ECOLOGICAL RESPONSES TO OCEAN ACIDIFICATION BY DEVELOPING MARINE FOULING COMMUNITIES

by

Norah Elizabeth Maclean Brown

B.Sc. (Hons), St. Francis Xavier University, 2011

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)

February 2018

Abstract

Increasing levels of CO_2 in the atmosphere are rapidly affecting ocean chemistry, leading to increased acidification (i.e., decreased pH) and reductions in calcium carbonate saturation state. This phenomenon, known as ocean acidification, poses a serious imminent threat to marine species, especially those that use calcium carbonate. In this dissertation, I use a variety of methods (field-based experiments, surveys, meta-analysis) to understand how marine communities respond to both natural and experimental CO_2 enrichment and how responses could be shaped by species interactions or food availability. I found that ocean acidification influenced community assembly, recruitment, and succession to create homogenized, low diversity communities. I found broadly that soft-bodied, weedy taxa (e.g., algae and ascidians) had an advantage in acidified conditions and outcompeted heavily calcified taxa (e.g., mussels, serpulids) that were more vulnerable to the effects of acidification, although calcified bryozoans and barnacles exhibited mixed responses. Next, I examined an important hypothesis of context dependency in ocean acidification research: that negative responses by calcifiers to high CO_2 could be reduced by higher energy input. I found little support for this hypothesis for species growth and abundance, and in fact found that, for some species, additional food supply exacerbated or brought out the negative effects of CO_2. Further, I found that acidification stress can tip the balance of community composition towards invasion, under resource conditions that enabled the native community to resist invasions. Overall, it is clear that acidification is a strong driving force in marine communities but understanding the underlying energetic and competitive context is essential to predicting climate change responses.

Lay Summary

Ocean acidification, the change in ocean chemistry caused by carbon dioxide emissions, is one of the most serious threats to marine ecosystems. In this thesis, I use both experiments in dock-side tanks where I manipulated CO_2 concentration in British Columbia and naturally occurring volcanic carbon dioxide seeps in the Mediterranean in order to understand the ecological effects of ocean acidification. I found that ocean acidification changed animal community assembly processes to create simplified communities with decreased biodiversity. I found that acidification altered and delayed the developmental trajectory of these communities. My results highlight the importance of considering animal communities as a whole, as responses are contingent on the interaction between different species. For example, I find that acidification stress can tip the balance towards invasive species and is dependent on supply of food item (prey species). Overall, it is clear that acidification is a strong driving force in marine communities.

Preface

Some of the work included in this dissertation has been published or submitted for publication to peer-reviewed journals:

Chapter 2: I designed the experiment, collected and analyzed the data, and wrote the first draft of the paper. I was advised throughout this process by Chris Harley and Tom Therriault.

Brown, N.E, Therriault, T.W., and Harley, C.D.G. 2016. Field-based experimental acidification alters fouling community structure and reduces diversity. Journal of Animal Ecology. 85(5): 1328-1339.

Chapter 3: I designed the experiment, collected and analyzed the data, and wrote the first draft of the paper. I was advised throughout this process by Chris Harley and Tom Therriault in Canada and Jason Hall-Spencer and Marco Milazzo during fieldwork in Italy. Sam Rastrick provided assistance in the field and edited the manuscript.

Brown, N.E., Milazzo, M., Rastrick, S.P.S., Hall-Spencer, J.M., Therriault, T.W., and Harley, C.D.G. 2017. Natural acidification changes the timing and rate of succession, alters community structure, and increases homogeneity in marine biofouling communities. Global Change Biology.

Chapter 4: I outlined the idea and designed the paper. I extracted the data along with Joey Bernhardt and Kat Anderson. Joey Bernhardt and I analyzed the data. I managed the database and produced the figures. I drafted the paper and all authors contributed to discussions, writing, and interpretation. I was advised through this process by Chris Harley.

Brown, N.E., Bernhardt, J.R., Anderson, K.M., and Harley, C.D.G. Increased food supply mitigates ocean acidification effects on calcification but exacerbates effects on growth. *In review*.

Chapter 5 is the result of a collaboration with Joey Bernhardt. Together, we designed the experiment. I conducted the experiment, analyzed the data, and drafted the paper. Chris Harley advised me through this process and he and Joey Bernhardt edited the paper.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ΩA Aragonite Saturation

ΩC Calcite Saturation

AT Total alkalinity

ATP Adenosine triphosphate

CO_2 Carbon dioxide

DIC Dissolved organic carbon

$HCO3^-$ Bicarbonate

OA Ocean acidification

***p*CO_2** Partial pressure of carbon dioxide

Acknowledgements

I am extremely grateful for the opportunity and privilege of being able to complete a PhD. I view experimental science as a unique undertaking that engages both curiosity and creativity and I consider myself very fortunate to be a part of such an endeavor.

First and foremost, I wish to thank my advisor Chris Harley for his guidance over the past six years. I am grateful for your insight, discussion, and thoughtful advice throughout this process. I have immensely benefitted from your mechanistic and experimental brilliance and natural history magic. Thank you for giving me the freedom to explore my ideas and for treating me as a collaborator. I am grateful for your emphasis on a work-life balance and you have set a wonderful example for this aspiring academic. I am especially grateful for your understanding, humour, and wisdom through the toughest parts of this process.

I would like to thank my fantastic committee, Colin Brauner, Mary O'Connor, and Tom Therriault, for their positivity and guidance throughout my PhD. Thank you to my mentor at StFX, David Garbary, for I wouldn't be where I am today without your enthusiasm for marine life and push in the right direction. I am grateful for your continued guidance.

I would like to thank my friends and peers in the Harley lab for providing hilarity and advice as needed. Thank you to my wonderful labmates/friends Kat Anderson, Fiona Beaty, Alyssa Gehman, Colin McLeod, Jocelyn Nelson, and Jenn Sunday for reading drafts of my chapters. Special thanks to fantastic volunteers turned labmates Graham Brownlee, Cassandra Konecny, and Emily Lim who were unwavering in their dedication to unpleasant tasks. Megan Vaughan was the most fantastic lab mate/field assistant/best friend I could ever hope for. Thank you for being ridiculous. I am grateful for my wonderful office mates, especially Shannon May McNally and Joey Bernhardt. Joey, I am so lucky that I have gotten to go through this whole journey with you since the beginning. Our chocolate snack breaks and songs listened to on repeat got me through the tough parts. Thank you to my friends here in Vancouver for forcing me to put down my laptop and head into the wilderness. The ladies adventure club: Lauren Hammond, Julia Lawson, Brianne Miller, Charlotte O'Hara, Ally Stocks, and Ali Stockwell, you girls have made me challenge myself in the backcountry and our friendship has been a rock (haha) throughout my thesis. I am so lucky to be surrounded by such badass independent women. Thank you to Alison Porter and Jess Lu for reminding me to take time to treatyoself and to take each day one step at a time. To my friends in Nova Scotia, your support and long-time friendship are so important to me. A special thank you to my boyfriend Alex Gap for your love and encouragement for the final push.

Finally, I am eternally grateful for the constant support from my family. Thank you to my brother Neil for countless words of encouragement, meals, and motivation through the years. It has been so great to be in the same city as you. To my parents, Daphne and Doug, this task would have been impossible to undertake without you. I am grateful for your wisdom on mental health (Mom) and academia (Dad) at every step of the way. I am so lucky to have such great role models, you have taught me how to appreciate the outdoors, be a feminist, have a work-life balance, and enjoy successes and learn from failures. I cannot describe how much your support means to me, I love you very much.

Chapter 1: Introduction

There are few areas of the ocean that are untouched by direct or indirect anthropogenic impacts (Halpern *et al.*, 2015). Coastal ecosystems are in close proximity to human influence and as such experience a combination of local, regional, and global stressors. These stressors on biological systems, while often unintended and undesired, can be seen as an opportunity to test our understanding of how communities and ecosystems work. Ecological communities are complex assemblages of interacting species that exist in a defined space and time and can vary in taxonomy, abundance, morphology, and function (Morin, 2011). Community ecology aims to generalize patterns (e.g., diversity, composition) and processes (i.e. mechanisms to explain patterns) by focusing on interspecific interactions, physiological and demographic responses to environmental conditions, and neutral or random processes (Jackson & Blois, 2015). By altering both patterns and processes within natural communities, local, regional, and global stressors can give us insight into what drives and maintains them.

My thesis addresses the ecological consequences of one of the most serious threats to marine ecosystems: ocean acidification (OA). Ocean acidification is the phenomenon by which changes in seawater carbonate chemistry occur as a result of increases in atmospheric CO_2 from burning fossil fuels and subsequent thermodynamic equilibration of CO_2 in air and seawater (Doney *et al.*, 2009a). Throughout my thesis, I interchange the terms “ocean acidification” and “acidification”, but in both cases I am implying CO_2-driven acidification, as opposed to acidification from other sources (e.g., changes in methane, nitrogen, or sulphur compounds, IPCC, 2013). Concentrations of carbon dioxide in the atmosphere have increased from ~280 parts per million (ppm) in the pre-industrial era (~year 1750) to over 400 ppm today; the oceans have buffered this change, by absorbing over a quarter of the CO_2 emitted in that time frame

(IPCC, 2013). Uptake of CO_2 by the ocean decreases ocean pH, reduces carbonate ion availability, and in some cases causes undersaturation of seawater with respect to aragonite and calcite (Doney *et al.*, 2009a). The different carbonate parameters play different physiological roles, but since pH is the most easily measured parameter, here I mainly consider the consequences of declines in seawater pH. Ocean acidification describes the direction of pH change, as it is predicted that global pH will decline by 0.3 to 0.5 units by the end of the century; since pH is on a logarithmic scale the change in one unit represents a change in order of magnitude (IPCC, 2013). The urgency of ocean acidification comes not only from the magnitude of expected pH change but also the astonishing pace of these changes which is unprecedented in geological history over the past 300 million years (Hönisch *et al.*, 2012). With many regions already experiencing declines in seawater pH (e.g., the northeast Pacific, Feely *et al.*, 2008, 2010; Cai *et al.*, 2011; Wootton & Pfister, 2012), there are concerns that acidification may outpace potential acclimatization and adaptation (Sunday *et al.*, 2014). Thus, there is an urgent need to understand how organisms and ecosystems will respond to projected acidification.

