixed with mercuric chloride for later dissolved inorganic carbon (DIC) processing. Water samples for DIC analysis were taken from all functioning tidepools on November 23, 2012, 33 haphazardly selected tidepools on October 18, 2013, and 30 times from each of the 6 tidepools sampled during the three-day intensive sampling during the last 4 days of the experiment (daytime low tide on October 29, 2013 to daytime high tide on November 1, 2013). DIC measurements were then combined with the associated pH, temperature and salinity data, before using CO_2Calc software (Robbins et al. 2010) with constants from Mehrbach et al. (1973) as adjusted by Dickson and Millero (1987) to determine the other carbonate parameters of the seawater, including: pCO_2; total alkalinity; carbonate and bicarbonate ion concentrations; as well as calcite and aragonite saturation states.

4.3.4 Data analysis

Tidepool pH, pCO_2, temperature, salinity, and oxygen saturation were analyzed using a two-factor linear mixed effects model in the *nlme* package in R. Aeration treatment and time of day (day versus night) were used as fixed factors and both date and tidepool as random effects.

To test the hypotheses that OA and herbivory affect community composition and diversity, I analyzed the community response variables in the following ways. To examine the effects of CO_2 and herbivore loss on a mature tidepool community, I used a single MANOVA on a Bray-Curtis dissimilarity matrix for the final survey timepoint using the

adonis function in the *Vegan* package in R. Additionally, I used principal response curves (PRC) to establish whether or not the community level difference in composition observed at the final time point could be seen throughout community assembly. All multivariate data was put through a Wisconsin double standardization before ordination and analysis. In this standardization, each species is first standardized by its maxima and then each sample is standardized to one. This standardization prevents very common species from having a larger effect than rare species while also controlling for the fact that some species were measured by percent cover and others by individual abundance. I used time series, repeated measures ANOVAs to examine the changes in species richness, Shannon diversity, and abundance of individual species and functional groups throughout the experiment. For these analyses, I focused on taxa that were spatial dominants in the tidepools. Gastropod herbivore abundance was removed from all community level measurements in order to ensure that any significant effect of these herbivores was measured in terms of their impacts on other species, not simply by the imposed differences in their abundances.

I carried out post hoc analyses on data where the main effects were significant for aeration treatment. For the chemical data, I ran pairwise comparisons using a Tukey adjustment in the *lsmeans* package. For the biodiversity data, I repeated the original analysis on subsets of data to accomplish post hoc contrasts; for example, when I compared high and ambient CO_2 addition to each other, I removed the no-air addition control, and so on. This was done for analyses with both multivariate and univariate responses. Further, the PRC method does not allow for multiple explanatory variables, so this analysis was run first using treatment as the sole explanatory variable (with all 6 unique treatment combinations) and then repeated for herbivory and then for each of the two pairs of air addition levels. To

control multiple comparisons within the same data set, I used a Bonferroni correction on all post hoc analyses. I adjusted alpha to 0.017 any time I used additional analyses to differentiate effects between aeration treatment levels ($\alpha = 0.05/3$, for the three analyses) and to $\alpha = 0.013$ for additional PRCs ($\alpha = 0.05/4$, for the four analyses – three for aeration treatment and one for herbivory).

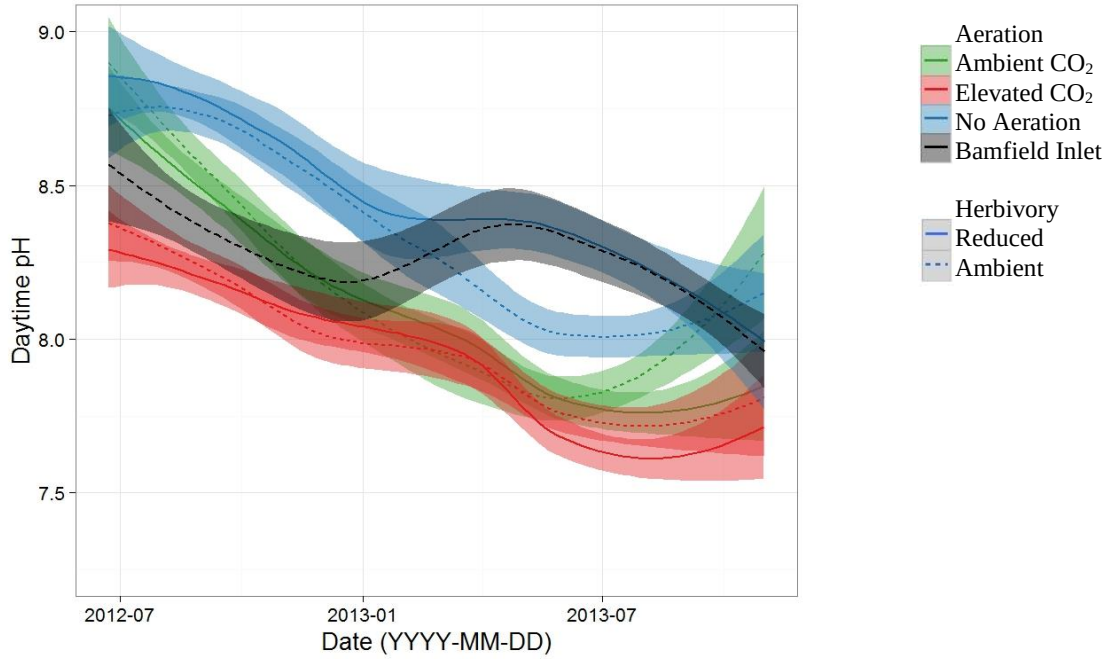
4.4 Results

4.4.1 Seawater chemistry

Tidepool pH treatments differences varied over time such that comparisons among tidepools did not reflect consistent abiotic differences over the 15-month experimental period, even though CO_2 addition was held constant throughout the experiment. Tidepool pH was significantly affected by aeration treatment, time of day and the interaction of herbivore abundance x aeration treatment x time of day (Table 4.1, Figure 4.1). Tidepool pH was lower at night for any given herbivore-aeration treatment combination (Table 4.1, Figure 4.1). Tidepool pH was highest in non-aerated tidepools during both day and night, while pH was lowest in the tidepools with high CO_2 aeration (effect of aeration; Figure 4.1, Table 4.1). This effect was significant at night regardless of herbivore treatment but not during the day (three-factor interaction; Figure 4.1, Table 4.1). During the day, tidepool pH was significantly affected by the interaction of herbivore abundance and aeration treatment: tidepools that received ambient aeration and reduced herbivory were similar in pH to tidepools that received elevated CO_2 aeration and reduced herbivore densities; as were tidepools receiving ambient aeration and ambient herbivory, and the tidepools receiving no aeration and ambient herbivore density (post hoc analysis, $p > 0.17$; Figure 4.1B). Daytime pH seemed to be more

consistently different between the two aeration treatments during the first third of the experiment than during the rest of the experiment (Figure 4.1).

A.



B.

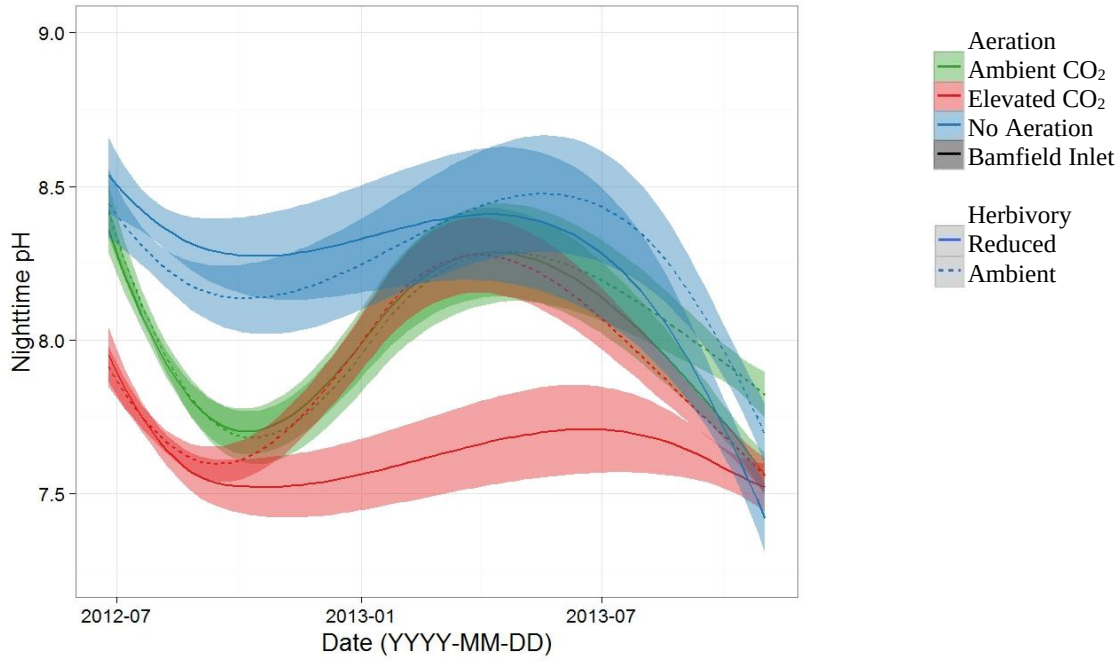


Figure 4.1. Tidepool pH over the duration of the experiment.

Daytime (A) and nighttime (B) pH are shown separately. Daytime ocean pH – taken from Bamfield inlet – is shown for reference, but was not included in the analysis. Solid lines indicate estimated mean and shading indicates the standard error of the mean (N = 3885).

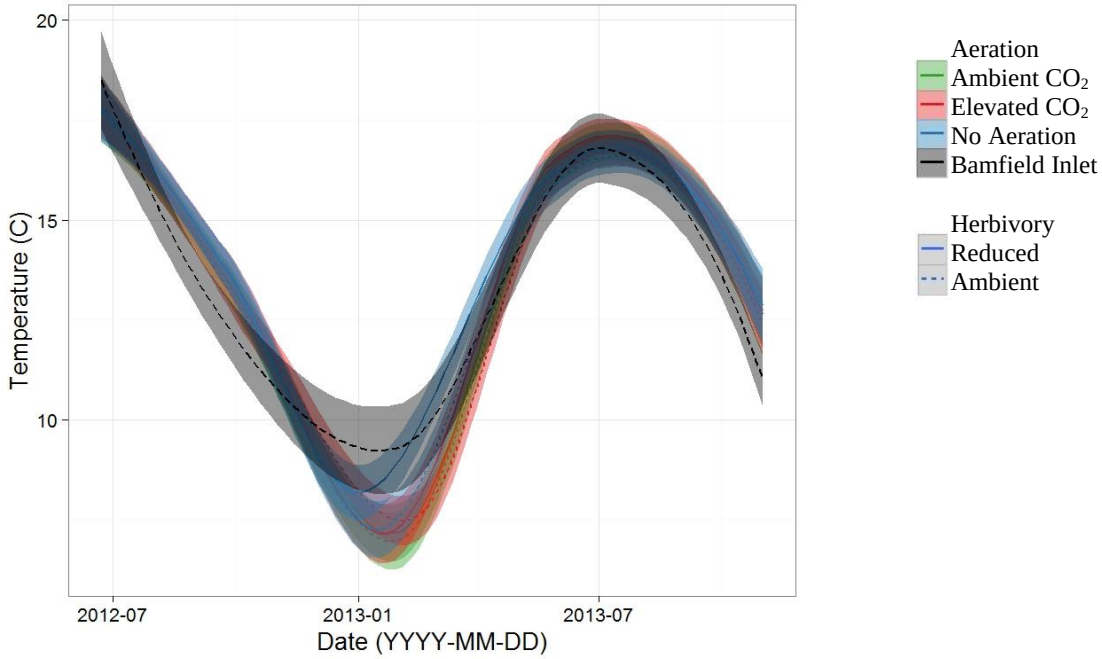
Table 4.1. Analysis of tidepool pH.

ANOVA table for a two-factor mixed-linear effect model showing the effects of time of day (daylight hours versus after dark) and CO_2 treatment on tidepool pH. Date and tidepool were used as random factors in this model.

	numDF	denDF	F-value	p-value
Random Factors	1	3825	187326	**<0.0001**
Aeration Treatment	2	54	67	**<0.0001**
Herbivores	1	54	0.1	0.75
Time of Day	1	404	150	**<0.0001**
Aeration Treatment x Herbivore	2	54	3.0	0.06
Aeration Treatment x Time of Day	2	404	1.5	0.23
Herbivore x Time of Day	1	404	0.1	0.74
3-way Interaction	2	404	5.0	**0.007**

Tidepools were significantly warmer during the day than at night (Table 4.2A). Tidepool temperature was not affected by aeration or herbivory treatments, nor were there any significant interactions among these factors (Table 4.2A). There was a strong seasonal effect on temperature with the lowest temperatures being measured in late January and highest temperatures falling in July both years (Figure 4.2A). Salinity was affected by a three-way interaction of time of day, aeration treatment, and herbivory treatment (Table 4.2B). The lowest average salinities were found during the day in tidepools with ambient aeration and reduced herbivory, while the highest average salinities were found at night in tidepools receiving no aeration and ambient herbivory (28.7 ± 0.1 and 29.6 ± 0.3 ppt, ± SE, respectively), despite pairwise comparisons of the tidepool treatments failing to show any significant differences (post hoc analysis, $p \geq 0.3$; Figure 4.2B). Salinity also fluctuated seasonality, with minimum values occurring in July both years, high values in January 2013, and again at the conclusion of the experiment in November 2013 (Figure 4.2B).

A.



B.

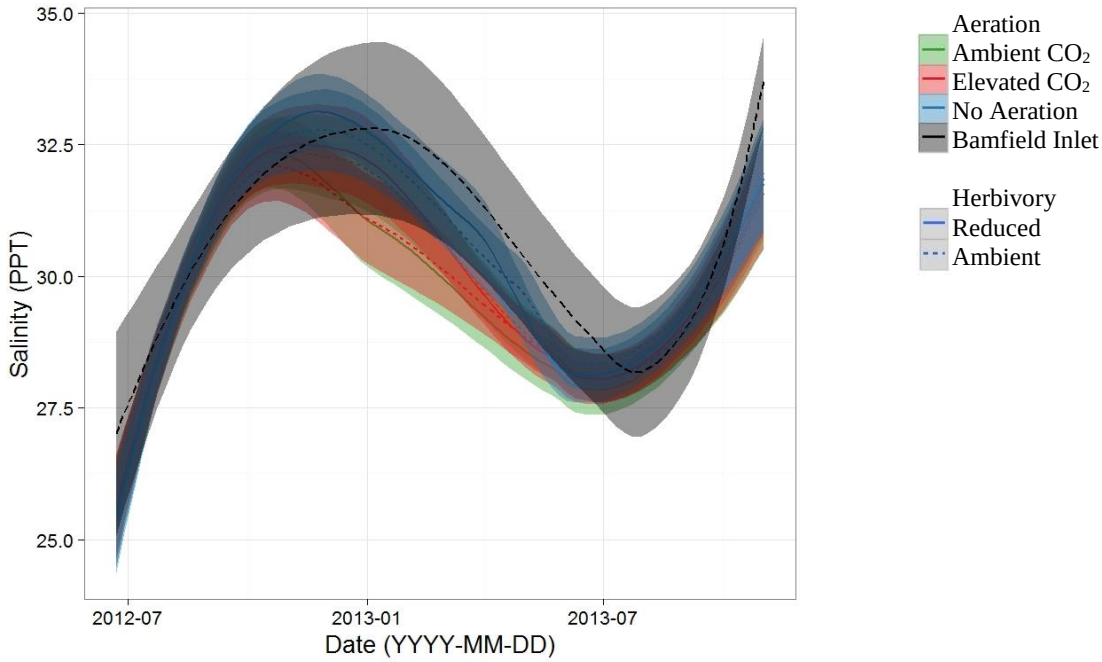


Figure 4.2. Tidepool temperature and salinity.

Temperature (A) and salinity (B) over the course of the experimental manipulation. Ocean temperature is shown for reference, but was not included in the analysis. Solid lines indicate estimated mean and shading indicates the standard error of the mean (N= 3885 and 2841, for temperature and salinity respectively).

Table 4.2. Analysis of tidepool temperature and salinity.

ANOVA table for a two-factor mixed-linear effect model showing the effects of time of day and CO_2 treatment on (A) tidepool temperature and (B) tidepool salinity. Date and tidepool were used as random factors in this model.

	numDF	denDF	F-value	p-value
A) Temperature				
Random Factors	1	3825	38356	**<0.0001**
Aeration Treatment	2	54	0.03	0.97
Herbivores	1	54	1.78	0.19
Time of Day	1	416	181	**<0.0001**
Aeration Treatment x Herbivore	2	54	0.41	0.67
Aeration Treatment x Time of Day	2	416	0.44	0.65
Herbivore x Time of Day	1	416	0.29	0.59
3-way Interaction	2	416	0.24	0.79
B) Salinity				
Random Factors	1	2789	128403	**<.0001**
Aeration Treatment	2	54	0.62	0.54
Herbivores	1	408	7.21	**0.008**
Time of Day	1	54	0.04	0.84
Aeration Treatment x Herbivore	2	408	0.03	0.97
Aeration Treatment x Time of Day	2	54	0.05	0.95
Herbivore x Time of Day	1	408	0.42	0.52
3-way Interaction	2	408	3.35	0.04

Tidepool pCO_2 was significantly affected by aeration treatment and time of day as well as the interaction of time of day and aeration, time of day and herbivory treatment, and a third order interaction (Figure 4.3, Table 4.3). The significant third order interaction (aeration, time of day, and herbivory) arose because the non-aerated, reduced herbivory pools fluctuated to a greater extent over the course of a day; this would be expected in situations where algal biomass is high and CO_2 concentrations are not constrained by continuous aeration. If we ignore the effect of herbivory, all aeration treatments experienced higher pCO_2 during the night than during the day (post hoc analysis, $p < 0.0001$; Figure 4.3), with

all the high CO_2 aeration tidepools having significantly higher pCO_2 than either the ambient or no aeration tidepools during the day (post hoc analysis, $p \leq 0.01$; Figure 4.3B). However, I found no statistically significant difference in pCO_2 between the ambient and no aeration tidepools, during the day, nor between any of the three aeration treatments during the night (post hoc analysis, $p \geq 0.07$; Figure 4.3). If we ignore the effect of aeration, tidepools with reduced herbivore density had higher pCO_2 than those with ambient herbivore density (Figure 3). However, this effect was only significant during the night (post hoc analysis, $p = 0.04$; Figure 3). I did not statistically analyze the other carbonate chemistry parameters for the tidepools, because I either calculated them from or used them to calculate the parameters already presented. However, these parameters are all summarized in Table 4.4.

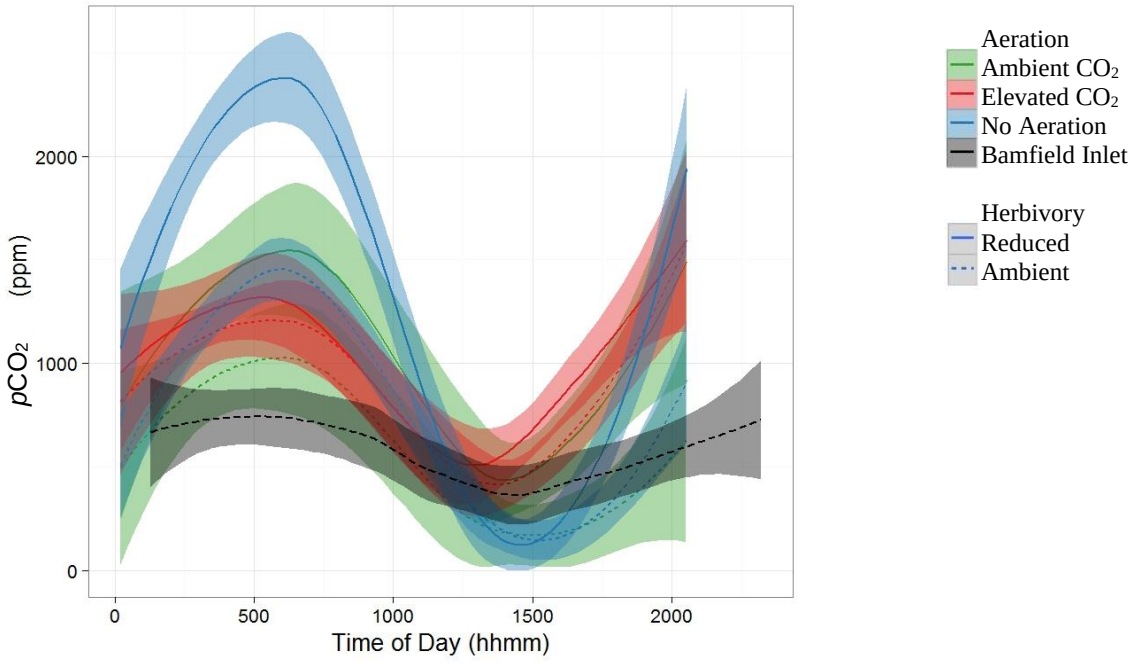


Figure 4.3. Tidepool *pCO₂* over a 24-hour period.

Curves are based upon sampling over multiple days. Ocean pCO_2 – taken from Bamfield Inlet – is shown for reference, but was not included in the analysis. Solid lines indicate estimated mean and shading indicates the standard error or the mean.

Table 4.3. Analysis of tidepool *pCO2*

ANOVA table for a two-factor mixed-linear effect model showing the effects of time of day (daylight hours versus after dark) and CO_2 treatment on tidepool pCO_2. Date and tidepool were used as random factors in this model.

	numDF	denDF	F-value	p-value
Random Factors	1	140	148	**<0.0001**
Aeration Treatment	2	49	9	**0.0005**
Herbivores	1	49	3	0.08
Time of Day	1	140	186	**<0.0001**
Aeration Treatment x Herbivore	2	49	<0.01	1.00
Aeration Treatment x Time of Day	2	140	13	**<0.0001**
Herbivore x Time of Day	1	140	5	**0.03**
3-way Interaction	2	140	8	**0.0006**

Table 4.4. Tidepool carbonate chemistry.

Carbonate chemistry parameters (mean ± (SE) indicated below) of the three tidepool CO_2 treatment conditions (ambient aeration, elevated CO_2 aeration, and no aeration control) during daylight hours (A) and after dark (B). All means are weighted to correct for the random effects of tidepool and date. Sample sizes vary according to parameters. Tidepool Salinity (Sal.), temperature (T), pH, O2 % saturation, and dissolved inorganic carbon (DIC) were all directly measured (N = 2849, 3885, 3885, and 111; respectively after correcting for repeated tidepool measures in the same tidepool on the same day). All other parameters were calculated from these measurements using CO_2Calc (n = 111, after correcting for repeated tidepool measures in the same tidepool on the same day).

Aeration	Herbivore	pH	T (°C)	Sal. (ppt)	O_2 ($\%_{Sat}$)	DIC (μmol/kgSW)	pCO_2 (ppm)	A_T (μmol/kgSW)	HCO_3^- (μmol/kgSW)	CO_3^{2-} (μmol/kgSW)	Ω_A	Ω_A
A) Day												
Ambient CO_2	Ambient	8.20 (0.05)	15.6 (0.02)	28.8 (0.02)	100 (5)	1466 (67)	246 (92)	1681 (78)	1306 (68)	139 (22)	3.4 (0.5)	2.1 (0.3)
	Reduced	8.13 (0.05)	15.7 (0.02)	28.7 (0.02)	98 (5)	1480 (67)	372 (94)	1638 (78)	1330 (66)	113 (21)	2.8 (0.5)	1.8 (0.3)
Elevated CO_2	Ambient	7.97 (0.05)	15.4 (0.02)	28.8 (0.02)	96 (4)	1565 (67)	550 (91)	1680 (78)	1437 (68)	86 (22)	2.1 (0.5)	1.3 (0.3)
	Reduced	7.91 (0.05)	15.8 (0.02)	28.9 (0.02)	94 (5)	1570 (65)	712 (94)	1627 (76)	1472 (66)	50 (21)	1.2 (0.5)	0.8 (0.3)
No Aeration	Ambient	8.40 (0.05)	15.5 (0.02)	29.0 (0.02)	99 (5)	1363 (71)	172 (100)	1643 (82)	1137 (71)	183 (23)	4.5 (0.6)	2.8 (0.4)
	Reduced	8.58 (0.05)	15.6 (0.02)	28.9 (0.02)	91 (5)	1346 (71)	218 (99)	1614 (82)	1135 (72)	173 (23)	4.2 (0.6)	2.7 (0.4)
B) Night												
Ambient CO_2	Ambient	7.95 (0.06)	13.5 (0.03)	28.7 (0.03)	90 (5)	1552 (70)	717 (139)	1582 (84)	1495 (74)	24 (28)	0.6 (0.7)	0.4 (0.4)
	Reduced	7.97 (0.06)	13.7 (0.03)	29.3 (0.03)	88 (5)	1553 (70)	874 (139)	1664 (84)	1394 (73)	93 (28)	2.3 (0.7)	1.4 (0.4)
Elevated CO_2	Ambient	7.71 (0.06)	13.6 (0.03)	28.9 (0.03)	94 (4)	1602 (70)	1109 (139)	1643 (84)	1493 (73)	46 (28)	1.1 (0.7)	0.7 (0.4)
	Reduced	7.71 (0.06)	13.8 (0.03)	29.2 (0.03)	90 (5)	1597 (69)	1122 (131)	1614 (83)	1499 (72)	31 (28)	0.8 (0.7)	0.5 (0.4)

Aeration	Herbivore	pH	T (°C)	Sal. (ppt)	O_2 ($\%_{Sat}$)	DIC (µmol/kgSW)	pCO_2 (ppm)	A_T (µmol/kgSW)	HCO_3^- (µmol/kgSW)	CO_3^{2-} (µmol/kgSW)	Ω_A	Ω_A
No Aeration	Ambient	8.20 (0.06)	13.9 (0.03)	29.6 (0.03)	68 (5)	1421 (74)	801 (141)	1590 (88)	1226 (77)	120 (29)	3.0 (0.7)	1.9 (0.4)
	Reduced	8.20 (0.06)	13.8 (0.03)	29.0 (0.03)	40 (5)	1449 (74)	1576 (141)	1482 (88)	1313 (78)	48 (29)	1.2 (0.7)	0.8 (0.5)

Tidepool oxygen saturation was influenced by aeration treatment, time of day, the interaction of aeration treatment x time of day, and the three-way interaction of aeration treatment x time of day x herbivore abundance (Figure 4.4, Table 4.5). Once again, these effects seem largely driven by the large fluctuation in the oxygen saturation of the non-aerated tidepools over the course of a day. During daylight hours, oxygen saturation was similar between all tidepools regardless of aeration treatment or herbivore pressure (post hoc analysis, $p \geq 0.95$; Figure 4.4). However, at night the oxygen saturation of the tidepools not receiving aeration and with reduced herbivory, dropped significantly below all other tidepools (post hoc analysis, $p \leq 0.01$; Figure 4.4). Similarly, at night the oxygen saturation of the tidepools not receiving aeration and with ambient herbivory, dropped significantly below all tidepools receiving elevated CO_2 aeration (post hoc analysis, $p \leq 0.05$) but not tidepools receiving ambient aeration (post hoc analysis, $p \leq 0.06$; Figure 4.4). There was no significant difference in the oxygen saturation levels of tidepools receiving ambient or elevated CO_2 bubbling – regardless of time of day. Although the oxygen saturation in the aerated tidepools seems to differ greatly from the non-aerated tidepools, they do appear to mimic the oxygen saturation of the ocean over the course of the day (Figure 4.4).