I examine the ecological consequences of ocean acidification by focusing on species- and community-level responses to ocean acidification using field-based experiments, surveys, and meta-analysis. Ecological questions are arguably best addressed in field-based experiments, as simplified studies in laboratory settings cannot fully elucidate the processes that underlie ecological pattern (Wernberg *et al.*, 2012). In addition to carefully controlled, field-based experiments, I use natural CO_2 seeps (emitting CO_2 for thousands of years as a result of volcanic activity, Hall-Spencer *et al.*, 2008) which are a promising forum for exploring the community- and ecosystem-level consequences of long-term acidification. Throughout this dissertation, I focus on how species interactions, especially competition with invasive species and consumption

of available food, can modify response to acidification. The following is a review of the effects of ocean acidification on physiological responses and energetics, species interactions, and community patterns leading to the specific objectives and hypotheses of my thesis.

1.1. Physiological Response and Energetics

The biological effects of ocean acidification have been documented for at least 180 marine species at various stages of ontogeny (Kroeker *et al.*, 2013a). Although neutral and even positive responses have been found, experimental ocean acidification has been shown to decrease calcification, photosynthesis, growth, and development across a number of species which likely contributes to noted reductions in survival and abundance (Kroeker *et al.*, 2013a). Calcified species that use calcium carbonate to build and maintain their shells have been shown, with some exceptions, to be overall more sensitive to ocean acidification than non-calcified species (Wittmann & Pörtner, 2013). This is likely due to challenges posed by ocean acidification in availability of carbonate ions (Doney *et al.*, 2009b), acid-base and ion regulation at sites of calcification (Melzner *et al.*, 2009a), and dissolution of carbonate and aragonite (Pickett & Andersson, 2015).

Many of the metabolic pathways to counter increases in CO_2 (or $[H^+]$) in extracellular or intracellular fluids in order to maintain homeostasis, such as acid-base and ion regulation and protein synthesis, are energetically costly (Melzner *et al.*, 2009a; Sokolova *et al.*, 2012; Stumpp *et al.*, 2012a). Some of the physiological responses to ocean acidification may therefore be a result of energetic trade-offs with basal maintenance costs that increase under stress (Sokolova *et al.*, 2012). The biochemical basis for this energetic trade-off is the allocation of energy in the

form of ATP, the production of which is limited both by food intake and metabolic rate (breakdown of food into ATP) (Pan *et al.*, 2015). While ocean acidification has been shown to increase overall ATP production in the short term (Turner *et al.*, 2015; Wang *et al.*, 2017), it also can increase the proportion of ATP allocated to protein synthesis and ion regulation from a limited ATP pool (i.e. no increase in metabolic rate) (Pan *et al.*, 2015). Further exploration into the effects of ocean acidification on energetic uptake, assimilation, and allocation, as well as a basic understanding of the metabolic costs of various biological processes (Frieder *et al.*, 2017) and the plasticity of metabolic pathways in the face of stress (Pan *et al.*, 2015), is needed to fully understand energetics of ocean acidification.

1.2. Species Interactions

As in many emerging fields, ocean acidification studies initially focused on the most easily answered questions, such as organism-level responses to changes in CO_2 in the laboratory. While documenting physiological sensitivities of a wide range of taxa to ocean acidification and providing explanations for differences in response (e.g., interspecific and ontogenetic variation in physiology, importance of calcification; Kroeker *et al.*, 2013a; Wittmann & Pörtner, 2013), many of these studies do not incorporate important aspects of natural ecosystems, namely other species that act as predators, competitors, habitats, or energy sources, all of which can influence species sensitivity to environmental conditions and stressors. Species interactions have been shown to alter physiological sensitivities: for example, predator-induced behavioural, morphological or physiological changes have resulted in changes to thermal performance curves (both optimum and maximum temperature) for individuals and populations (Culler *et al.*, 2014; Katzenberger *et al.*, 2014; Luhring & Delong, 2016). Further, habitat forming species can alter

realized abiotic conditions such that species inside these habitats (e.g., mussel beds, kelp forests) experience dramatically different conditions than those on the outside, or on the edge (Frieder *et al.*, 2012; Jurgens & Gaylord, 2016). Finally, the realized outcome of environmental change on a given species could depend on the physiological sensitivity of another species with which it interacts (Kordas *et al.*, 2011). Indeed, response measures from species in community settings show more variability, indicating that predictability of species responses might decrease when considered in a multi-species context (Hale *et al.*, 2011; Kroeker *et al.*, 2013a). Thus, testing sensitivities of species to ocean acidification in a functioning community context is essential to improving projections for a species of interest.

Species interactions can be particularly unpredictable when one species lacks a shared evolutionary history with the other, i.e. an introduced species. Invasive species, introduced species that have established and spread in a new region (Ricciardi & Cohen, 2007), have been recognized as drivers of biodiversity loss and can pose a large and long-term threat to native communities (Lodge, 1993; Mainka & Howard, 2010; Sorte *et al.*, 2010). Once established, introduced species can have profound effects on community structure; for example, invasive species are often exceptional competitors for food and space and thus reduce resources otherwise available to native species (Dukes & Mooney, 1999; Stachowicz *et al.*, 1999; Tilman, 2004; Dijkstra & Harris, 2009; Grey, 2011). Invaders can also alter fundamental properties of the ecosystem including energy flow between trophic groups, benthic-pelagic coupling, nutrient cycling, habitat structure, and ecosystem functioning (Vitousek, 1990; Stachowicz *et al.*, 2002a; Occhipinti-Ambrogi, 2007; Brook *et al.*, 2008; Walther *et al.*, 2009). Species and community responses to environmental variability suggest that there are multiple consequences of climate change for biological invasions, notably new introductions via increased transport, propagule

pressure, and biotic resistance, and exacerbation of ecological effects of already established non-native species via changing reproductive phenology, shifting community composition, and geographic range shifts (Dukes & Mooney, 1999; Occhipinti-Ambrogi, 2007; Rahel & Olden, 2008; Walther *et al.*, 2009; Mainka & Howard, 2010). Thus far, ocean acidification has been shown to increase the abundance of some invasive species (Vaz-Pinto *et al.*, 2013; Hall-Spencer & Allen, 2015) but changes in invasion processes, impact or biotic resistance under acidification have not been explored.

Another form of interaction, consumption, can mediate all aspects of organismal fitness in heterotrophs. Under food limitation, energetic allocation can result in trade-offs between basal maintenance and fitness-related metabolic processes (Bradley *et al.*, 1991; Sokolova, 2013). Sensitivity to environmental stress can therefore be influenced by changes in resource supply via changes in energetic allocation (McLean *et al.*, 2015). Since ocean acidification imparts an energetic cost on organisms in order to maintain homeostasis (as described above; Sokolova *et al.*, 2012; Stumpp *et al.*, 2012b), a number of studies have proposed that sensitivity to ocean acidification could be modified by food availability (Edmunds, 2011; Comeau *et al.*, 2013; Thomsen *et al.*, 2013; Crook *et al.*, 2013; Drenkard *et al.*, 2013; Hettinger *et al.*, 2013; Pansch *et al.*, 2014; Ramajo *et al.*, 2016; Towle *et al.*, 2015; Pan *et al.*, 2015; Ramajo *et al.*, 2015; Maier *et al.*, 2016; Büscher *et al.*, 2017). Beyond this hypothesis, which I address in more detail below, changes in climate variables (e.g., pH, temperature) alter consumptive interactions in a number of ways, such as mismatch in timing of consumer and resource (Winder & Schindler, 2004), changes in feeding rate of the consumer (Clements, 2016), changes in palatability or defenses of the resource (Arnold *et al.*, 2012; Poore *et al.*, 2013) or changes in abundance or quality of the resource (Rossoll *et al.*, 2012). Therefore, disentangling how climate change affects the energetic

context of an organism and how this in turn influences the response to climate change will likely prove important for improved predictions.

1.3. Community Patterns

There is growing interest in how the differential responses to changes in pH by various taxonomic and life history stages combined with species interactions could produce changes in structure, diversity, and function at the community level (Nagelkerken & Connell, 2015). Processes such as community assembly, succession, and the maintenance of diversity are important at a number of scales: from shaping the niche for a species of interest to setting the stage for ecosystem processes. A given species of interest or a whole ecosystem are often focal points for management and conservation and therefore community-level studies are necessary for linking and broadening our understanding of these levels (Harley *et al.*, 2006; Wernberg *et. al*., 2012). To date, substantial changes in algal, seagrass, and benthic invertebrate community composition and structure have been demonstrated in response to experimental and natural acidification, noting increased dominance of organisms with little dependence on calcification and overall drops in species richness (Martin *et al.*, 2008; Dashfield *et al.*, 2008; Hall-Spencer *et al.*, 2008; Widdicombe *et al.*, 2009; Porzio *et al.*, 2011; Hale *et al.*, 2011; Kroeker *et al.*, 2011, 2013b; Hofmann *et al.*, 2012; Alsterberg *et al.*, 2013; Christen *et al.*, 2013; Campbell & Fourqurean, 2014; Fabricius *et al.*, 2014; Nogueira *et al.*, 2017; Vizzini *et al.*, 2017). Candidate groups of organisms likely to be winners (e.g., fleshy weedy species) and losers (e.g., heavily calcified species) in sessile communities have been identified. Planktonic communities, in contrast, show mixed responses to elevated CO_2: both resistance to acidification (Aberle *et al.*, 2013; Langer *et al.*, 2017) and substantial changes in abundance and composition (Smith *et al.*,

2016; Algueró-Muñiz *et al.*, 2017). Finally, there is limited information on processes that link planktonic and benthic invertebrate communities: namely community assembly, settlement, and recruitment. For example, both natural and experimental acidification have been shown to decrease early recruitment of calcified invertebrates onto artificial substrata, resulting in species-poor homogenized communities (Kroeker *et al.*, 2013b; Donnarumma *et al.*, 2014; Peck *et al.*, 2015; Allen *et al.*, 2016; Cox *et al.*, 2017). However, temporal dynamics of settlement and implications for subsequent recruitment and succession (Menge & Sutherland, 1987; Roughgarden *et al.*, 1988) have yet to be explored in an acidification context.