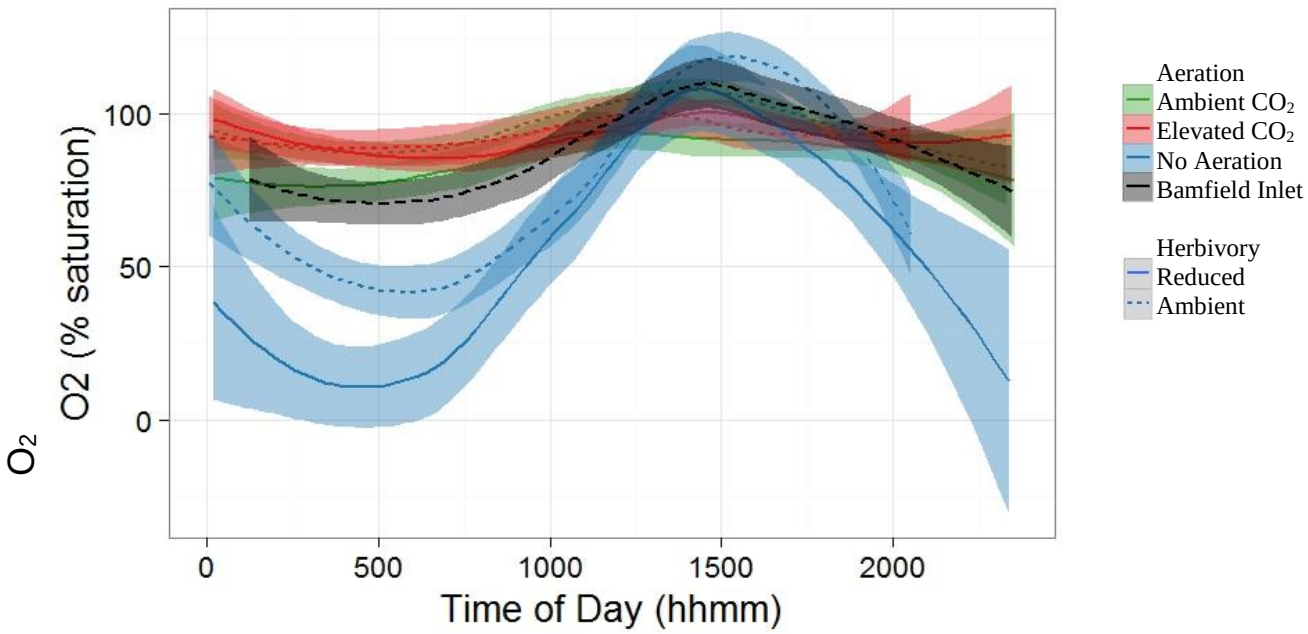


Figure 4.4. Oxygen % saturation of tidepools over a 24-hour day.

Curves are generated from data collected over multiple days. Oxygen saturation in the ocean (Bamfield Inlet) is shown for reference, but was not included in the analysis. Solid lines indicate estimated mean and shading indicates the standard error of the mean.

Table 4.5. Analysis of tidepool oxygen saturation.

ANOVA table for a two-factor mixed-linear effect model showing the effects of time of day (daylight hours versus after dark) and CO_2 treatment on oxygen percent saturation of the tidepools. Date and tidepool were used as random effects in this model.

	numDF	denDF	F-value	p-value
Random Factors	1	260	2914	**<0.0001**
Aeration Treatment	2	53	10	**0.0002**
Herbivores	1	53	4	0.06
Time of Day	1	260	88	**<0.0001**
Aeration Treatment x Herbivore	2	53	2	0.19
Aeration Treatment x Time of Day	2	260	42	**<0.0001**
Herbivore x Time of Day	1	260	3	0.07
3-way Interaction	2	260	3	**0.05**

4.4.2 Effectiveness of herbivore exclusion

Painted copper rings on the exterior slowed gastropod colonization of tidepools, but regular removal was needed in order to keep the densities low. Maximum gastropod herbivore abundance in the herbivore-reduced tidepools was on average 65% lower than in tidepools exposed to ambient herbivore abundances on sampling days (Figure 4.5). There was no effect of aeration on gastropod abundance, nor second order interaction of aeration x time or time x gastropod reduction (Table 4.6). However, there were survey occasions where, in some aeration treatments, the gastropod abundance in some tidepools receiving the reduced herbivore treatment was similar to the gastropod abundance in the tidepools receiving the ambient herbivore treatment; this was particularly common during the early recruitment portion of the experiment (third-order interaction of date x aeration treatment x herbivore treatment; Figure 5, Table 4.6). This effect was relatively transient as treatments diverged after more gastropods had colonized tidepools in the ambient herbivory treatment and as I removed gastropod herbivores from tidepools in the reduced herbivory treatment following each biodiversity survey.

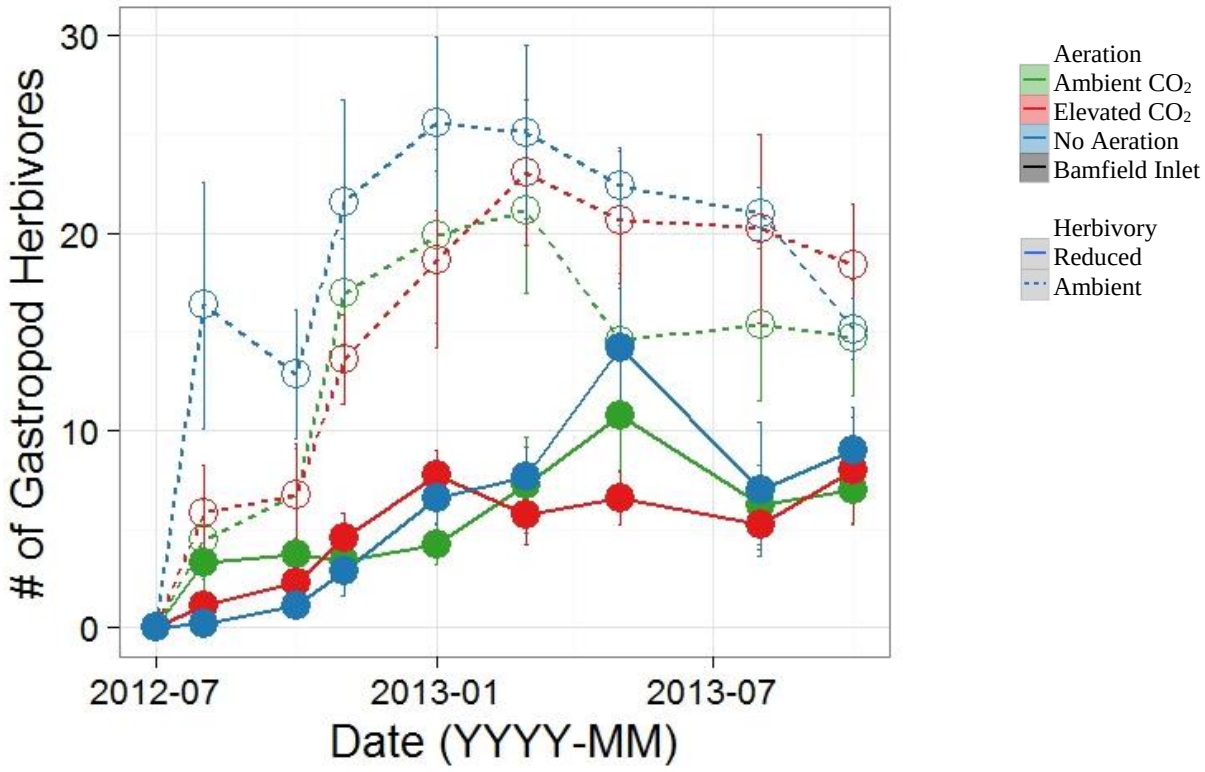


Figure 4.5. Gastropod herbivore density.

Number of gastropod herbivores present in tidepools at the time of biodiversity surveys depending on herbivory and aeration treatment over 15 months of succession. Herbivore reduction tidepools are shown by solid lines and closed circles, while tidepools experiencing ambient herbivore pressure are shown by dashed lines and open circles. Non-aerated, ambient bubbling, and elevated CO_2 bubbling are shown in blue, green, and red, respectively. Error bars indicate SE.

Table 4.6. Analysis of gastropod herbivore density.

Results of repeated measures ANOVA testing the effect of herbivore reduction and aeration treatment on the number of gastropod herbivores present in tidepool communities. ANOVA tables are divided into two sections to show analysis between and within tidepools, *Error: Tidepool* and *Error: Within* respectively.

	Df	**SumSq**	**MeanSq**	**F-value**	**Pr(>F)**
Error: Tidepool					
Aeration Treatment	2	806	403	2	0.11
Herbivores	1	12294	12294	70	**<0.0001**
Date	1	226	226	1	0.26
Aeration Treatment x Herbivores	2	606	303	1	0.19
Aeration Treatment x Date	2	757	378	2	0.13
Herbivores x Date	1	105	105	1	0.44
3- Way interaction	2	1431	716	4	**0.03**
Residuals	48	8384	175		

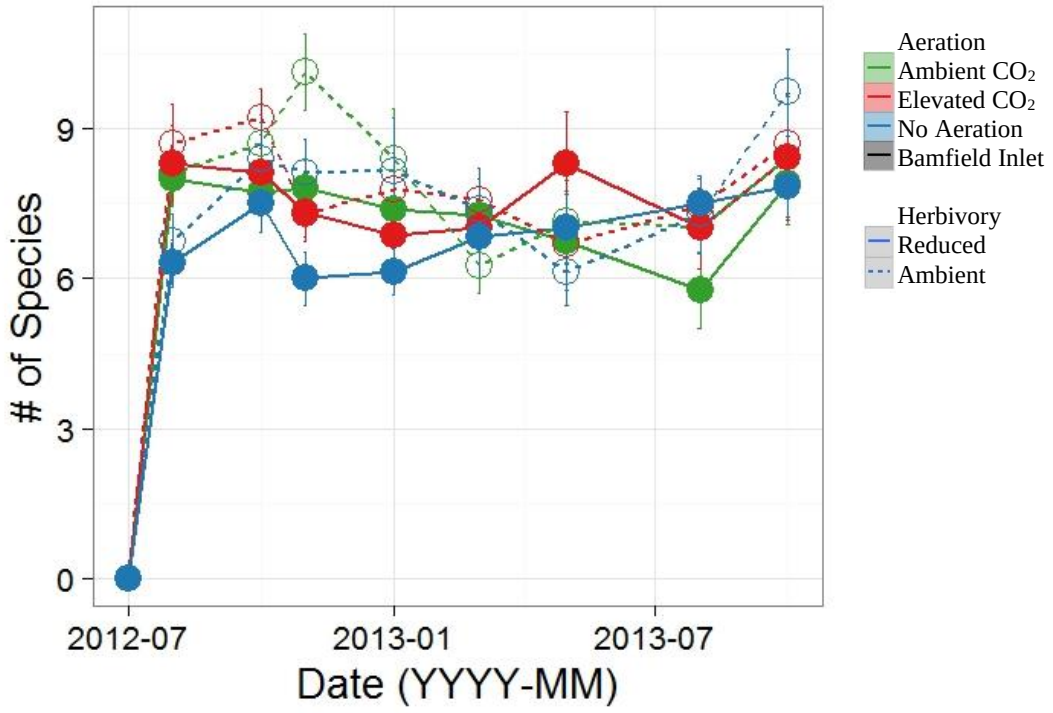
Error: Within					
Date	1	2574	2574	49	**<0.0001**
Date x Aeration Treatment	2	187	94	2	0.17
Date x Herbivores	1	94	94	2	0.18
3- Way interaction	2	746	373	7	**0.0008**
Residuals	336	17473	52		

4.4.3 Community response

Shannon diversity was significantly lower when herbivore abundance was reduced (Figure 4.6B, Table 4.7B). Because, I observed no effect of herbivore loss on community evenness (Table 4.7C), this reduction in diversity was likely driven by the decrease in species richness when herbivore abundance was reduced: from an average richness of 7.9 ± 0.2 to 7.3 ± 0.1 species over the entire duration of the experiment (mean ± SE; Figure 4.6A, Table 4.7A). There was no effect of aeration treatment on any of these three diversity indices, nor interactive effects. In terms of space utilization within the pools, I observed higher total percent cover in the tidepools in the reduced herbivore treatments that in the ambient herbivore treatment; 52 ± 2 % of the substratum was covered at ambient herbivore densities compared to 68 ± 2 % (± SE) with

the reduction of herbivores (Figure 4.7, Table 4.8). There was also a significant effect of aeration treatment and an interactive effect of time x aeration treatment on total percent cover in the tidepools, resulting in the total cover in aerated tidepools being higher than cover in non-aerated tidepools during parts of the experiment (Post hoc analyses; Figure 4.7, Table 4.8).

A.



B.

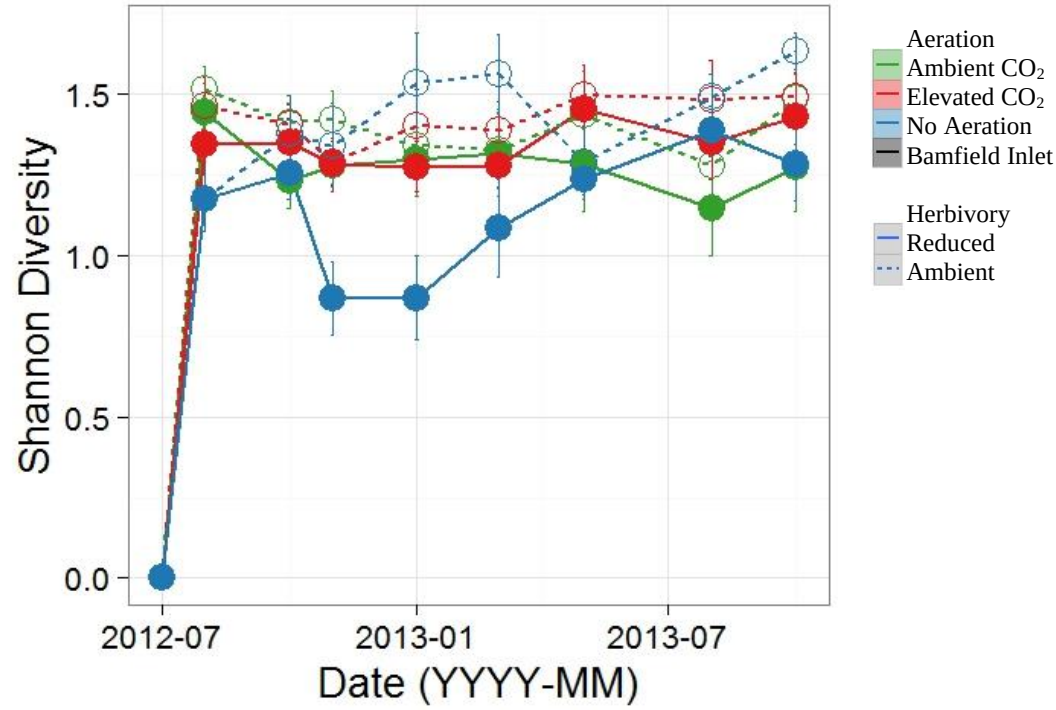


Figure 4.6. Tidepool species richness and diversity.