1.4. Study System

I addressed questions about community change under ocean acidification using field-based marine fouling assemblages. These sessile invertebrate communities colonize anthropogenic structures and some natural rock outcroppings. Sessile fauna include colonial and solitary tunicates, barnacles, bryozoans, polychaetes, hydroids, and mussels, many of which are filter feeders. Marine fouling communities have long been used as model systems for studying patterns and processes in communities (e.g., stability, Sutherland, 1974). These quickly developing communities are ideally suited to studying recruitment and succession (Chalmer, 1982). Further, these communities typically have a high proportion of invasive to native species (Stachowicz & Byrnes, 2006), which make them well suited to studying species interactions.

1.5. Structure of this Dissertation

In Chapter 2, I set out to answer the following questions about the ecological effects of ocean acidification:

2.1. Does ocean acidification alter recruitment of fouling species from the plankton?

2.2. Which biofouling species are winners and losers in the face of ocean acidification? Are traits of the winners and losers predictable?

2.3. Does ocean acidification reduce fouling community diversity and richness? And if so, are these changes primarily driven by direct effects of ocean acidification or indirect effects (i.e. species interactions)?

By manipulating CO_2 in unique *in-situ* mesocosms, I captured recruitment and early successional dynamics of invertebrate communities from plankton while conserving natural fluctuations in environmental variables. I found that high CO_2 promoted homogenized, low diversity communities.

For Chapter 3, I moved from mesocosms to naturally acidified volcanic vent sites, where I observed the development of fouling communities along a pH gradient and asked if responses to the questions from Chapter 2 hold in naturally acidified sites. Specifically:

3.1. Is order of arrival or subsequent succession between species different at the acidified site vs. the control site?

3.2. If communities from the acidified site are transplanted to control conditions mid-succession, do past acidification effects persist, or are they reversed by alleviation of CO_2 stress?

Using reciprocal transplants along a shallow water volcanic CO_2 gradient in the Mediterranean, I assessed the importance of timing and history of OA on patterns of succession and community structure. I observed that succession at the acidified site was initially delayed but then caught up over the next four weeks. These changes in succession led to homogenization of communities maintained in or transplanted to acidified conditions, and altered community structure in ways that reflected both short- and longer-term acidification history. These community shifts were likely a result of interspecific variability in response to increased CO_2 and changes in species interactions.

With this baseline of understanding of changes in communities, I set out to see if energetic context could modify the responses to acidification in Chapter 4. I asked:

4.1. Are the effects of OA and food supply additive?

4.2. If effects are non-additive, does food addition alleviate the negative effects of OA?

A recent meta-analysis concluded that food supply confers resistance to ocean acidification (Ramajo *et al.*, 2016). If true, this has far-reaching implications for how we both understand and study OA. In Chapter 4, I addressed this question by improving the meta-analytic methodology and updating the relatively small dataset. I first clarified the hypothesis put forward by the authors and others, and then examined the available data in a more appropriate framework. I

found that for calcification, food addition did indeed reduce CO_2 impacts. Surprisingly, however, I found that food addition actually exacerbated the effects of acidification on growth, which may be due to the increased scope for CO_2 effects in food-replete, high-growth situations. There has been little consideration beyond the individual level in understanding the relationship between food supply and response to acidification. Therefore, in Chapter 5 I set out to answer the questions:

5.1. Do species in a community setting respond to food supply and acidification in the same way as in isolation?

5.2. Does the pattern of interaction between food and acidification change when moving from the organismal to the community level?

In Chapter 5, I examined species and community-level responses to OA and food addition (supplementation with one of two species of phytoplankton) in *in-situ* mesocosms. I used the interaction between dominant hydroids and invasive ascidians to illustrate how competition might change responses to OA and food addition using data from the experiment and a conceptual model. Community level responses reflect both a summation of the species-level responses within the community context and traits of the dominant species. Species richness and community structure demonstrated that the negative effects of acidification are not overcome by food availability. Overall, the proposed hypothesis regarding the ability for food addition to mitigate the negative effects of acidification is thus far not widely supported at species or community levels.

Overall in this dissertation, I use novel field-based experiments to show that ocean acidification acts throughout the community assembly process to create homogenized, low diversity

communities. I found that species interactions, especially with invasive species, are key moderators of the effects of acidification and energetic supply. I demonstrated that working within the context of a functioning community allows for examination of mechanistic underpinning of response to CO_2 and is essential in order to improve ocean acidification predictions.

Chapter 2: Field-based Experimental Acidification Alters Fouling Community Structure and Reduces Diversity

2.1. Introduction

Climate change, largely attributed to the rapid increase in atmospheric carbon since the industrial revolution, is having a considerable impact on marine ecosystems (Hoegh-Guldberg & Bruno, 2010). Predicted consequences of greenhouse gas emissions range from alterations of ocean currents, upwelling patterns and storm frequencies, to salinity changes, increased temperatures, and changes in ocean chemistry (IPCC, 2013). Among climate change impacts, changes in the carbonate chemistry of the global ocean, or ocean acidification (OA), may be one of the most important for ecological systems (Halpern *et al.*, 2008). Ocean pH is declining at a rate unobserved in the fossil record over the past 300 million years (Hönisch *et al.*, 2012). While global surface ocean pH is predicted to decline by 0.3 to 0.5 units by the end of the century (IPCC, 2013; Doney *et al.*, 2014), in some regions the rate of change may be faster due to shifts in upwelling intensity, eutrophication, or freshwater inputs (e.g., the northeast Pacific, Feely *et al.*, 2008, 2010; Cai *et al.*, 2011; Wootton & Pfister, 2012). In regions currently experiencing elevated CO_2 conditions, environmental suitability for some marine organisms may already be declining (e.g., pteropods in the California Current Ecosystem; Bednaršek *et al.*, 2014).

Over the past decade, experimental research has largely focused on organismal responses to OA. Effects on multiple performance metrics, including metabolic rate, growth, development, behaviour, and overall survival have been documented for a wide diversity of species (Widdicombe & Spicer, 2008; Dupont & Thorndyke, 2009; Kroeker *et al.*, 2013a; Wittmann & Pörtner, 2013). Documented effects of OA range from negative to neutral and even positive,

depending on interspecific and ontogenetic variation in physiology and the cost of and reliance on calcified structures (Kroeker *et al.*, 2013a; Wittmann & Pörtner, 2013). Because there is considerable variation in responses among species, there is growing interest in how the differential responses to changes in pH among and within species could influence community diversity, structure, and function. Importantly, OA could modify outcomes of competition, predation, and facilitation through effects on population growth rate, resource availability, production and efficacy of defensive structures, and behaviour among other factors (Leduc *et al.*, 2013; Sanford *et al.*, 2014; Gaylord *et al.*, 2015). Multi-species approaches are urgently needed to better understand how acidification will influence species interactions and cascading indirect effects at the community and ecosystem scale (Godbold & Solan, 2013).

Community-scale questions are arguably best addressed in field-based (e.g., shallow subtidal CO_2 vents) or pseudo-field-based (e.g., flow through-mesocosms) systems that encompass biological and physical complexities of the natural environment (Wernberg *et al.*, 2012; Stewart *et al.*, 2013). Recent experiments in field-based mesocosms show changes in community structure in seagrass (Campbell & Fourqurean, 2014) and phytoplankton communities (Schulz *et al.*, 2013) following exposure to elevated CO_2. Work in CO_2 vent systems has demonstrated CO_2 effects on successional patterns (Kroeker *et al.*, 2013b, 2013c; Porzio *et al.*, 2013) and algal community structure (Kroeker *et al.*, 2011; Porzio *et al.*, 2011; Johnson *et al.*, 2013). Vent sites are often dominated by species with little reliance on calcification, and are often lower in diversity and complexity than nearby reference sites (e.g., Hall-Spencer *et al.*, 2008; Fabricius *et al.*, 2014). These vent studies provide insight into the potential consequences of long-term acidification, including shifts in species interactions (Garrard *et al.*, 2014) and local adaptation (Kroeker *et al.*, 2013c).

One important determinant of species interactions and community structure is the dynamics of settlement and recruitment (Menge & Sutherland, 1987; Roughgarden *et al.*, 1988). Although many studies to date identify species and community responses to acidification, most lack information on community assembly (i.e., settlement and recruitment). A few studies have followed recruitment and development of juveniles spawned from adults during laboratory acidification experiments (e.g., coralline algae, Roleda *et al.*, 2015, oysters, Parker *et al.,* 2012). Only a small number of studies have addressed recruitment under acidified but otherwise natural conditions. Both Kuffner *et al.* (2007) and Jokiel *et al.* (2008) showed decreased recruitment of crustose coralline algae as a result of experimental HCl acidification in flow-through mesocosms (but see Gattuso *et al.* (2011) for reasons why HCl addition is an imperfect proxy for OA). Milazzo *et al.* (2014) found that transplanted vermetid gastropods had fewer successful brooded recruits in elevated CO_2 areas at a natural CO_2 vent site, due to poor attachment related to dissolution of settlement discs. Peck *et al.* (2015) measured colonization of Mediterranean microalgal and invertebrate biofouling organisms onto both pre-colonized and bare polyethylene pipes in flow-through tanks with filtered water supplied from a nearby lagoon. They found reduced numbers of spirorbid worms and higher abundance of diatoms and filamentous algae under acidified conditions. Although these studies hint at the potentially broad significance of acidification for settlement and recruitment processes, there is little information on the direct effect of experimentally manipulated CO_2 concentration on the development of invertebrate communities from plankton in natural settings. Realistic field experiments that allow for natural recruitment dynamics are a necessary step forward for developing accurate climate change predictions for marine benthic communities.

In this study I tested how experimental acidification affects community diversity and structure in the context of a naturally recruited community. Specifically, I manipulated CO_2 concentration in field-based, flow-through mesocosms that supported fouling communities composed of both calcified and non-calcified taxa. I hypothesized that ocean acidification would affect community structure both by directly altering settlement and growth of sensitive organisms (Wittmann & Pörtner, 2013) and by indirectly altering survival of one organism via the success of another (i.e., a competitive effect, McCormick *et al.*, 2013a). In particular, I expected the settlement and growth of calcifying organisms to be more susceptible to low pH than soft-bodied organisms (Kroeker *et al.*, 2013a; Wittmann & Pörtner, 2013). I predicted that these acidification-driven changes in recruitment and competition might shift the community as a whole from an assemblage with a diverse range of calcified species, to an assemblage containing a reduced number and proportion of calcified species (as seen at vent sites, e.g., Kroeker *et al.*, 2013c). These changes in composition and structure could drive corresponding reductions in overall richness and diversity, especially if uncompensated by increases in non-calcified species. If the predicted community changes are driven mainly by acidification effects on recruitment from larvae, then I expect the signal to be detectable early on in the experiment. In contrast, if the community responses to CO_2 are driven by sensitivity of recruited juveniles or changes in species interactions, then I would expect community responses to emerge only later on in the experiment.

2.2. Materials and Methods

2.2.1. Study Site and Species

Experiments were conducted at the Reed Point Marina in Port Moody, British Columbia (49°17′31″N, 122°53′25″W). The marina is located near the sheltered eastern terminus of Burrard Inlet. The site is seasonally brackish with salinities ranging from 16 ppt in late spring to 26 ppt in winter due to seasonal patterns in precipitation, snowmelt, and inputs from local streams and rivers (Thomson, 1981). The experiments were conducted over a ten-week period (25 July 2012 to 3 October 2012). Manipulations involved the sessile, invertebrate-dominated community that colonizes the undersides of anthropogenic structures and some natural rock outcroppings, which is widely referred to as the marine fouling community. Species found in the fouling community at this site included bryozoans (*Membranipora membranacea* Linnaeus 1767), mussels (*Mytilus trossulus* Gould 1850), hydroids (*Obelia dichotoma* Linnaeus 1758), barnacles (*Balanus crenatus* Bruguiere 1789), and ascidians, both native (*Corella inflata* Huntsman 1912) and introduced (*Botryllus schlosseri* Pallas 1776).

2.2.2. Experimental Set-up

The ten-week experiment was conducted in a novel field-deployed flow-through mesocosm system. Twenty-four polyethylene mesocosms (52 L, 55 x 39 x 33 cm) were deployed between two floating docks and each mesocosm was partially submerged such that ~ 5 cm of the mesocosm container remained above water (Fig. 2.1). Individual aquarium pumps (Eheim 600 Compact, Germany) with intakes at 1 m depth continuously supplied unfiltered seawater from below each mesocosm at a rate sufficient to replace the volume of the mesocosm approximately

four times per hour (~ 4 L min^{-1}). The use of continuously flowing seawater in semi-submerged mesocosms preserved the natural temporal variation in pH, temperature, and salinity experienced

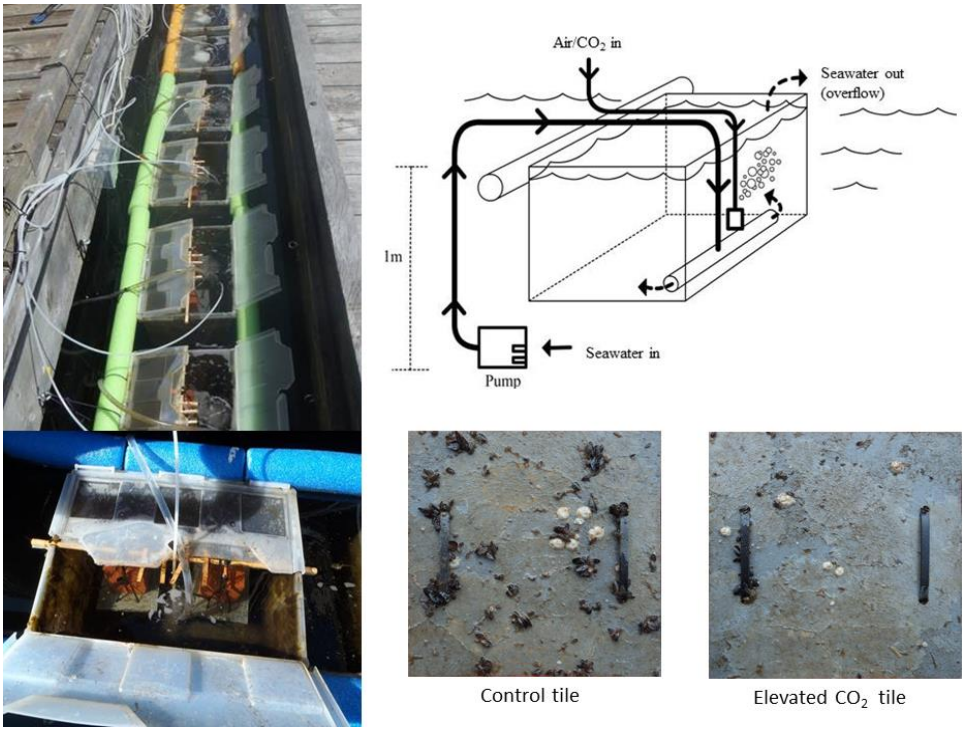

Fig. 2.1. Photographs and schematic of the experimental mesocosms and tiles. Seawater is pumped in from 1 m depth and then overflows over the top of the mesocosm with a ~15 min turnover rate. Air or CO_2 enriched air was continuously bubbled into each mesocosm to maintain treatment differences.

In Port Moody. All pumps were equidistant to the source communities along the undersides of the docks. Each mesocosm had a clear lid to retain natural diel light cycles and to minimize the off-gassing of CO_2. Mesocosm walls were scraped clear weekly to avoid diatom buildup, which along with associated bacterial buildup, might have influenced oxygen consumption and nitrate production. The mesocosms were randomly assigned to one of two treatments: ambient or elevated CO_2. In the ambient CO_2 treatment, air was bubbled in via an air compressor (GAST

Inc) at ~1.67 L min ¯1. In the elevated CO_2 treatment, a compressed 80% CO_2: 20% O_2 mix was bubbled in at ~0.035 L min $^{-1}$ in addition to air at ~1.67 L min^{-1}. This created a 680 ppm difference in dissolved CO_2 in seawater between treatments which is within projections for the year 2100 (IPCC, 2013). Seawater within the mesocosms was well mixed and pH did not vary with space inside the tanks. Mass flow controllers and gang valves were used to control the volume of air entering each mesocosm and wooden airstones created fine bubbles for rapid equilibration. The mesocosms were continuously supplied with these air sources except for two occasions due to system failure where the tanks were without aeration for periods ≤ 48 hours. To avoid changes in water flow, the pump water intakes were cleaned every 3 to 4 days.

Temperature and salinity were measured (Yellowstone Scientific Instruments Model 85, Montana USA) in each mesocosm and the adjacent marina at least twice per week at midday. Weekly pH measurements were taken at midday (Oakton 6 pH meter, Oakton Instruments, Illinois USA), calibrated using National Bureau of Standards (NBS) buffers. NBS buffers are not specifically designed to be used for calibration of pH meters for seawater measurement and can result in pH measures with accuracy of +/- 0.05 pH. Many others have used this technology, accepting the associated loss of accuracy (e.g., Wootton & Pfister 2012, Sanford *et al.,* 2014). A water sample from each mesocosm was taken once per week and preserved with mercuric chloride for dissolved inorganic carbon (DIC) analysis following procedures in Dickson (2007) (Dissolved Inorganic Carbon Analyzer model AS-C3 Apollo SciTech Inc. Bogart, Georgia USA). The DIC and pH measurements were used to estimate the remaining parameters of the carbonate system using the CO2SYS program (Pierrot *et al.,* 2006) with dissociation constants from Millero (2010) and Dickson (1990).

Each mesocosm contained two roughened PVC tiles (14.5 cm x 14.5 cm, N=46) that served as recruitment surfaces. Tiles were weighted and suspended side by side in each mesocosm, with the recruitment surface facing down. Over the course of the experiment, propagules of fouling organisms were allowed to settle naturally on the tiles. Each tile was photographed once per week. Photographs were analyzed for percent cover of all species by overlaying a grid of 100 points. The number of mussels, barnacles, and bryozoan colonies on each tile were quantified from photographs at the final (week 10) sampling date. I further classified bryozoans and barnacles as alive or dead/senescent; barnacles were determined to be dead if their test was empty and bryozoans were categorized as senescing if the test appeared to be dissolving, part of the natural seasonal attrition for this species. I also measured the size of the largest barnacle on each tile at the 10^{th} week and the size of the three largest bryozoan colonies from weeks 0 to 4, before overlap began to affect colony growth, shape, and size. One randomly selected tile from each mesocosm was preserved in 3% formalin for fine-scale species identification; organisms were identified to the lowest taxonomic unit possible in the laboratory under a dissecting microscope. All intact mussels from these preserved tiles were photographed to determine a size spectrum for mussels on each tile (method verified with calipers to ensure accuracy). All photographic analyses were conducted in ImageJ (Schneider *et al.*, 2012). The mussels from each tile were then dried for 48 hours and weighed together. One mesocosm out of the 24 initially deployed was excluded from analysis due to consistent pump failure throughout the experiment; therefore, the final sample size was 23 (ambient conditions, n=11; elevated CO_2, n=12).