Effect of herbivore reduction and aeration treatment on species richness (A) and Shannon diversity (B) of tidepool communities over 15 months of succession. Herbivore reduction tidepools are shown by solid lines and closed circles, while tidepools experiencing ambient herbivore pressure are shown by dashed lines and open circles. Non-aerated, ambient bubbling, and elevated CO_2 bubbling are shown in blue, green, and red, respectively. Data shown represents all species except gastropod herbivores, such that any differences seen relate to species other than the manipulated species. Error bars indicate SE.

Table 4.7. Analysis of tidepool species richness, evenness, and diversity.

Results of repeated measures ANOVA testing the effect of herbivore reduction and aeration treatment on species richness (A), evenness (B), and Shannon diversity (C) of tidepool communities. The table is divided into two sections to show analyses between and within tidepools, *Error: Tidepool* and *Error: Within*, respectively.

	Df	SumSq	MeanSq	F-value	Pr(>F)
A) Species Richness					
Error: Between					
Aeration Treatment	2	20.9	10.5	0.97	0.39
Herbivores	1	44.2	44.2	4.11	**0.048**
Date	1	25.9	25.9	2.41	0.13
Aeration Treatment x Herbivores	2	6.6	3.3	0.31	0.74
Aeration Treatment x Date	2	8.3	4.2	0.39	0.68
Herbivores x Date	1	0.8	0.8	0.08	0.79
3- Way interaction	2	11.5	5.7	0.53	0.59
Residuals	48	516.4	10.8		

Error: Within					
Date	1	546	545.9	59.50	**<0.00001**
Date x Aeration Treatment	2	20	9.8	1.07	0.35
Date x Herbivores	1	1	0.5	0.06	0.81
3- Way interaction	2	3	1.6	0.17	0.84
Residuals	394	3615	9.2		
B) Evenness					
Error: Between					
Aeration Treatment	2	0.001	0.0006	0.18	0.84
Herbivores	1	0.005	0.005	1.67	0.20
Date	1	0.009	0.009	2.67	0.11
Aeration Treatment x Herbivores	2	0.005	0.003	0.79	0.46
Aeration Treatment x Date	2	0.005	0.003	0.79	0.46
Herbivores x Date	1	0.007	0.007	2.06	0.16
3- Way interaction	2	0.008	0.004	1.26	0.29
Residuals	48	0.16	0.003		

Error: Within					
Date	1	0.02	0.02	10.65	**0.001**
Date x Aeration Treatment	2	0.0002	0.00012	0.08	0.92
Date x Herbivores	1	0.002	0.002	1.64	0.20
3- Way interaction	2	0.0002	0.00008	0.05	0.95

	Df	SumSq	MeanSq	F-value	Pr(>F)
Residuals	336	0.50	0.001		
C) Shannon Diversity					
Error: Tidepool					
Aeration Treatment	2	0.78	0.39	1.73	0.19
Herbivores	1	2.70	2.70	12.00	**0.001**
Date	1	2.08	2.08	9.27	**0.004**
Aeration Treatment x Herbivores	2	0.59	0.29	1.30	0.28
Aeration Treatment x Date	2	0.05	0.03	0.12	0.89
Herbivores x Date	1	0.25	0.25	1.12	0.30
3- Way interaction	2	0.07	0.03	0.15	0.86
Residuals	48	10.79	0.22		

Error: Within					
Date	1	22.77	22.77	97.75	**0. <0.00001**
Date x Aeration Treatment	2	0.59	0.30	1.27	0.28
Date x Herbivores	1	0.09	0.09	0.37	0.54
3- Way interaction	2	0.03	0.02	0.07	0.93
Residuals	394	91.78	0.23		

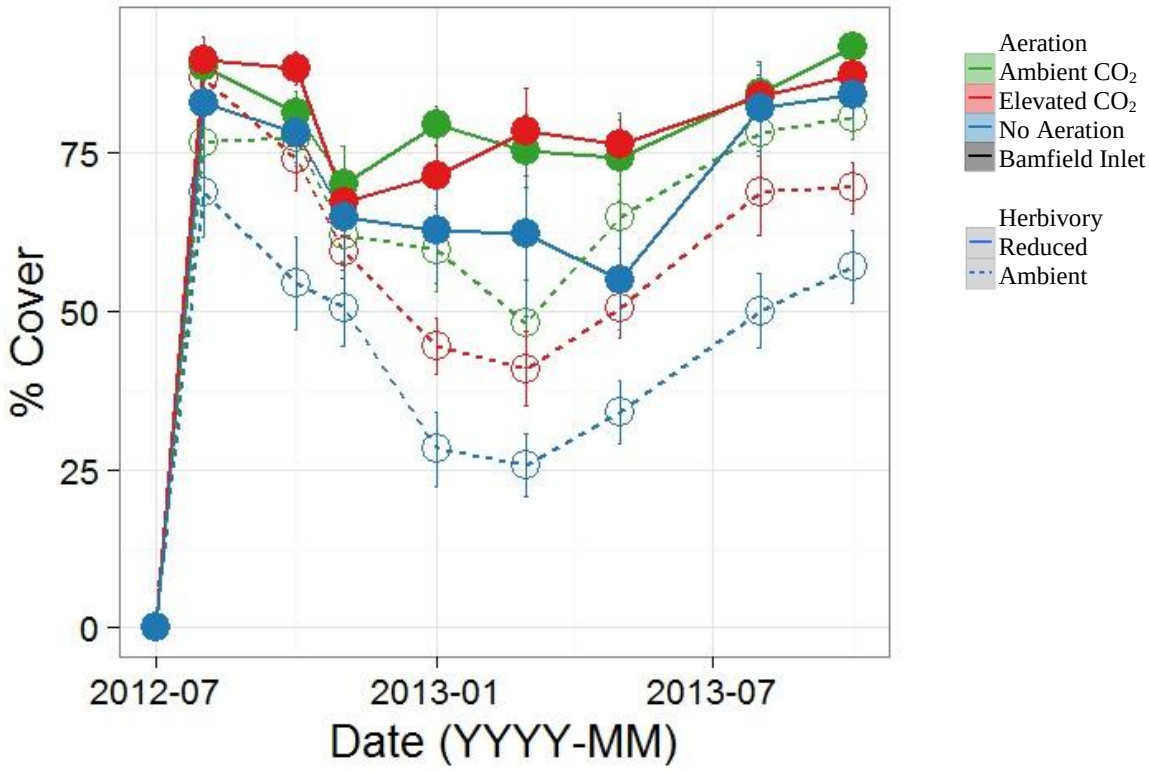


Figure 4.7. Tidepool percent cover.

Effect of herbivore reduction and aeration treatment on percent cover of tidepool all organism over 15 months of succession. Herbivore reduction pools are shown by solid lines and closed circles, while tidepools experiencing ambient herbivore pressure are shown by dashed lines and open circles. Non-aerated, ambient bubbling, and elevated CO_2 bubbling treatments are shown in blue, green, and red, respectively. Error bars indicate SE.

Table 4.8. Analysis of tidepool percent cover.

Results of repeated measures ANOVA testing the effect of herbivore reduction and aeration treatment on the percent cover of the tidepool community. ANOVA tables are divided into two sections to show analysis between and within tidepools, *Error: Tidepool* and *Error: Within* respectively. Post hoc analyses on aeration treatment were carried out by repeating the analysis on subsets of the data for pairwise comparisons of the three treatment levels. We used a Bonferroni correction ($\alpha = 0.017$) on post hoc analyses to control for multiple comparisons (*).

	Df	SumSq	MeanSq	F-value	Pr(>F)
Whole Model					
Error: Tidepool					
Aeration Treatment	2	18654	9327	15.725	**<0.0001**
Herbivores	1	33782	33782	56.957	**<0.0001**
Date	1	104	104	0.175	0.6772
Aeration Treatment x Herbivores	2	3379	1690	2.849	0.0678
Aeration Treatment x Date	2	3966	1983	3.344	0.0437
Herbivores x Date	1	401	401	0.676	0.4149
3- Way interaction	2	1553	776	1.309	0.2795
Residuals	48	28469	593		

Error: Within					
Date	1	516	516	1.798	0.181
Date x Aeration Treatment	2	469	234.7	0.818	0.442
Date x Herbivores	1	454	454	1.582	0.209
3- Way interaction	2	99	49.4	0.172	0.842
Residuals	336	96411	286.9		
Post Hoc: High vs Ambient*					
Error: Tidepool					
Aeration Treatment	2	5294	2647	4.228	0.0211
Date	1	14769	14769	23.591	**<0.0001**
Aeration Treatment x Date	2	301	151	0.241	0.7871
Residuals	43	26920	626		

Error: Within					
Date	1	57	56.8	0.27	0.6041
Aeration Treatment x Date	2	1231	615.4	2.923	0.0559
Residuals	212	44636	210.5		
Post Hoc: No Air Control vs Ambient*					
Error: Tidepool					

	Df	SumSq	MeanSq	F-value	Pr(>F)
Aeration Treatment	2	19512	9756	12.484	**<0.0001**
Date	1	140	140	0.179	0.6739
Aeration Treatment x Date	2	11329	5664	7.248	**0.0017**
Residuals	51	39857	782		

Error: Within					
Date	1	3	2.6	0.009	0.923428
Aeration Treatment x Date	2	5058	2528.8	8.985	**0.000191**
Residuals	179	50379	281.4		
Post Hoc: No Air Control vs High*					
Error: Tidepool					
Aeration Treatment	2	29522	14761	18.051	**<0.0001**
Date	1	2652	2652	3.243	0.0776
Aeration Treatment x Date	2	4010	2005	2.452	0.0961
Residuals	52	42523	818		

Error: Within					
Date	1	683	682.9	2.234	0.136
Aeration Treatment x Date	2	330	164.8	0.539	0.584
Residuals	240	73365	305.7		

My multivariate analyses of the effects of aeration treatment and herbivore loss on community structure painted a similar picture. The final sampling date (15 months after the start) showed a significant effect of both herbivore loss and aeration treatment on community structure, but no interaction (Figure 4.8, Table 4.9). Once again, the patterns in community structure observed were driven by differences between aerated and non-aerated treatments, not by changes in CO_2 concentrations between the two aeration treatments. These same effects of herbivore loss and aeration were observable throughout the duration of our manipulation (Figure 4.9, Table 4.10).

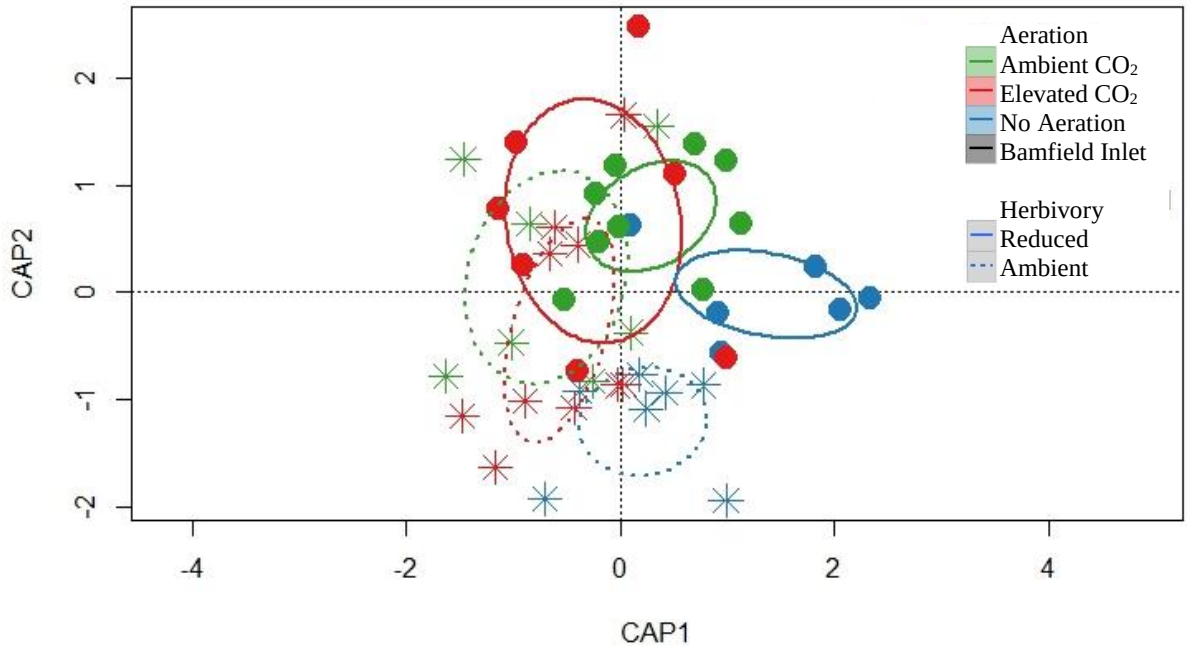


Figure 4.8. Capscale ordination of community composition data taken at the end of the 15-month field experiment.

Because gastropod herbivores were experimentally manipulated, they are not included in this ordination. Herbivore reduction treatments are shown by filled circles and solid lines, tidepools experiencing ambient herbivore pressure are shown by dashed lines and stars. No air controls, ambient bubbling, and elevated CO_2 bubbling are shown in blue, green, and red, respectively.

Table 4.9. Multivariate analysis of diversity.

Multivariate analysis on the effects of aeration treatment and herbivore reduction on community structure was on a Bray-Curtis dissimilarity matrix for the final survey timepoint. Post hoc analyses on aeration were carried out by repeating the analysis for pairwise comparisons of the three treatment levels. We used a Bonferroni correction (α = 0.017) on post hoc analyses to control for multiple comparisons (*).

	Df	SumsSqs	MeansSqs	F Model	R^2	Pr(>F)
Full Model						
Aeration Treatment	2	0.65	0.33	1.88	0.08	**0.009**
Herbivore	1	0.44	0.44	2.55	0.05	**0.004**
Interaction	2	0.17	0.09	0.50	0.02	0.98
Residuals	40	6.93	0.17		0.85	
Post Hoc: High vs Ambient*						
Aeration Treatment	1	0.18	0.18	1.05	0.03	0.38
Residuals	31	5.24	0.17		0.97	
Post Hoc: Ambient vs No Air Control*						
Aeration Treatment	1	0.41	0.41	2.29	0.08	**0.007**
Residuals	27	4.78	0.17		0.92	
Post Hoc: High vs No Air Control*						
Aeration Treatment	1	0.41	0.41	2.28	0.08	**0.009**
Residuals	28	5.07	0.18		0.92	

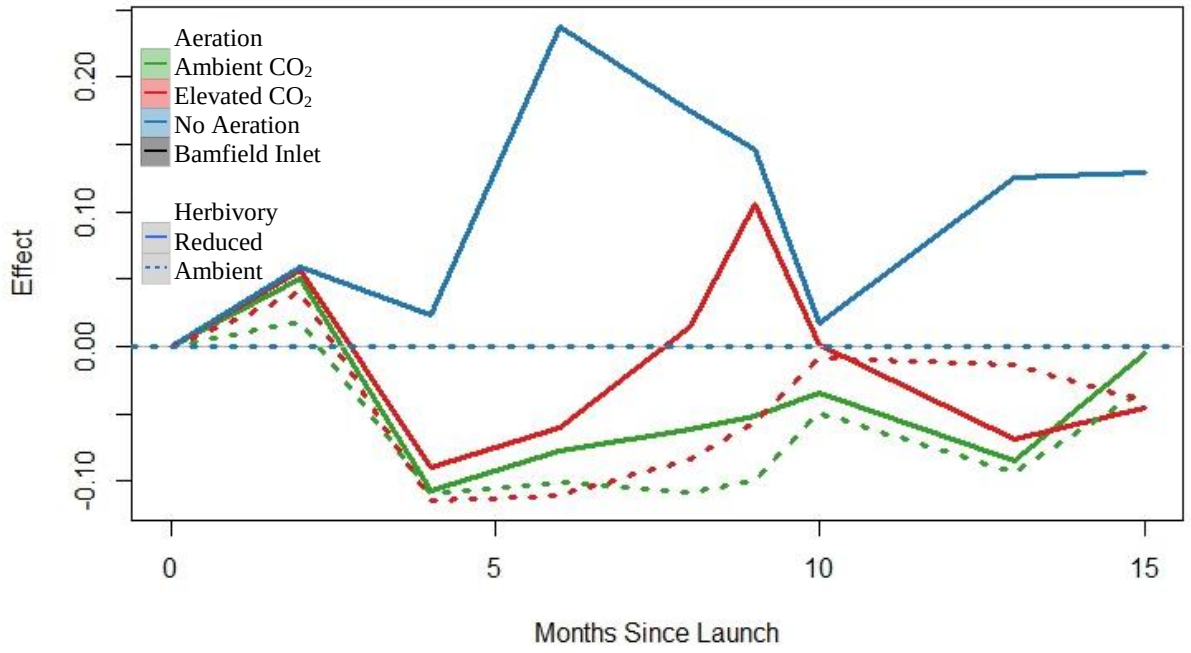


Figure 4.9. Principal response curves showing multivariate diversity over 15-month manipulation.

Principal response curves of each treatment group showing the differential effect of each treatment over the 15-month duration of manipulation. Effect is shown relative to no air addition control with ambient herbivory levels – the zero-effect line – as this treatment represents present-day tidepool conditions. Because gastropod herbivores were experimentally manipulated, they are not included in this ordination. Herbivore reduction pools are shown by solid lines, while tidepools experiencing ambient herbivore pressure are shown by dashed lines. Non-aerated, ambient bubbling, and elevated CO_2 bubbling treatments are shown in blue, green, and red, respectively.

Table 4.10. Multivariate analysis on the effects of aeration treatment and herbivore reduction on community structure over the entire 15-month experiment.

We used a Bonferroni correction ($\alpha = 0.013$) on post hoc analyses to control for multiple comparisons (*).

	Df	Variance	F - Value	Pr(>F)
Full Model				
RDA	1	0.01	34	**0.010**
Residuals	406	0.10		
Post Hoc: Herbivory*				
RDA	1	0.004	15	**0.010**
Residuals	442	0.12		
Post Hoc: High vs Ambient*				
RDA	1	0.002	6	0.04
Residuals	304	0.010		
Post Hoc: Ambient vs No Air Control*				
RDA	1	0.001	19	**0.010**
Residuals	279	0.12		
Post Hoc: High vs No Air Control*				
RDA	1	0.006	13	**0.010**
Residuals	283	0.12		

4.4.4 Responses of individual taxa

The main space occupiers in the experimental tidepools were diatoms, mussels, and barnacles. These three taxa were also the largest contributors to observed differences in community structure as analyzed by our multivariate statistics. There was an effect of aeration treatment, herbivore abundance and an interactive effect of herbivore abundance x aeration treatment on diatom cover (Figure 4.10, Table 4.11). While diatoms responded positively to herbivore reduction by a near doubling of diatom cover averaged across the experiment, this effect was more extreme in non-aerated tidepools than it was in tidepools aerated with ambient air (Post hoc analyses; Figure 4.10, Table 4.11). Further, I observed a significant main effect of

CO_2 aeration on diatom growth whereby the aeration of high CO_2 air led to significantly higher diatom cover compared to aeration by ambient air (24.5 ± 1.4 and 12.4 ± 1.0, % ± SE, respectfully), indicating that CO_2 also facilitated diatom growth. By contrast, there was an insignificant trend for higher mussel density under ambient aeration than under high CO_2 aeration (20.4 ± 1.8 and 12.4 ± 1.3, % ± SE, respectfully; Figure 4.11, Table 4.12). Instead, the observed effect of aeration treatment was driven by lower mussel abundance in the absence of any aeration (2.6 ± 0.2, % ± SE; Figure 4.11, Table 4.12). Additionally, tidepools with reduced herbivore density were more likely to see continued increases in mussel cover later into the experiment than tidepools with ambient herbivore densities (15.1 ± 1.4 and 9.5 ± 1.0, % ± SE; respectively over the course of the experiment; Figure 4.11, Table 4.12). Barnacle cover increased throughout the duration of the experiment, as did the difference in barnacle cover under current and reduced herbivore densities (Figure 4.12, Table 4.13). Barnacle cover was significantly higher at ambient herbivore densities than at reduced herbivore densities, with a 45% reduction in average barnacle cover under conditions of reduced herbivory at the end of the 15-month manipulation. There was no main or interactive effect of aeration on barnacle cover. Finally, aerated tidepools built up larger amounts of dead material, a mix of broken shells and apparent microbial mat, here forward referred to as mat cover (Figure 4.13, Table 4.14). Mat cover was not affected by herbivore abundance or CO_2 addition, but it did increase over the 15-month manipulation (Table 4.14).

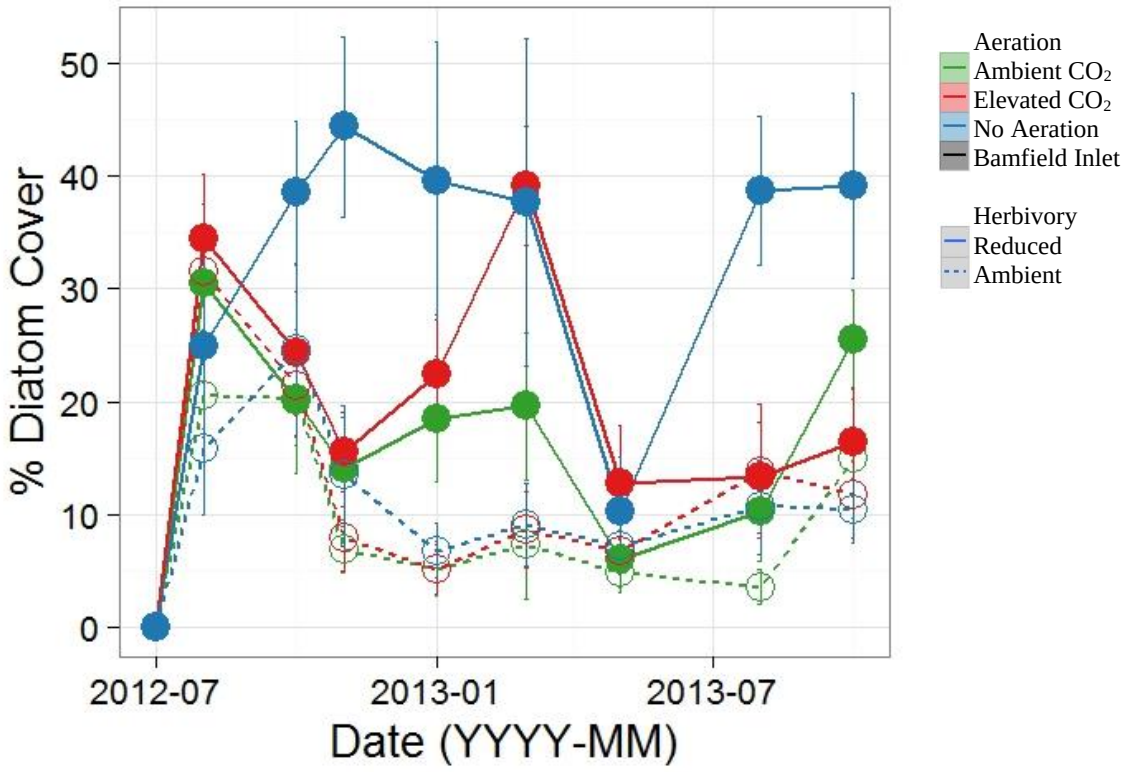


Figure 4.10. Tidepool diatom cover.

Effect of herbivore reduction and aeration treatment on diatom cover in tidepool communities over 15 months of succession. Herbivore reduction tidepools are shown by solid lines and closed circles, while tidepools experiencing ambient herbivore pressure are shown by dashed lines and open circles. Non-aerated, ambient bubbling, and elevated CO_2 bubbling are shown in blue, green, and red, respectively. Error bars indicate SE.

Table 4.11. Analysis of tidepool diatom cover.

Results of repeated measures ANOVA testing the effect of herbivore reduction and aeration treatment on percent diatom cover in the tidepool community. ANOVA tables are divided into two sections to show analysis between and within tidepools, *Error: Tidepool* and *Error: Within* respectively. Post hoc analyses on aeration treatment were carried out by repeating the analysis on subsets of the data for pairwise comparisons of the three treatment levels. We used a Bonferroni correction ($\alpha = 0.017$) on post hoc analyses to control for multiple comparisons (*).

	Df	SumSq	MeanSq	F-value	Pr(>F)
Whole Model					
Error: Tidepool					
Aeration Treatment	2	4074	2037	4.81	**0.013**
Herbivores	1	15157	15157	35.80	**0.0000003**
Date	1	519	519	1.23	0.27
Aeration Treatment x Herbivores	2	3415	1707	4.03	0.02
Aeration Treatment x Date	2	193	96	0.23	0.80
Herbivores x Date	1	126	126	0.30	0.59
3- Way interaction	2	691	346	0.82	0.45
Residuals	48	20320	423		

Error: Within					
Date	1	3738	3738	15.17	**0.00012**
Date x Aeration Treatment	2	702	351	1.42	0.24
Date x Herbivores	1	97	97	0.39	0.53
3- Way interaction	2	203	102	0.41	0.66
Residuals	336	82814	246		
Post Hoc: High vs Ambient*					
Error: Tidepool					
Aeration Treatment	2	10908	5454	19.96	**<0.00001**
Herbivores	1	3614	3614	13.22	**0.0008**
Date	1	3	3	0.01	0.92
Aeration Treatment x Herbivores	1	57	57	0.21	0.65
Aeration Treatment x Date	2	554	277	1.01	0.37
Herbivores x Date	1	85	85	0.31	0.58
3- Way interaction	1	237	237	0.87	0.36
Residuals	39	10658	273		

Error: Within					
Date	1	1482	1482	7.15	**0.008**
Date x Aeration Treatment	2	1940	970	4.68	**0.010**
Date x Herbivores	1	49	49	0.24	0.63

	Df	SumSq	MeanSq	F-value	Pr(>F)
3- Way interaction	1	937	937	4.52	0.03
Residuals	210	43514	207		
Post Hoc: No Air Control vs Ambient*					
Error: Tidepool					
Aeration Treatment	2	9666	4833	11.65	**0.0001**
Herbivores	1	9227	9227	22.25	**0.00002**
Date	1	2501	2501	6.03	0.018
Aeration Treatment x Herbivores	2	3813	1907	4.60	**0.015**
Aeration Treatment x Date	2	949	475	1.15	0.33
Herbivores x Date	1	193	193	0.47	0.50
3- Way interaction	1	638	638	1.54	0.22
Residuals	46	19080	415		

Error: Within					
Date	1	126	126	0.49	0.48
Date x Aeration Treatment	2	1381	690	2.70	0.07
Date x Herbivores	1	846	846	3.30	0.07
3- Way interaction	1	1164	1164	4.55	0.03
Residuals	177	45314	256		
Post Hoc: No Air Control vs High*					
Error: Tidepool					
Aeration Treatment	2	168	84	0.22	0.80
Herbivores	1	8099	8099	21.17	**0.00003**
Date	1	33	33	0.09	0.77
Aeration Treatment x Herbivores	2	3272	1636	4.28	0.020
Aeration Treatment x Date	2	234	117	0.31	0.74
Herbivores x Date	1	714	714	1.87	0.18
3- Way interaction	2	957	478	1.25	0.30
Residuals	46	17603	383		

Error: Within					
Date	1	3786	3786	15.25	**0.0001**
Date x Aeration Treatment	2	293	146	0.59	0.56
Date x Herbivores	1	16	16	0.07	0.80
3- Way interaction	2	1	1	0.00	1.00
Residuals	237	58839	248		

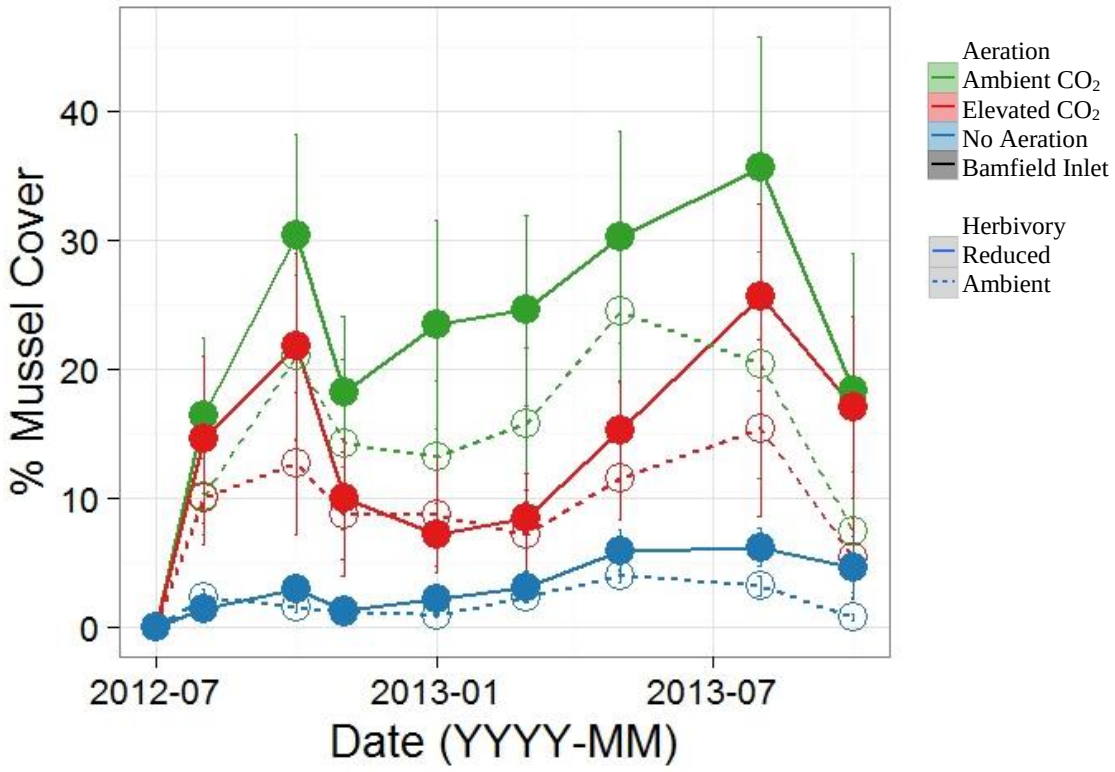


Figure 4.11. Tidepool mussel cover.