2.2.3. Statistical Analyses

To test my hypothesis about changes in community structure, permutational routines were performed on the data to understand multivariate community structure (using PERMANOVA, permutational multivariate analysis of variance) and community dispersion (using PERMDISP, a test for homogeneity of multivariate dispersions). As a percent cover of a given species is ecologically different than a percent cover of the next, all variables (i.e., species) were standardized by total percent cover of that species across tiles, putting abundances on the same scale. Next, I calculated a Bray-Curtis resemblance matrix on the standardized data. The PERMANOVA routine used the BC matrix to compare community structure, using 9999 permutations and a type III sum of squares. To compare dispersion (i.e., variability) among the two tiles within mesocosms between treatments, I first calculated a resemblance matrix among centroids for mesocosms using Euclidean distance and then used the PERMDISP routine. To compare dispersion among tiles, ignoring mesocosms, between treatments I used PERMDISP on the BC matrix. All multivariate community analyses were conducted in PRIMER 6.0 (PRIMER-E, Ivybridge, UK).

The effect of acidification on the percent cover of each species (using the average % cover for the two tiles) and on size of bryozoan colonies (using the average size of 3 bryozoan colonies per tile, averaged across the two tiles in each mesocosm) over time was determined using repeated measures ANOVAs in JMP 10.0 (SAS Institute, Cary, North Carolina, USA). The majority of the data was normally distributed (within species, treatment and a given time point) and as RMANOVAs are typically robust against violations of normality (Vallejo *et al.*, 2010), I chose not to perform transformations to this dataset. RMANOVAS do require compound symmetry or sphericity and I tested for this using Mauchly's test. If sphericity was found to be violated, I used

the Greenhouse-Geisser correction (Field, 2013). I used generalized linear mixed-effects models (GLMMs) to analyze photographic data from both tiles in each mesocosm (i.e., the tile was the level of replication), with mesocosm included as a random effect (glmmADMB package, Skaug *et al.* 2013). Specifically, I used GLMMs to test for an acidification effect on the number of bryozoans, mussels, and barnacles at the end of the experiment, and for differences in the relative proportion of senescent bryozoans and dead barnacles. I graphically assessed the distribution fit using probability plots in R and then used the most appropriate distribution in analysis using GLMMs. The numerical response variables were assumed to be Poisson distributed and the mortality proportions were treated as binomial responses.

GLMMs were also used to test for the effect of acidification on the size of the largest barnacle assuming a gamma distribution for the response. A gamma distribution was also assumed when analyzing the effect of acidification on the overall size of mussels and the largest 5% of mussels from a single preserved tile from each mesocosm. I used Generalized Linear Models (GLMs) with assumed gamma distributions to analyze the effect of acidification on size of the single largest mussel and gamma-corrected variance of mussel size per tile. To analyze the effect of acidification on the summed dry biomass of the preserved mussels, I used quantile regression (QR) of the median (tau = 0.5) because GLMs using a calculated mean of this measurement had heterogenous residuals that could not be resolved, and QR does not require homogeneity of the residuals (see Cade & Noon, 2003).

2.3. Results

2.3.1. Seawater Parameters

DIC and pH naturally fluctuated through time but communities in the elevated CO_2 treatment experienced consistently lower pH over the course of the experiment than those in the ambient treatment (ΔpH_{NBS} = 0.35, Table 2.1, Fig. 2.2a). Temperature also fluctuated during the experimental period and ranged from a peak of 20.4°C in August down to 13.1°C in October with a negligible difference between treatments (Δtemperature = 0.04°C; Fig. 2.2b). Salinity increased slowly throughout the experimental period from a minimum of 17.5ppt in July to

Table 2.1. Seawater carbonate chemistry in ambient (n=11) and elevated (n=12) CO_2 treatments. Mean midday values averaged over time from July 25th – Oct 3, 2012. Asterisks indicate calculated values in the CO2-SYS program (Pierrot *et al.*, 2006).

Seawater parameter	Ambient	Elevated CO_2
Temperature (°C)	16.45 +/- 1.84	16.41 +/- 1.82
Salinity	22.46 +/- 2.20	22.45 +/- 2.20
pH_{NBS}	8.15 +/- 0.18	7.80 +/- 0.20
DIC (μmol kg^{-1})	1574.42 +/- 142.11	1649.63 +/- 143.73
pCO_2 (μatm)*	396.18 +/- 173.07	1077.67 +/- 456.55
Alkalinity (μmol kg^{-1})*	1695.84 +/- 122.10	1679.86 +/- 125.03
HCO_3^- (μmol kg^{-1})*	1465.17 +/- 155.82	1558.89 +/- 140.85
Ω Calcite*	2.43 +/- 0.88	1.27 +/- 0.60
Ω Aragonite*	1.50 +/- 0.54	0.78 +/- 0.37

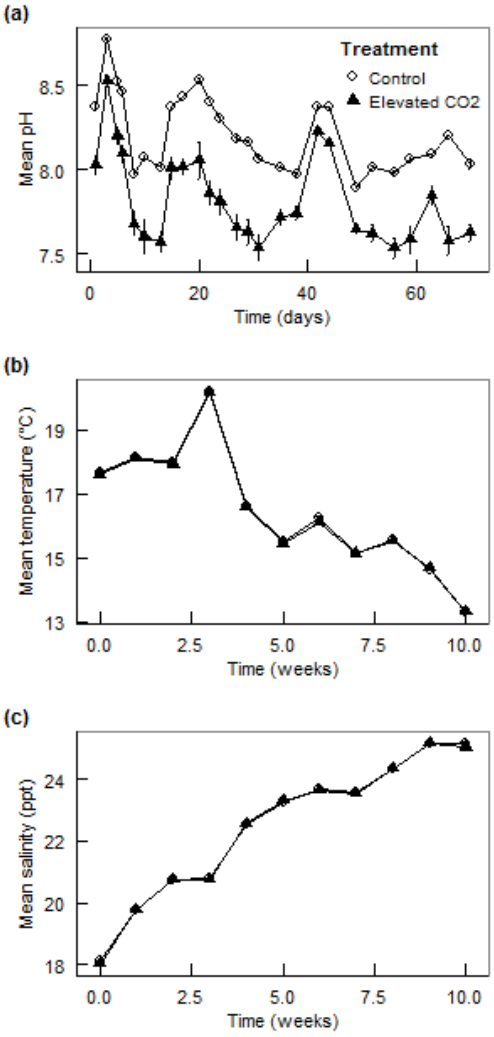

Fig. 2.2. Mean water (a) pH_{NBS}, (b) temperature, and (c) salinity in elevated CO_2 and ambient mesocosms over the course of the experiment. Error bars represent standard error.

25.3ppt in October; there was little difference between treatments (Δsalinity = 0.01; Fig. 2.2c). Environmental conditions in ambient mesocosms were broadly similar to source water at 1 m depth, although the mesocosms were half of a degree cooler and slightly less acidic (ΔpH_{NBS} = +0.08, Δtemperature = -0.49°C, Δsalinity= -0.17ppt).

2.3.2. Community Responses

Acidification significantly altered community structure after 10 weeks (PERMANOVA, Bray Curtis Similarity, pseudo-F=4.26, P=0.006; Fig. 2.3) primarily due to differences in abundances of sessile species. This response could be a result of the finding that communities that developed under elevated CO_2 conditions were less variable than those under ambient conditions, both among the two tiles within each mesocosm (PERMDISP, F=6.56, P=0.038, Fig. 2.3), and among tiles across all mesocosms (PERMDISP, F=6.56, P=0.046).

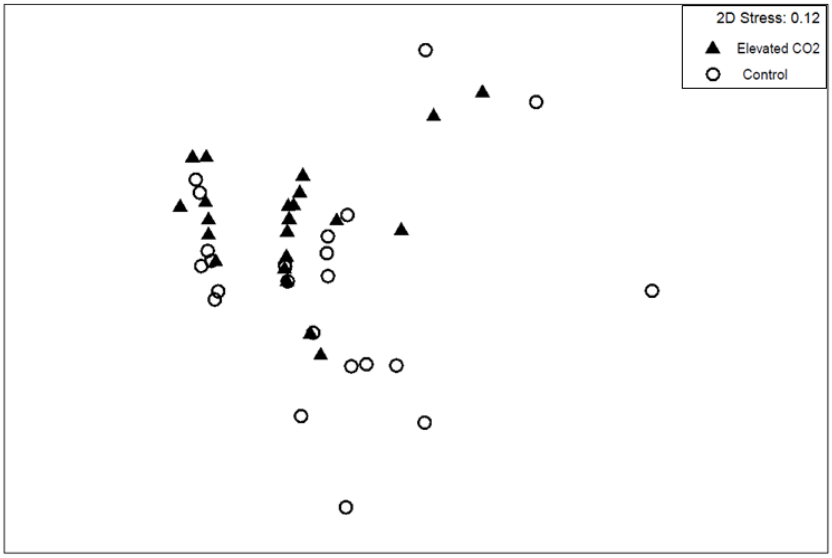

Fig. 2.3. MDS plot showing the relationship between communities in elevated CO_2 conditions (n=24 tiles nested within 12 mesocosms) and ambient conditions (n=22 tiles nested within 11 mesocosms) after 10 weeks. PERMANOVA, Bray Curtis Similarity, P=0.006.

Shannon's diversity index (which incorporates the number and relative abundances of living species) was significantly lower in the elevated CO_2 treatment compared to ambient conditions and was over 40% lower by the 10th week (Fig. 2.4a; MANOVA, Treatment F=19.8, P=0.0002, Time F=16.0, P<0.0001, Time*Treatment F=3.8, P=0.044). Conversely, species richness increased over time but did not differ between treatments (Fig. 2.4b; MANOVA, Treatment F=0.10, P=0.76, Time F=28.09, P<0.0001, Time*Treatment F=1.12, P=0.34), indicating that there were similar numbers of species in both treatments but the relative abundances of the species within a treatment differed, i.e. dominance increased under acidification.

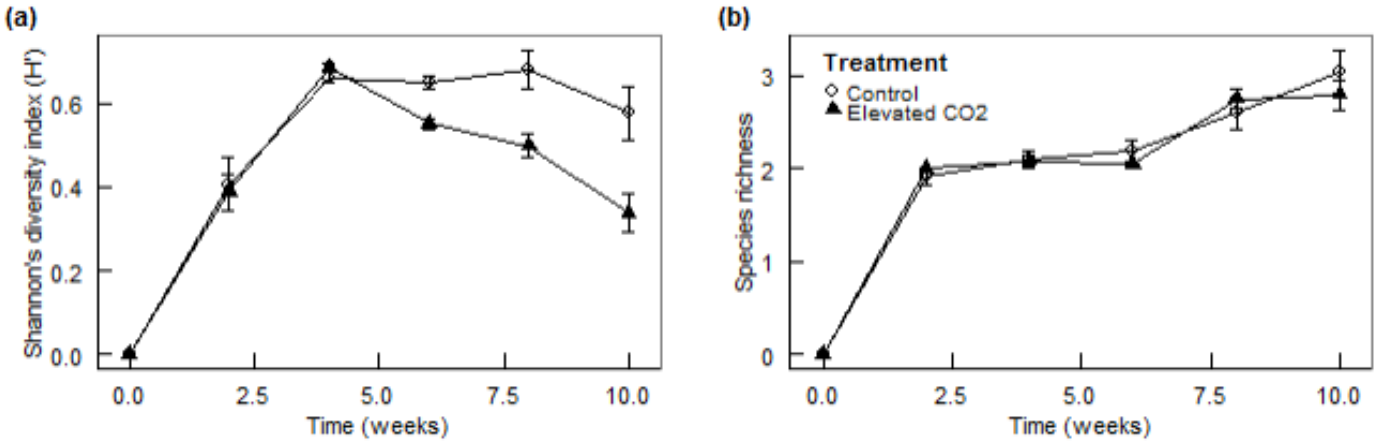

Fig. 2.4. Shannon's diversity (a) and species richness (b) in elevated CO_2 and ambient mesocosms over time. Error bars indicate standard error.

2.3.3. Species-specific Responses

As organisms recruited to the tiles, the amount of space occupied rose from 0% to 80% in 10 weeks, with no difference between treatments in total space occupied by the communities (RMANOVA, Treatment F=0.67, P=0.42, Time F=483.21, P<0.0001, Time*Treatment F=0.42, P=0.63). In both treatments, the bryozoan *Membranipora membranacea* was the dominant space-holder, covering about 60-70% of the tiles by the 10th week, while mussels *Mytilus trossulus* contributed ~25% cover at their peak (Fig. 2.5).

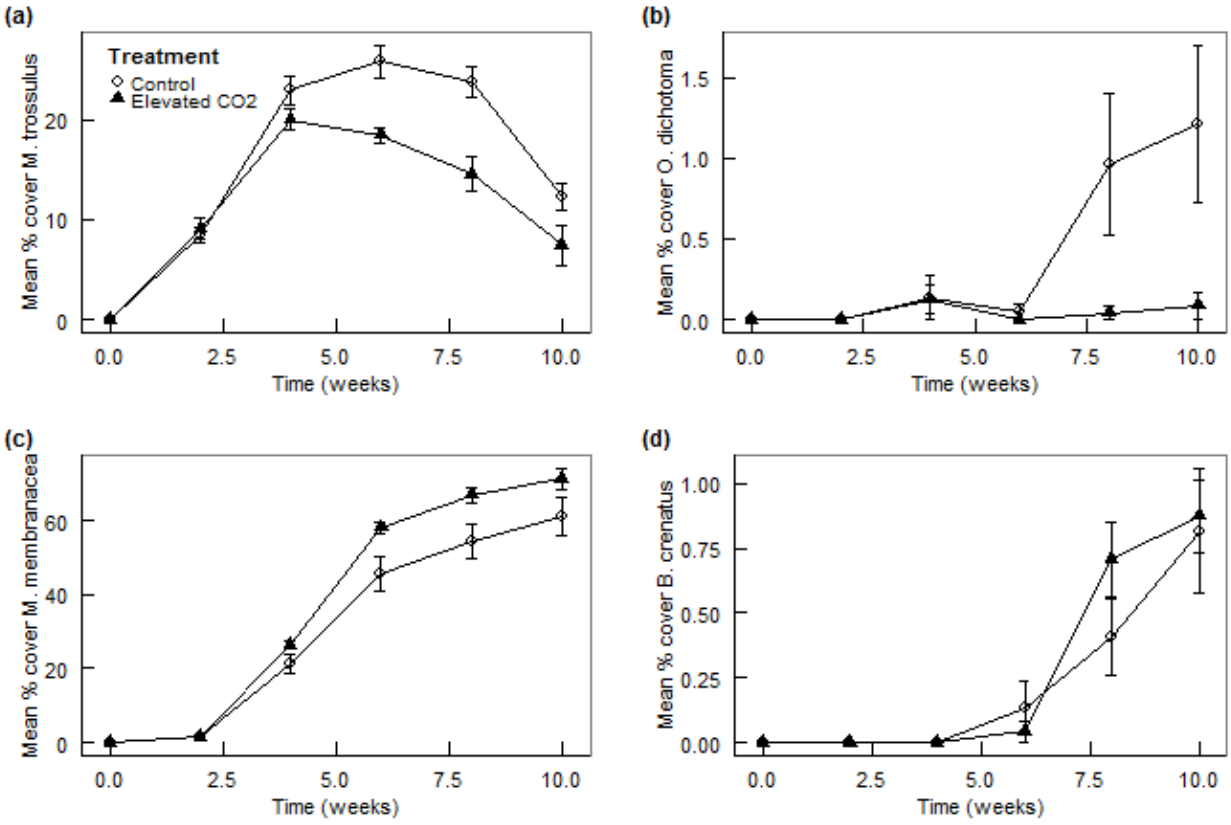

Fig. 2.5. Percent cover of *Mytilus trossulus* (a), *Obelia dichotoma* (b), *Membranipora membranacea* (c), and *Balanus crenatus* (d) in elevated CO_2 and ambient mesocosms over time. Error bars indicate standard error.

Barnacles and hydroids occurred in low numbers on almost all tiles, while both colonial and solitary tunicates occurred in low numbers on only a few tiles. Mussel cover was initially similar between treatments, but differences among treatments began to emerge after several weeks and mussel cover was decidedly lower in the elevated CO_2 treatment over the second half of the experiment (RMANOVA, Treatment F=12.31, P=0.0021, Time F=67.48, P<0.0001, Time*Treatment F=6.01, P=0.0008, Fig. 2.5a). After 10 weeks, mussel cover had decreased by almost 40% and there were over 30% fewer mussels recruited per tile in the elevated CO_2 conditions compared to ambient conditions (GLMM, z=-2.37, P=0.018, Appendix A, Fig. S.2.1a). Acidification did not alter the average size of mussels per tile (GLMM, z=-0.86, P= 0.39, Appendix A, Fig. S2.2a), nor the size of the largest 5% of mussels per tile (GLMM, z=-1.58, P=0.11, Appendix A, Fig. S.2.2b), but did reduce the size of the single largest mussel on each tile (GLM, z=2.32, P=0.031, Appendix A, Fig. S2.2c). The variance of mussel size per tile was not different between treatments (GLM, z=0.98, P=0.34, Appendix A, Fig. S2.2d), nor was the summed dried biomass of mussels (QR (tau=0.5), t=-0.052, P=0.96, Appendix A, Fig. S2.1b).

Total cover of the hydroid *Obelia dichotoma* remained low throughout the experiment (averages between 0 and 1.5 %). Nevertheless, cover of hydroids was significantly reduced by the end of the experiment in the elevated CO_2 treatment (RMANOVA, Treatment F=6.21, P=0.021, Time F=4.87, P=0.011, Time*Treatment F=4.30, P=0.018, Fig. 2.5b).

Bryozoan colonies covered more space under acidified conditions over the course of the experiment with differences becoming apparent after six weeks (RMANOVA, Treatment F=5.19, P=0.033, Time F=470.54, P<0.0001, Time*Treatment F=4.91, P=0.015; Fig. 2.5c). By the 10^{th} week, colonies in the elevated CO_2 treatment covered 10% more of the total space

than those in the ambient treatment (71% and 61%, respectively). At the 10th week, there were similar numbers of healthy (non-senescent) bryozoan colonies per tile in both treatments (mean 18.1 vs. 20.9 for ambient and elevated CO_2, respectively; GLMM, z=0.98, P=0.33; Appendix A, Fig. S2.3a) but there were fewer senescing colonies in the ambient CO_2 treatment (mean 0.9 vs. 2.3; GLMM, z=3.33 P=0.0009; Appendix A, Fig. S2.3b). The proportion of senescent bryozoans in the elevated CO_2 treatment was more than double that in ambient conditions (mean 5.0 % vs. 10.9% for ambient and elevated, respectively; GLMM, z= 2.91, P=0.0036). The size of young *M. membranacea* colonies was not different between treatments over the first four weeks (RMANOVA, Treatment F=0.42, P=0.66, Time F=516.41, P<0.0001, Time*Treatment F=0.74, P=0.39, Appendix A, Fig. S2.3c). After 4 weeks, colonies began to collide and compete for space, and growth could no longer be reliably quantified.