Effect of herbivore reduction and aeration treatment on mussel cover in tidepool communities over 15 months of succession. Herbivore reduction tidepools are shown by solid lines and closed circles, while tidepools experiencing ambient herbivore pressure are shown by dashed lines and open circles. Non-aerated, ambient bubbling, and elevated CO_2 bubbling are shown in blue, green, and red, respectively. Error bars indicate SE.

Table 4.12 Analysis of mussel cover.

Results of repeated measures ANOVA testing the effect of herbivore reduction and aeration treatment on percent mussel cover in the tidepool communities. ANOVA tables are divided into two sections to show analysis between and within tidepools, *Error: Tidepool* and *Error: Within* respectively. Post hoc analyses on aeration treatment were carried out by repeating the analysis on subsets of the data for pairwise comparisons of the three treatment levels. We used a Bonferroni correction ($\alpha = 0.017$) on post hoc analyses to control for multiple comparisons (*).

	Df	SumSq	MeanSq	F-value	Pr(>F)
Whole Model					
Error: Tidepool					
Aeration Treatment	2	20386	10193	7.68	**0.001**
Herbivores	1	2615	2615	1.97	0.17
Date	1	751	751	0.57	0.46
Aeration Treatment x Herbivores	2	626	313	0.24	0.79
Aeration Treatment x Date	2	5045	2522	1.90	0.16
Herbivores x Date	1	63	63	0.05	0.83
3- Way interaction	2	369	185	0.14	0.87
Residuals	48	63692	1327		

Error: Within					
Date	1	194	194	2.64	0.10
Date x Aeration Treatment	**2**	**54**	**27**	**0.37**	0.69
Date x Herbivores	**1**	**462**	**462**	**6.29**	**0.01**
3- Way interaction	**2**	**59**	**30**	**0.41**	0.67
Residuals	**336**	**24688**	**74**		
Post Hoc: High vs Ambient*					
Error: Tidepool					
Aeration Treatment	**2**	**9498**	**4749**	**3.55**	0.04
Date	**1**	**4443**	**4443**	**3.32**	0.08
Aeration Treatment x Date	**2**	**1801**	**900**	**0.67**	0.52
Residuals	**43**	**57575**	**1339**		

Error: Within					
Date	**1**	**14**	**14**	**0.14**	0.71
Aeration Treatment x Date	**2**	**0**	**0**	**0.00**	1.00
Residuals	**212**	**21330**	**101**		
Post Hoc: No Air Control vs Ambient*					
Error: Tidepool					
Aeration Treatment	**2**	**13070**	**6535**	**10.00**	**0.0002**

	Df	SumSq	MeanSq	F-value	Pr(>F)
Date	**1**	**2221**	**2221**	**3.40**	0.07
Aeration Treatment x Date	**2**	**3399**	**1699**	**2.60**	0.08
Residuals	**51**	**33320**	**653**		

Error: Within					
Date	**1**	**36**	**36**	**0.74**	0.39
Aeration Treatment x Date	**2**	**640**	**320**	**6.47**	**0.002**
Residuals	**179**	**8850**	**49**		
Post Hoc: No Air Control vs High*					
Error: Tidepool					
Aeration Treatment	**2**	**14000**	**7000**	**8.11**	**0.001**
Date	**1**	**243**	**243**	**0.28**	0.60
Aeration Treatment x Date	**2**	**4629**	**2315**	**2.68**	0.08
Residuals	**52**	**44908**	**864**		

Error: Within					
Date	**1**	**301**	**301**	**5.55**	0.019
Aeration Treatment x Date	**2**	**235**	**117**	**2.16**	0.12
Residuals	**240**	**13042**	**54**		

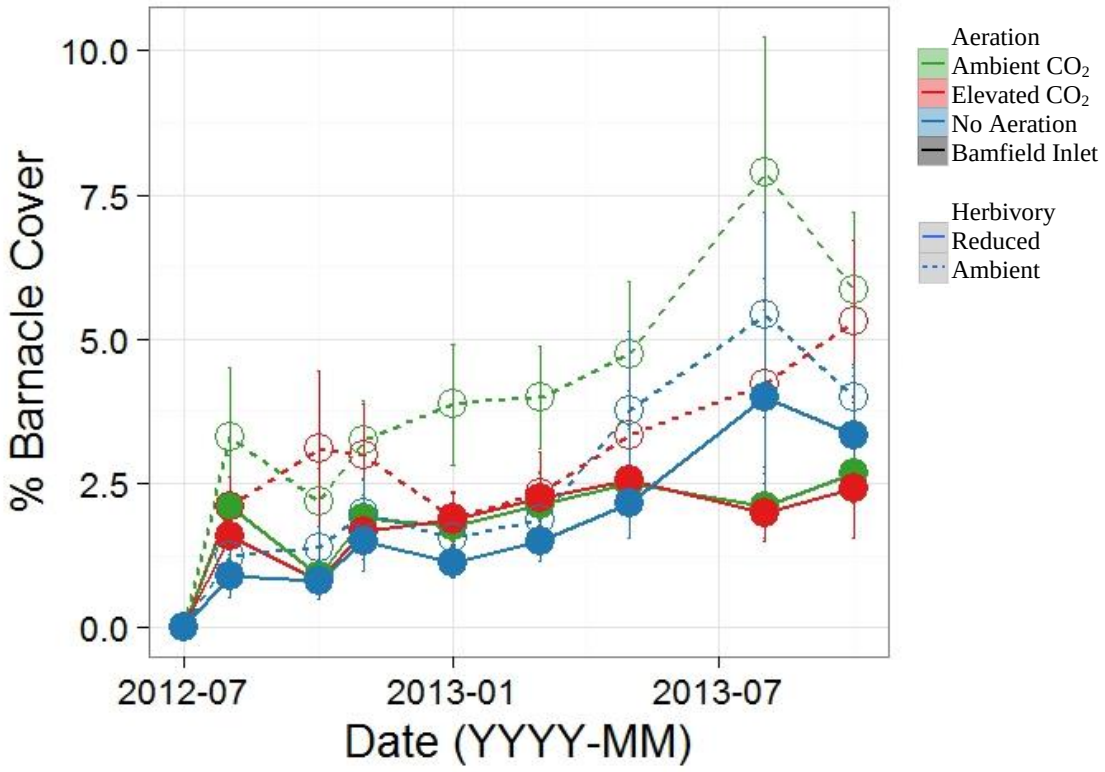


Figure 4.12. Tidepool barnacle cover.

Effect of herbivore reduction and aeration treatment on barnacle cover in tidepool communities over 15 months of succession. Herbivore reduction tidepools are shown by solid lines and closed circles, while tidepools experiencing ambient herbivore pressure are shown by dashed lines and open circles. Non-aerated, ambient bubbling, and elevated CO_2 bubbling are shown in blue, green, and red, respectively. Error bars indicate SE.

Table 4.13. Analysis of barnacle cover.

Results of repeated measures ANOVA testing the effect of herbivore reduction and aeration treatment on percent barnacle cover in the tidepool communities. ANOVA tables are divided into two sections to show analysis between and within tidepools, *Error: Tidepool* and *Error: Within* respectively.

	Df	**SumSq**	**MeanSq**	**F-value**	**Pr(>F)**
Error: Tidepool					
Aeration Treatment	2	57.5	28.76	1.94	0.16
Herbivores	1	237.6	237.55	15.99	**0.0002**
Date	1	4.5	4.46	0.30	0.59
Aeration Treatment x Herbivores	2	38.7	19.35	1.30	0.28
Aeration Treatment x Date	2	0.7	0.35	0.02	0.98
Herbivores x Date	1	0.3	0.30	0.02	0.89
3- Way interaction	2	3.6	1.80	0.12	0.89
Residuals	48	713.1	14.86		

Error: Within					
Date	1	273.5	273.53	55.84	**<0.00001**
Date x Aeration Treatment	2	12.7	6.37	1.30	0.27
Date x Herbivores	1	42.4	42.37	8.65	**0.004**
3- Way interaction	2	14.8	7.42	1.51	0.22
Residuals	336	1645.9	4.90		

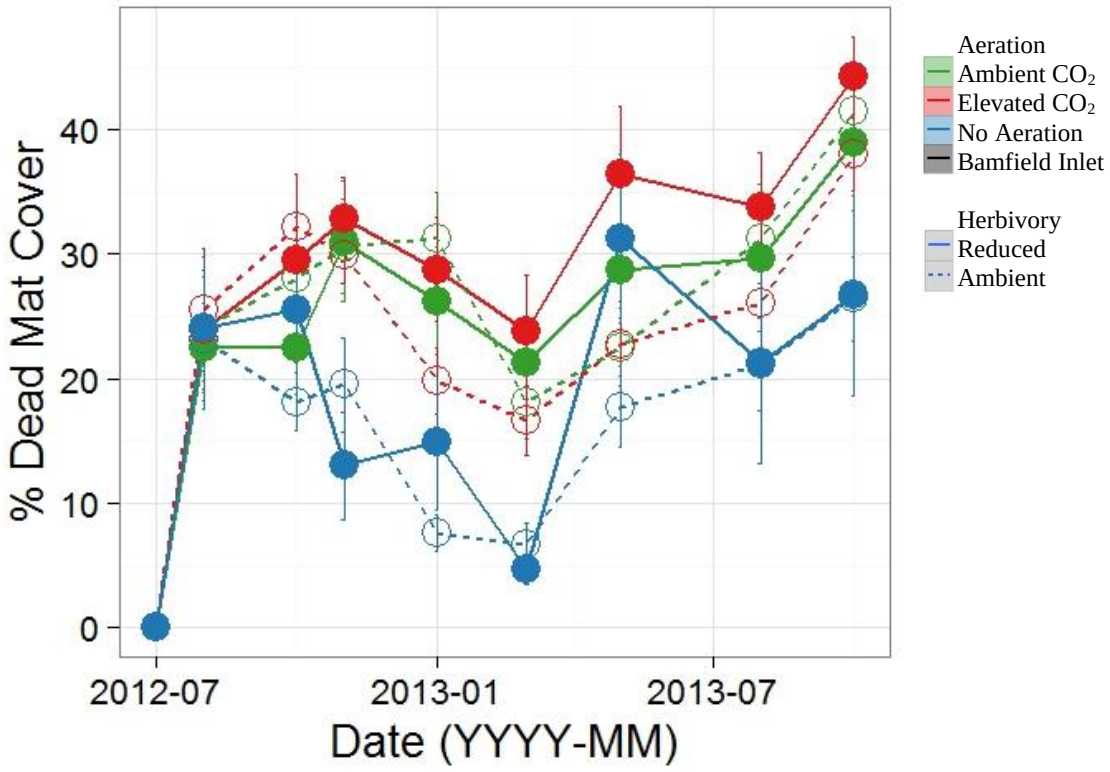


Figure 4.13. Tidepool dead mat cover.

Effect of herbivore reduction and aeration treatment on microbial mat build up in tidepool communities over 15 months of succession. Herbivore reduction tidepools are shown by solid lines and closed circles, while tidepools experiencing ambient herbivore pressure are shown by dashed lines and open circles. Non-aerated, ambient bubbling, and elevated CO_2 bubbling are shown in blue, green, and red, respectively. Error bars indicate SE.

Table 4.14. Analysis of tidepool dead mat cover.

Results of repeated measures ANOVA testing the effect of herbivore reduction and aeration treatment on mat build-up in the tidepool communities. ANOVA tables are divided into two sections to show analysis between and within tidepools, *Error: Tidepool* and *Error: Within* respectively. Post hoc analyses on aeration treatment were carried out by repeating the analysis on subsets of the data for pairwise comparisons of the three treatment levels. We used a Bonferroni correction ($\alpha = 0.017$) on post hoc analyses to control for multiple comparisons (*).