Acidification had no effect on barnacle *Balanus crenatus* percent cover over time (RMANOVA, Treatment F=0.31, P=0.58, Time F=28.53, P<0.0001, Time*Treatment F=1.09, P=0.34, Fig. 2.5d), number of live barnacles (GLMM, z=0.71, P=0.48, Appendix A, Fig. S2.4a), number of dead barnacles (GLMM, z=1.95, P=0.051, Appendix A, Fig. S2.4b), nor the proportion barnacles that had died by the end of the experiment (GLMM, z=1.42, P=0.15). Furthermore, maximum barnacle size after 10 weeks was not affected by acidification (GLMM, z=0.05, P=0.96, Appendix A, Fig. S2.4c).

2.4. Discussion

I demonstrated that an experimental increase in CO_2, with the accompanying reduction in seawater pH, can result in significant shifts in community diversity and structure. In addition to changes in community structure (nMDS), elevated CO_2 reduced community variability (permDISP), resulting in more similar biofouling communities from one experimental tile to the next both among and within the acidified mesocosms. This indicates that there was a wider variety in the potential assemblages that could form under ambient conditions, whereas the potential community outcomes for any given tile under acidification was more restricted. The observed community structural changes were primarily attributable to differences in relative abundances of recruited species, as species richness and total use of free space were similar between treatments. The communities under acidified conditions typically had lower abundances of mussels and hydroids (three-dimensional and erect) and higher abundance of bryozoans (flat, primarily two-dimensional) compared to communities in ambient conditions. These community level changes could be the sum of both direct impacts on recruitment, growth, and space occupancy of single species and any indirect effects of CO_2 resulting from shifts in interactions among species (Garrard *et al.*, 2014; Gaylord *et al.*, 2015). Fouling communities have a long history of use as model systems for studying emergent community patterns (e.g., stability, Sutherland, 1974) and although community structure and composition can be sensitive to environmental conditions including temperature and salinity (Dijkstra *et al.*, 2010; Needles & Wendt, 2013), information on the effects of OA on fouling communities remains limited. To date, only a single study has specifically examined OA effects on a fouling community; Peck *et al.* (2015) monitored colonization of Mediterranean fouling species into indoor mesocosms that maintained stable (as opposed to naturally fluctuating) pH, temperature, food, and regular cycles

of light. Notably, they found that community composition changed significantly under acidification, with lower cover of calcified spirorbids and increased cover of soft-bodied ascidians under low pH conditions (Peck *et al.*, 2015).

2.4.1. Efficacy of CO_2 Manipulations

Whole-community manipulations provide insight for a suite of ecological properties including the effects of OA on single species within a community context, on the outcome of species interactions, and on community-level measures like diversity and function. Several community-level OA studies have taken advantage of either the feasibility and degree of control offered by the laboratory setting (Dashfield *et al.*, 2008; Russell *et al.*, 2009; Widdicombe *et al.*, 2009; Hale *et al.*, 2011; Hofmann *et al.*, 2012), or the natural setting of existing spatial pH gradients at volcanic vent sites (Kroeker *et al.*, 2011, 2013b, 2013c, Porzio *et al.*, 2011, 2013; Johnson *et al.*, 2013). However, both the lack of realism in laboratory studies and extremely high temporal variability in pH (Hofmann *et al.*, 2011; Kroeker *et al.*, 2011), issues with pseudoreplication (Wernberg *et al.*, 2012), and potential for confounding with other biologically important factors (e.g., metal contamination, Boatta *et al.*, 2013) at vent sites pose notable problems.

Some of the weaknesses of laboratory and vent studies can be overcome with properly replicated field-based approaches, which are increasingly viewed as essential to progressing experimental marine climate science (Wernberg *et al.*, 2012). Field-based mesocosm experiments can incorporate natural variability in pH, which is important for capturing realistic responses from organisms with high plasticity (Shaw *et al.*, 2013a; Johnson *et al.*, 2014). In my experiment, the CO_2 manipulation reduced pH by ~0.35 pH units, a shift similar to that predicted to occur by the end of this century (0.3 – 0.5 pH units, IPCC, 2013) and already within the range of shallow

water (< 10 m) variation in coastal systems – including coastal British Columbia – due to inputs from coastal runoff, primary production / respiration, and upwelling (Feely *et al.*, 2008; Bednaršek *et al.*, 2014). Natural fluctuations in pH, temperature, and salinity were conserved in the mesocosms and for these environmental parameters, the water in ambient mesocosms was very similar to source water. Furthermore, the natural setting and length of my experiment allowed us to capture seasonal (summer to autumn) change in organism response to CO_2 (Godbold & Solan, 2013; Campbell & Fourqurean, 2014).

There are, of course, limitations to experimental manipulation in mesocosms. Reference tiles placed outside the mesocosms developed with similar trajectories and species compositions, although higher biomass, than those inside the mesocosms (N. Brown *pers. obs.*). Water flow in the mesocosms did not mimic natural hydrodynamics of the marina; communities outside the mesocosms likely experience higher average mass flux rates and tidally-driven variation in current velocities not present within the mesocosms. Lower flow rates within mesocosms may have reduced larval supply and phytoplankton delivery, potentially limiting recruitment, growth, and survival of recruiting species. In addition, bubbling within the mesocosms could have affected settlement or development of some species. Furthermore, because acidification only occurred within the confines of the mesocosm, both propagules and food entering the mesocosms were not exposed to high CO_2 prior to their arrival, and as such the effects of acidification on the settlement process and food quality may not be fully captured. Finally, my relatively short-term experiment necessarily exposed organisms to acute changes in pH and did not take into account transgenerational effects of CO_2 nor the potential for adaptation, both of which are important for making realistic predictions about species response to climate change (Sunday *et al.*, 2014; Rodríguez-Romero *et al.*, 2015). In all field experiments, there is a tradeoff between realism and

feasibility and despite some limitations, my design allows us to observe natural community assembly under manipulated but naturally varying conditions.

2.4.2. Direct and Indirect Effects of CO_2 on the Community

One of the most ecologically important calcified taxa in northeast Pacific fouling communities are mussels in the genus *Mytilus*. Although mussels have heavily calcified shells, research on the effects of OA on *Mytilus* spp. has yielded mixed results, suggesting that there may be species, population, and ontogenetic differences in growth responses to ocean acidification within the genus. There is evidence that acidification slows somatic growth rates in both *M. trossulus* and closely related *M. edulis*, *M. californianus*, and *M. galloprovincialis* larvae (Kurihara *et al.*, 2008; Gazeau *et al.*, 2010; Gaylord *et al.*, 2011; Sunday *et al.*, 2011) and scope for growth and weight in juvenile *M. chilensis* (Navarro *et al.*, 2013; Duarte *et al.*, 2014). However, some studies found neutral (*M. galloprovincialis*, Range *et al.*, 2012) or positive effects of acidification on somatic growth rates of *Mytilus* juveniles (*M. edulis*, Thomsen *et al.*, 2013). In my experiments I did not find differences in biomass, nor size of juvenile *M. trossulus* (both average size and size of the largest 5%) that recruited into the mesocosms. The single largest mussel from each mesocosm was significantly smaller in the elevated CO_2 treatment, indicating that pH-driven size limitation might still be important, at least at the upper limits of growth for this species. However, the ecological relevance of this result is questionable, especially given that there is a higher probability of finding a large individual on a tile with more mussels (i.e., in the control treatment, which tended to have more mussels). Given the ecological importance of *Mytilus* spp. (Buschbaum *et al.*, 2009), a better understanding of how mussels will perform in the face of OA in the field is a research priority.

There were significantly fewer mussels in the elevated CO_2 treatment than in the ambient treatment after six weeks and continuing until the end of the experiment. This pattern may have been due to pre-settlement impairment of larvae, pre-settlement avoidance based on chemical cues emitted from settled organisms after the sixth week, and/or early post-settlement mortality of juveniles. Although residence time of larvae may not have been sufficient to result in physiological impairment (water turnover time ~15 minutes), settlement rates could have been reduced in larvae unable to recover from the acute pH shock. Under a longer larval exposure period I might expect to see settlement effects on larvae and carry-over effects on recruitment (Ross *et al.*, 2011). Indirect effects may also have been important; settling larvae are often sensitive to the resident community (Osman & Whitlach, 1995), which shifted due to acidification, and ocean acidification can affect larval settlement behavior (e.g., in corals) via response to altered chemical cues from settlement substrates with associated microbial communities (Doropoulos *et al.*, 2012a; Doropoulos & Diaz-Pulido, 2013; Webster *et al.*, 2013).

Early mortality of juveniles likely contributed to the observed reduction in recruitment, as peak mussel recruitment occurred before differences between treatments became apparent and mussels in both treatments declined in numbers after this point (N. Brown, *pers. Obs.*). This mortality could be due to detachment, as some studies have noted byssal threads of attached mussels weakening and breaking in response to OA (O'Donnell *et al.*, 2013). The response to elevated CO_2 during the second half of the experiment could also reflect seasonal change in the interaction between CO_2 and other environmental factors (Reum *et al.*, 2014; Breitburg *et al.*, 2015). Shifts in temperature (Harvey *et al.*, 2013), hypoxia (Gobler *et al.*, 2014), salinity (Zhang *et al.*, 2014), and food availability (Thomsen *et al.*, 2013) can all modulate the sensitivity of organisms to acidification. Seasonal changes in these variables may have magnified responses to

CO_2 in the last half of the experiment as organisms passed physiological thresholds of pH (Shaw *et al.*, 2013b). In addition to changes in environmental variables, seasonal effects could include changes in larval supply and quality, although variation in the latter are poorly understood for this seasonal estuarine system.