	Df	SumSq	MeanSq	F-value	Pr(>F)
Whole Model					
Error: Tidepool					
Aeration Treatment	2	7432	3716	15.33	**0.00001**
Herbivores	1	521	521	2.15	0.15
Date	1	258	258	1.07	0.31
Aeration Treatment x Herbivores	2	575	288	1.19	0.31
Aeration Treatment x Date	2	708	354	1.46	0.24
Herbivores x Date	1	101	101	0.42	0.52
3- Way interaction	2	95	47	0.20	0.82
Residuals	48	11636	242		

Error: Within					
Date	1	2149	2149	14.03	**0.0002**
Date x Aeration Treatment	2	306	153	1.00	0.37
Date x Herbivores	1	107	107	0.70	0.40
3- Way interaction	2	95	47	0.31	0.73
Residuals	336	51463	153		
Post Hoc: High vs Ambient*					
Error: Tidepool					
Aeration Treatment	2	1290	645	2.89	0.07
Date	1	1208	1208	5.42	0.025
Aeration Treatment x Date	2	345	173	0.77	0.47
Residuals	43	9593	223		

Error: Within					
Date	1	1862	1862	12.74	**0.0004**
Aeration Treatment x Date	2	452	226	1.55	0.22
Residuals	212	30989	146		
Post Hoc: No Air Control vs Ambient*					
Error: Tidepool					

	Df	SumSq	MeanSq	F-value	Pr(>F)
Aeration Treatment	2	6677	3339	14.96	**0.00001**
Date	1	17	17	0.08	0.78
Aeration Treatment x Date	2	109	55	0.25	0.78
Residuals	51	11382	223		

Error: Within					
Date	1	700	700	4.28	0.04
Aeration Treatment x Date	2	488	244	1.49	0.23
Residuals	179	29304	164		
Post Hoc: No Air Control vs High*					
Error: Tidepool					
Aeration Treatment	2	6256	3128	14.67	**0.00001**
Date	1	205	205	0.96	0.33
Aeration Treatment x Date	2	470	235	1.10	0.34
Residuals	52	11091	213		

Error: Within					
Date	1	1261	1261	8.56	**0.004**
Aeration Treatment x Date	2	33	17	0.11	0.89
Residuals	240	35365	147		

4.5 Discussion

Species interactions have long been shown to influence community structure across a wide variety of systems. Consumer impacts on benthic community structure have been particularly well documented (Lubchenco 1978, Shurin et al. 2002, Paine 2002, Bracken and Stachowicz 2007, Hughes et al. 2007, Altieri et al. 2009). Here, I found that reductions in gastropod herbivore abundance – which may accompany OA – led to larger impacts on tidepool community structure than direct effects of CO_2 addition, the primary driver of OA. In addition to changes in community structure both at the end of the experiment and throughout the 15-month manipulation, tidepools with reduced herbivore abundances showed lower species richness and Shannon diversity. Reduced herbivore abundance also led to higher total area coverage in the

tidepools driven primarily by increased diatom and mussel cover despite decreased barnacle cover. By contrast, I saw no effect of CO_2 addition on community composition – either over the course of the manipulation or at the final time point – nor did I see effects of CO_2 on species richness or Shannon diversity, as compared to the ambient aeration treatment. While there was a trend towards lower mussel cover under high CO_2 aeration as compared to ambient aeration, this trend was not statistically significant after the Bonferroni correction. The only taxa that showed a significant response to high CO_2 aeration, as compared to ambient aeration, were diatoms, which show a higher percent cover under conditions of high CO_2.

While there were only minimal effects of CO_2 on the tidepool community, there were substantial effects of aeration on the structure of the tidepool communities as well as the abundance of individual species. Composition of tidepool communities was significantly different between aerated treatments and the non-aerated control both throughout the course of the experiment and at the final sampling point. These effects were not observable through differences in species richness or Shannon diversity. Rather tidepools not receiving aeration had lower total cover, lower mussel cover, and lower build-up of microbial mat. This is likely driven by the considerably different oxygen environment experienced by the non-aerated tidepools. The non-aerated tidepools showed the largest fluxes in O_2, with a much larger drop in O_2 saturation at night than any of the tidepools receiving aeration. Further, there is also a large difference in O_2 saturation between the non-aerated tidepools with and without herbivores, where tidepools with low herbivore abundance experienced lower O_2 saturation at night. This is likely driven by the respiration of higher biomass in the herbivore excluded tidepools. Given these substantial differences between aerated and non-aerated tidepools, it is important to understand that any

effect observed in response to increased CO_2 is seen in terms of existing after the organisms have been relieved of nighttime oxygen limitation.

Changes in herbivore abundance often drive alteration of species richness and diversity in both marine and terrestrial ecosystems (Lubchenco 1978, 1980, Manier and Hobbs 2006, Hughes et al. 2007, Gruner et al. 2008, Altieri et al. 2009, Dyer et al. 2010). In areas of high productivity, this is often because herbivores perform the role of creating space for species that might otherwise not be able to compete with spatial dominants – this mechanism is known as consumer-mediated coexistence. This mechanism was directly observable in my experiment, as I observed both an average of 46% less bare space and 8% lower species richness in tidepools with reduced herbivore abundance; the herbivores are creating bare space for other species by decreasing the abundance of spatial dominants (in this case, diatoms and mussels). As I found no single species that was exclusively found in tidepools with ambient herbivore densities, it seems that, in this system, herbivory increases the likelihood of multiple rare species being found in any given tidepool, but it does not seem to universally facilitate the presence of any particular species that is otherwise fully absent from the community. Further, herbivory facilitated barnacle recruitment, a common yet not particularly abundant tidepool taxa. This pattern has been observed previously in a geographically close intertidal system (Farrell 1988).

It was unexpected to observe an effect of herbivory on species richness, diversity, and community structure in response to herbivore abundance (an indirect effect of CO_2), but not a direct effect of CO_2 addition on any of these same parameters. Most research conducted on the response of the whole community to increased CO_2 have found it to have a significant effect on community structure and often a negative effect on either, if not both, species richness and diversity; this is true both in manipulative (Brown et al. 2016) and observational studies (Hall-

Spencer et al. 2008, Porzio et al. 2011, Kroeker et al. 2011). A notable exception to this trend can be seen in another study that looks at the impact of increased CO_2 on tidepool communities (Sorte and Bracken 2015). This similarity may be due to the relative robustness of tidepool communities to increases in CO_2, owing to the large natural fluctuations in pCO_2 that were observed in tidepools without aeration (Vargas et al. 2017).

Because of the large differences in O_2 saturation, it is difficult to compare the non-aeration treatment directly to the high CO_2 aeration treatment, as there are potentially effects of both aeration and CO_2 addition. Importantly, there were very few effects of elevated CO_2 observed when comparing directly between the two aeration treatments, indicating that these tidepool communities are relatively robust to these levels of CO_2 addition. Also, it is important to note that there were no interactive effects of herbivore reduction and aeration on any of the response variables, permitting the interpretation of herbivore reduction independently from that of aeration and CO_2 addition. The principal effect of CO_2 addition was an increase in diatom cover. This effect was detected regardless of herbivore density, suggesting that diatoms will receive two benefits from OA: (1) reduced grazing pressure from calcified herbivores, and (2) higher productivity through carbon fertilization.

Conversely, mussel cover, which was significantly higher when herbivore abundance was low, seemed to be negatively affected by increased CO_2. While this trend was not significant following the Bonferroni correction of alpha, it does line up well with what has been seen in earlier field manipulations of CO_2 (Brown et al. 2016). If the effects of herbivore reduction and CO_2 addition are opposite and additive, any reduction in herbivore pressure with increased CO_2 may mitigate the negative effect of increases CO_2 on mussels. Such a buffer would be important for maintaining the diverse infaunal community within the mussel beds which, although not

quantified in this study, may be vulnerable to OA indirectly via biogenic habitat loss (e.g. the mussel bed; Sunday et al. 2016).

In addition to helping us understand the potential indirect effects of OA via herbivore loss, this research demonstrates the often-underestimated ability of organisms to alter their abiotic environment. One of the most dramatic cases of this can be seen by the way the night time pH of high CO_2 aerated tidepools with ambient herbivore abundances diverged in the winter from the night time pH of tidepools receiving the same aeration treatment but with reduced herbivore densities, despite CO_2 addition remaining consistent throughout the manipulation. I suspect this is because tidepools with higher herbivore abundances experienced large reductions in total cover as the herbivores continued to graze down the light limited algae. This likely decreased tidepool biomass and therefore total respiration in those tidepools, making the baseline pH higher in tidepools with lowered herbivore abundances. While I was aware of this effect during the manipulation, I chose not to increase the CO_2 being added to the high CO_2 aeration tidepools with ambient herbivory to make their pH more similar to their reduced herbivore counterparts. I made this decision because this effect was real and driven by the biological community interacting with the environment I had already manipulated. Experiments that tightly control pH using auto-adjusters, may actually be interfering with this process and thus exposing the study organism and community to more extreme conditions than would be realistic if biological feedbacks were allowed to play out.

Similarly, it was surprising that the pH of the non-aerated tidepools remained the highest, as I might have predicted those tidepools to be the most affected by respiration at night given the low oxygen levels in the tidepool. However, there was significantly less total surface area covered and almost no mussel cover in non-aerated tidepools. While hypoxia seemed to drive the

low percent cover in non-aerated tidepools, this low percent cover is likely why pH remained higher in non-aerated tidepools at night. Further, significant differences in tidepool pCO_2 between the two aeration treatments (ambient and high CO_2 addition) are only detectable during daylight hours, likely due to the large amounts of respiration in tidepools after dark, which may swamp the CO_2 addition. Tidepool pCO_2 measurements could only be calculated for sampling points when DIC measurements were taken (late October and November of each year). Thus, tidepool pCO_2 was never calculated for tidepools without existing respiring communities affecting the measurements.

4.6 Conclusions

Communities are structured by a combination of direct and indirect effects. Unfortunately, the latter are more difficult to detect and understand, as they may require empirical manipulations in multi-species assemblages over relatively long timescales. Nevertheless, it is important that researchers understand the summation of both direct and indirect effects to understand communities in nature and the ways in which they are responding to environmental forcing. Here I found that the indirect effects of potential gastropod herbivore loss, driven by increased pCO_2, may have a larger impact on community structure than CO_2 addition directly. This is because, in tidepools, herbivores maintain biodiversity by reducing the abundance of competitively dominant taxa, diatoms and mussels. This may hold a key for being able to identify important points of vulnerability to global change in many different ecological communities. Communities whose richness and diversity are maintained by a species or guild are likely only as resistant to abiotic change as that species or guild is itself resistant to abiotic change.

Chapter 5: Concluding remarks

5.1 Synopsis

Many impacts of global change are already being documented in almost every known ecosystem in real time, ranging from changes in species' life history and phenological traits to widespread changes in community assembly and ecosystem function. While each of the many abiotic factors associated with global change (increased CO_2, shifts in nutrient availability and rainfall patterns, increased air and water surface temperature, etc.) come with their own suite of physiological and ecological consequences, the ultimate hope is that by understanding some fundamental ecological rules, ecologists will be able to make generalizations and predictions about how communities will change over time. The silver lining in this global change storm cloud is that it has provided ecologists and physiologists with a new set of conditions through which we can test our existing knowledge about how the biological world works. Our understanding of how biological communities work should frame how we set up expectations for communities and organisms to respond to global change (Gaylord et al. 2015). By exposing organisms and communities to these new conditions, scientists are able to test the existing knowledge of how biological systems work, while making predictions about what ecological communities will look like in the future.

This thesis aims to understand the mechanisms and community-level consequences of changes to consumer-resource interactions in response to environmental change. In my general introduction (Chapter 1), I argued that many of the existing conceptual models for explaining community assembly do a poor job considering the way in which the abiotic environment can directly alter interactions between species. I therefore proposed an alternative form to the filter model of community assembly (Keddy 1992), where I combine the abiotic and biotic filter into a

single step (Figure 5.1). The thesis chapters that followed used projected OA conditions to explicitly demonstrate mechanisms by which the abiotic environment can affect biological interactions and what the consequences of those changes may be for biological communities.

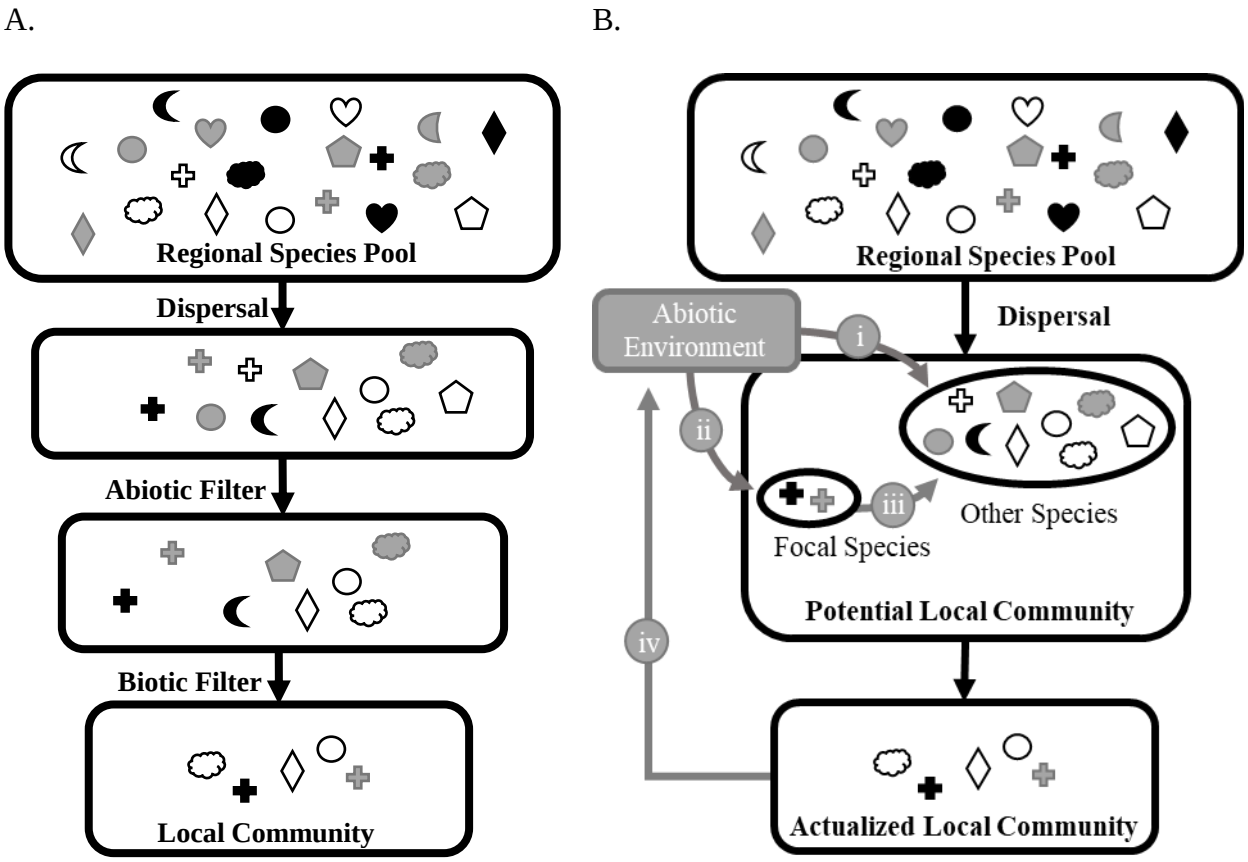


Figure 5.1. Revisiting a traditional way to present the filter model of community assembly and an updated conceptual model for community assembly.

This figure shows a traditional way to present the filter model of community assembly (A; proposed by Keddy 1992 and modified from Vellend 2016) and an updated conceptual model for community assembly first shown in chapter 1 (B). In the traditional filter model (A), the local community is determined by a series of filters which sequentially prevent species from moving from the regional species pool into the local community. In the updated model (B), the intermediate stages between the regional species pool and the actualized species pool are combined to consider the abiotic and biotic environment simultaneously. This is because, while the abiotic environment can directly impact every species in the potential local community (i), by impacting an intermediate focal species (ii, or guild), the abiotic environment may indirectly impact the rest of the species in the community via the focal species impact on the rest of the community (iii). While neither model explicitly details changes in abundance, it is notable that changes in the abundance of each species are possible as a result each arrow. Unlike the version of this figure from

Chapter 1, I have added line *iv* to indicate the impact the actualized local community can have on the abiotic environment.

In Chapter 2, I set out to describe the processes by which changes in a basic species trait (growth rate) can lead to altered species interactions. I hypothesized that both changes in size-specific feeding rate and body size (due to reduced growth rate) would drive declines in *per capita* feeding rates of highly calcified herbivores (e.g. urchin's and snails), but not of less calcified crustacean herbivores (e.g. crabs and isopods). Although I found no change in feeding rates of any herbivores under conditions of high CO_2, I did find that there were significant reductions in the growth rate of the highly-calcified herbivore species, but not of the less calcified herbivore species. In itself, reduced growth rates were not a surprising result. Meta-analyses consistently show that highly calcified taxa such as mollusks and echinoderms are more likely to experience reduced growth in response to OA than less calcified arthropods (Kroeker et al. 2010, 2013), and dwarfed gastropod herbivores have been documented in naturally occurring CO_2 vent systems (Garilli et al. 2015). It was surprising to find no effect of increased CO_2 on size-specific feeding rates, as it indicates that those herbivores are unable to compensate for the negative impacts of CO_2 by feeding more (Carey et al. 2016). However, because I also demonstrated that smaller body size leads to lower *per capita* consumption rates, I predict an overall decrease in herbivore pressure by highly calcified herbivores, which means that changes in body size may be a suitable proxy for changes in *per capita* feeding rates of herbivores under high CO_2.

In addition to potential effects on body size and mass-specific feeding rates, I hypothesized that OA may also affect trophic relationships via changes in herbivore abundance. Given the time-scales of the experiments in Chapter 2, it was not possible to assess the

importance of changes in herbivore abundance. However, in Chapter 3, I directly addressed the issue of changes in abundance using long-term outdoor mesocosm manipulations of seawater CO_2. Amphipod abundance increased in response to increased CO_2. When I allowed the abundance of amphipods in my herbivory assays to vary according to their experimental source population density, I observed an increase in population-level herbivory, but only when temperatures were elevated along with CO_2, as is expected in most future scenarios.

Chapter 3 also demonstrates the importance of considering multiple stressors and multiple species simultaneously. I hypothesized that changes in algal palatability in response to increased CO_2 and temperature would play just as large of a role in determining feeding rates as herbivore responses to the same treatment conditions. I observed impacts of temperature and CO_2 on feeding rates when the herbivore and algae were manipulated separately: temperature had a significant negative effect on herbivore *per capita* feeding rates and CO_2 addition had a significant negative effect on algal palatability, but only at ambient temperatures. However, when both the herbivore and algae were exposed to the same treatment conditions, there was no change in *per capita* feeding rates. While ecologists recognize that species responses to multiple stressors are often not additive, it is important to also recognize that species interactions may not be predictable based upon the sum of their parts either. For this reason, the more contextual realism researchers can bring to their experiments, the more likely scientists are to find these ecological surprises and be able to incorporate these non-additive interactions into models. Further, while these *per capita* effects are illuminating, they should only be considered in the context of population density, which increased dramatically under high CO_2 and fully overshadowed *per capita* level effects. Thus, moving forward it may be appropriate to use

abundance metrics as reasonable first approximations for how herbivory pressure may change in novel environments.

In Chapter 4, I presented data from a 15-month *in situ* manipulation in tidepool communities where I simultaneously tested the direct effects of elevated CO_2 along with the predicted indirect effects of CO_2 addition via gastropod herbivore loss. I predicted that OA would have negative effects on the tidepool community both directly through the addition of CO_2 and indirectly through reductions in gastropod herbivore abundance. However, I found that the indirect effects of CO_2 had a larger impact (and in this case the only impact) on community structure and diversity than the direct effects of CO_2 addition. This occurred because gastropod herbivores reduce the abundance of diatoms in tidepools, which enhanced diversity by creating space for other species. Without the herbivores fulfilling this ecological function, there was a decrease in mean species richness and a significant shift in community structure. This chapter explicitly builds on the previous two: Chapters 2 and 3 I evaluated the mechanism by which the abiotic environment is most likely to impact herbivory, and Chapter 4 shows that these changes in herbivory levels can have greater impacts on the biological community than the direct effects of the abiotic factor itself.

5.2 Ocean acidification, herbivory, and ecological resilience

Ecological resilience refers to an ecological community's ability to maintain or return to a known state in the face of a disturbance or environmental change, and is important in considering both the implications and the limitations of my results. Because herbivores have the capacity to greatly impact community structure, they can be important in preserving the ecological resilience of communities that are impacted by increased CO_2. This particularly may be the case if increased CO_2 results in the increased abundance of highly palatable macroalgae

(Falkenberg et al. 2012, Ghedini et al. 2015, also seen in Chapter 4). Mechanisms that underpin ecological resilience can potentially be seen within a single species or through multiple species in in a single functional group.

In Chapter 2, I proposed a framework for thinking about the three ways in which abiotic conditions can impact feeding rates: (1) by affecting size specific feeding rates; (2) by affecting individual size; and (3) by affecting population size. I simultaneously showed that because calcified herbivores grow more slowly under conditions of increased CO_2, we would likely expect to see a decrease in the average body size of individuals within a cohort, and thus an overall decrease in herbivory. However, if we assume a single consumer-resource pair and no change in resource production, this decrease in herbivory levels may lead to under-exploited resources, resulting in a potential increase in carrying capacity – in terms of number of individuals, but not biomass. Thus, a single species may compensate for decreased *per capita* feeding rates by increasing in abundance, this is known as the energy equivalence rule for size-abundance relationships (summarized by White et al. 2007). In this way, a single species may be able to maintain a community's resiliency to abiotic change by maintaining the impact of its presence through increased abundance. I do not think this will apply broadly in the case of increased CO_2; the same taxonomic groups that tend to respond negatively to increased CO_2 as adults are also quite sensitive at other life-stages, showing reduced fertilization and developmental rates, as well as reduced survival and recruitment (Kroeker et al. 2010, 2013). In this way, it is likely that a species with reduced *per capita* feeding rates (due to decreased growth at high CO_2) would also suffer from reduced propagule pressure, making it more difficult for it to take advantage of the additional resource by increasing its abundance.

An alternative mechanism for ecological resilience may be found through ecological redundancies, i.e. multiple species that serve the same function. Thus, if a single species is lost, its ecological roll can be provided by another species (Walker 2015). This may be a more promising mechanism through which herbivores may aid in the resilience of marine communities. When there is an increase in primary production (as predicted with OA) or simply increased resource availability due to the reduction of a certain herbivore species, another herbivore species that is more robust to increased CO_2 may increase in abundance to utilize that additional resource. This bottom up effect has already been argued to be the cause of increased abundance of amphipods in long-term mesocosm manipulations (Heldt et al. 2016) and one case of increased micro-gastropods at naturally occurring CO_2 vents (Connell et al. 2017).

The results from this thesis provide mixed support for the prediction that redundancy within the herbivore guild may be instrumental in maintaining ecological resilience. On one hand, Chapter 2 demonstrated that the *per capita* consumption rates of less calcified herbivores are less likely to decrease with increased CO_2 than the *per capita* consumption rates of highly calcified species. Additionally, in Chapter 3, the increased abundance of amphipods in response to increased CO_2 drove an increase in population level herbivory. Both of these chapters thus provide evidence that a subset of herbivores may remain, and potentially even thrive, under conditions of increased CO_2. On the other hand, in Chapter 4, when gastropod herbivores were reduced in tidepools, there was no evidence of other herbivore taxa (e.g., amphipods or other crustaceans) filling that ecological niche. In other words, there was no indication that functional redundancy within the tidepools communities would provide a mechanism for ecological resilience.

While I did not find evidence of mechanisms promoting resilience in my tidepool communities, there is a clear case to be made for context dependence. In South Australian kelp forests, it has been found that gastropod herbivores may increase their size specific feeding rate in response to increased CO_2, and by doing so help control the growth rates of weedy turf algae (Falkenberg et al. 2012, Ghedini et al. 2015, Ghedini and Connell 2016). This is the opposite of what I found in Chapter 2, and the interpretations of these findings may change once they have been integrated with changes in growth rates and abundance, as gastropods tend to be vulnerable to increased CO_2. However, in Chapter 3, I show evidence that functional redundancy may offset the negative impacts of increased CO_2 and potential herbivore loss in Australian kelp forest communities via increased amphipod abundance. In this way, herbivores in some ecosystems may be more likely to serve as a mechanism for maintaining resilience than in others.

5.3 Incorporating realistic variability in long-term manipulations

Scientists, by training, have a strong affinity for control, this tends to lead to fixed treatment levels, rather than levels that can vary through time. Indeed, it is only by executing well-controlled experiments that one can precisely pinpoint the consequence of the variable of interest. However, occasionally it is only by propagating realized variation at one level through to the next level of biological organization that scientists are able to describe important mechanisms. Specifically, in Chapters 2 and 3, it was through allowing CO_2 addition to drive variation in size and abundance that I was able to demonstrate the mechanisms by which CO_2 addition can change consumer resource interactions. This demonstrates the need to consider multiple time scales while incorporating the variation observed at each scale when conducting community ecology.

Further, Chapter 4 brings focus to the pervasiveness of variability in the environment. Of particular importance to this thesis is the high degree of variability in seawater pH and the degree to which pH and CO_2 are controlled by biological processes. While it would theoretically have been possible to minimize the temporal variability in these biotic factors, the amount of CO_2 that would have been required to keep a low pH during daylight photosynthesis would have been much greater than the levels predicted to occur in the next 100-150 years, unrealistically increasing the chance of observing a CO_2 effect. Fluxes in CO_2 concentration are not the only abiotic factor that can be altered by an existing community; Chapter 4 also brought to light the importance of oxygen in determining the structure of tidepool communities, which is highly reduced at night when there is no photosynthesis to counter respiration. The effect of this nightly drop in oxygen availability on community structure was so drastic that I have added another line on my updated filter model of community assembly (Figure 1.5B, Line iv), by which the actualized community alters the abiotic environment that the community is experiencing and thus can indirectly alter the composition of the realized community.

5.4 Future directions

Based upon the results of this thesis, I recommend two areas where future research effort should be particularly focused. The first relates to the prediction of climate variables in fluctuating environments. It is imperative that we continue to increase our understanding of the biogeochemical feedback loops responsible for carbon flux in the nearshore environment. Without this piece of the puzzle, not only do scientists lack a clear understanding of current environmental pH and CO_2 patterns and fluxes, but also of how these fluxes will change with increased CO_2. When, Cornwall and colleagues accounted for diurnal fluctuations in pH while studying the effects of OA on algal growth, they found that diurnal fluctuations had as large an

impact on algal growth as changes in mean pH (2013). It has also been shown that species from more variable pH environments may be more robust to changes in mean pH (Vargas et al. 2017) and that macrophyte dense communities can increase local pH and potentially buffer the communities from OA during daylight (Chapter 4; Krause-Jensen et al. 2016). However, scientists still lack a clear understanding of how pH and CO_2 fluctuations will change in the future and without that understanding scientists may be missing key tipping points and impacts from short term extreme events. Collaborations with oceanographers and biogeochemists will likely be useful in continuing to shed light on these processes, as well as developing more appropriate in better generating treatment levels for future experiments.

Secondly, this thesis strongly demonstrates the importance of considering populations and species density when attempting to understand the implications of environmental change on ecological communities. In light of this information, it is important that scientists focus on multigenerational studies, particularly those that allow for evolution to occur in subsequent generations. Research into whether evolution and transgenerational acclimation can buffer the negative impacts of OA has been mixed (Sunday et al. 2011, 2014, Lohbeck et al. 2012, Pespeni et al. 2013, Welch et al. 2014, Thor and Dupont 2015). Thus, it is unclear when/if evolutionary rescue will buffer the negative impacts of OA and other climate variables. It is also uncertain how impacts on the individual will transfer to changes in abundance and distribution at the population and species level. Future research should focus on how environmental change will alter multigenerational processes that drive species abundance, as changes in species abundance are central to understanding the community level impacts of environmental change.

5.5 Final conclusions

Ecologists use simplified models for explaining the drivers of community structure to help formulate a functional understanding of the mechanisms that determine and maintain biodiversity. However, over-simplified models, such as the filter model of community assembly, often miss important mechanisms through which communities are impacted by changes in the environment. This thesis outlines mechanisms wherein changes in the abiotic environment can cause changes in species interactions via changes in body size and abundance. Additionally, this thesis demonstrates how these changes can alter community structure and diversity. This research demonstrates the importance of considering species interactions holistically, by allowing individual size and population abundance to vary in response to abiotic factors. In order to most effectively understand the potential outcomes of global change, it is imperative that ecologists focus on ecological interactions that are important for maintaining diversity and community structure, such as herbivory. This is especially relevant in cases where the loss of a particular interaction in response to environmental change may have larger implications on the community than the environmental change itself.

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