Percent cover of the hydroid *Obelia dichotoma* was significantly reduced after eight weeks in the elevated CO_2 treatment. Although rare during my experimental period, populations of *O. dichotoma* can be abundant and important members in these fouling communities (Standing, 1976). This is the first report of the effects of acidification on hydrozoans and further study is needed to elucidate the mechanism behind the observed response to elevated CO_2.

Percent cover of bryozoan colonies (*Membranipora membranacea*) increased under acidified conditions over time, with differences becoming apparent after six weeks. As there was no difference in the total number of colonies at the tenth week and no detectable differences in growth (by week 4, in the absence of intraspecific competition for space), the observed percent cover increase could be caused by release from competition with other organisms negatively affected by OA (e.g., mussels). Although seemingly neutral direct effects of OA early in the experiment and positive net effects of OA by week 10 may be surprising since *M. membranacea* is a calcified bryozoan, results from other calcified encrusting bryozoan species suggest CO_2 enrichment can enhance growth (e.g., *Electra pilosa*, Saderne & Wahl, 2013). Allocation of energy to rapid growth may be at a cost to other processes, e.g., defense in *Schizoporella errata* (Lombardi *et al.*, 2011). Although *M. membranacea* cover was higher in acidified treatments, I found a higher proportion of senescing *Membranipora* colonies in the acidified treatment, suggesting a fitness cost. This negative effect (likely direct), taken together with the positive effects of OA on bryozoan cover (potentially indirect), demonstrates the complexity of OA

effects on a functioning community. More work will need to be done to understand both the balance and the mechanisms of these effects. *M. membranacea*, although native to the Pacific coast, is a common invader elsewhere, with particularly important impacts on kelps where it can weaken blades, making kelps more susceptible to mechanical damage (Krumhansl *et al.*, 2011) and inhibit native herbivores (O'Brien *et al.*, 2013). Therefore, if the net outcome for *M. membranacea* is positive, the ecological impacts of *M. membranacea* as an invasive species might be enhanced as a result of ocean acidification.

Not all members of the biofouling community responded to ocean acidification. Neither recruitment (percent cover and number) nor final size of barnacles (*B. crenatus*) was affected by acidification. There was a non-significant trend towards a higher number of dead barnacles (empty shells) on the high CO_2 tiles when compared to those under ambient conditions. The trend in barnacle survival could be caused both by direct (e.g., physiological) and indirect effects (e.g., increased predation by nudibranchs, flatworms) of OA and warrants further investigation. In general, though, barnacles in this study and others appear to be resistant to the effects of OA (e.g., *Balanus glandula* & *Semibalanus cariosus* (Wootton *et al.*, 2008), *Amphibalanus improvisis* (Pansch *et al.*, 2012) and *A. amphitrite* (McDonald *et al.*, 2009), *Semibalanus balanoides* and *Elminius modestus* (Findlay *et al.*, 2010b)). However, not all barnacle populations show high resistance to acidification and some may be susceptible to shortened larval duration (Pansch *et al.*, 2012) and reduced growth rates (Findlay *et al.*, 2010a, 2010b), both of which could have ecosystem-level repercussions.

Overall, my results suggest that responses to acidification include a combination of direct effects on particular species and indirect effects mediated by changes in species interactions, particularly competition. Although communities did not reach 100% cover in either treatment, competition

for space is thought to become important at ~50% cover for sessile benthic species such as barnacles (Menge, 2000). Furthermore, I observed both intraspecific (e.g., bryozoan colonies colliding) and interspecific competition (e.g., mussels recruiting on top of bryozoan colonies) during the experiment. Therefore, it is likely that the differences I observed can be attributable to a combination of changing competitive outcomes under OA, as observed by others (e.g., Russell *et al.*, 2009; Kroeker *et al.*, 2012; Connell *et al.*, 2013) and direct effects of pH on specific species.

2.4.3. Conclusions

Contrary to the widely stated prediction (Wittmann & Pörtner, 2013), some calcified species were robust to changes in pH (barnacles, bryozoans), although others did poorly for some but not all performance metrics (mussels). Although calcification is clearly a key component in sensitivities of various taxa to acidification (Wittmann & Pörtner, 2013), I did not find the reliance on calcified structure to be a strong predictor variable in this species-poor biofouling community. However, the trend towards simplified, homogenous communities among tiles in the acidified mesocosms observed here is consistent with previous work (Kroeker *et al.*, 2013c) which noted similar homogeneity among algal communities at low pH sites. This trend towards simplification may underlie the transformation from communities with erect or 3-dimensional species (like mussels and hydroids) to communities dominated by prostrate species (e.g., bryozoans), which may have implications for habitat complexity and associated species diversity (Fabricius *et al.*, 2014). A growing concern for these ecosystems made up of low diversity, homogenized communities is the potential of compromised compensatory responses and reduced functional breadth (Gaylord *et al.*, 2015). My results add to a small but growing list of studies

that have identified changes in community diversity and structure in response to elevated CO_2 (Hale *et al.*, 2011; Kroeker *et al.*, 2013c; Campbell & Fourqurean, 2014). The acidification-induced shifts in diversity and species composition observed in this and other studies would have been difficult to predict on the basis single-species studies alone. Community-level studies such as this one are needed to link information at the individual and population levels, which make up the majority of climate change experiments, to ecosystem level impacts (Harley *et al.*, 2006; Wernberg *et al.*, 2012). With an improved understanding of how interconnected assemblages of species will respond to ocean acidification, we will be better positioned to understand and anticipate changes ranging from the distribution and abundance of species to the functioning of entire ecosystems (Widdicombe *et al.*, 2009; Kroeker *et al.*, 2013b).

Chapter 3: Natural Acidification Changes the Timing and Rate of Succession, Alters Community Structure, and Increases Homogeneity in Marine Biofouling Communities

3.1. Introduction

Anthropogenic carbon dioxide enrichment of the atmosphere and subsequent decreases in pH in the ocean are well documented (Feely *et al.*, 2004; Tans, 2009). The rate of change of ocean pH is unprecedented in recent geological history (Hönisch *et al.*, 2012) and the biological implications of these rapid chemical changes are being realized across a wide range of taxa (Kroeker *et al.*, 2013a; Wittmann & Pörtner, 2013). In coastal marine ecosystems, changes in seawater CO_2 are occurring on a number of different timescales: diurnally with photosynthesis and respiration, days to weeks with the lunar cycle and upwelling dynamics, seasonally due to both biotic and abiotic forcing, and over many decades with anthropogenic forcing (Hofmann *et al.*, 2011; Boyd *et al.*, 2016; Henson *et al.*, 2017). It is clear that incorporating natural fluctuations in CO_2 is necessary for making better predictions of the biological response to ocean acidification (Shaw *et al.*, 2013a; Small *et al.*, 2015; Boyd *et al.*, 2016). Although a few studies have addressed this point at diurnal timescales (e.g., Clark & Gobler, 2016; Li *et al.*, 2016), it is unclear how marine ecosystems respond to longer term (weeks to months) CO_2 fluctuations and whether these effects can be reversed (transient vs. persistent effects) if CO_2 stress is relieved (but see Vaz-Pinto *et al.*, 2013).

In communities influenced by disturbance (i.e., most communities), the effects of fluctuating CO_2 are mediated by direct effects of acidification on settlement and recruitment and by indirect effects mediated by the interactions between early and later successional species. Ocean acidification can influence settlement of planktonic stages and recruitment into benthic populations (Cigliano *et al.*, 2010; Brown *et al.*, 2016), and resident organisms influence

subsequent settlement (i.e. secondary colonization, Osman *et al.*, 1989). However, the importance of colonization history and priority effects in shaping community structure and succession in light of CO_2 or pH heterogeneity at a variety of temporal scales has yet to be addressed. One challenge to understanding the effects of acidification on the succession and development of marine communities is that, to date, our understanding of biological effects of ocean acidification is primarily informed by studies of single species in isolation. Such studies show how ocean acidification might influence organisms through changes in energetic demand (Garilli *et al.*, 2015; Harvey *et al.*, 2016), reproduction and development (Ross *et al.*, 2011), growth rate (Kroeker *et al.*, 2013a), development of defensive structures (Sanford *et al.*, 2014), and behaviour (Milazzo *et al.*, 2016). Extrapolating from single-species studies to assess the effects of ocean acidification on community development, structure, and function requires understanding how changes propagate across these scales, and often the links between performance, demography and community structure are simply not known (but see Enquist *et al.*, 2015). To anticipate ecosystem-level changes, it is essential to understand responses of multi-species assemblages to acidification. Early studies exposed pre-settled communities to acidification in laboratory conditions (e.g., Hale *et al.*, 2011), but deeper understanding of recruitment and settlement processes requires *in-situ* CO_2 manipulation (e.g., Brown *et al.*, 2016) or observation where continued recruitment can occur.

Shallow water CO_2 seeps allow the study of intact communities and have been increasingly used as natural laboratories, providing insights into the community- and ecosystem-level effects of acidification. Changes in community composition, structure, and losses in diversity have been documented along natural CO_2 gradients for both macro-algal (Kroeker *et al.*, 2011; Porzio *et al.*, 2011; Johnson *et al.*, 2012; Connell *et al.*, 2013; Baggini *et al.*, 2014, 2015; Linares *et al.*, 2015)

and macroinvertebrate (Hall-Spencer *et al.*, 2008; Cigliano *et al.*, 2010; Fabricius *et al.*, 2011, 2014; Donnarumma *et al.*, 2014; Goodwin *et al.*, 2014) communities. A consistent pattern of community change over decreasing pH gradients is reduced abundance and diversity of calcifying taxa (e.g., coralline algae, molluscs) and increased dominance by non-calcified species (e.g., fleshy brown algae, anemones). These patterns are driven by a combination of direct effects on growth and survival, such as the dissolution of calcareous shells/skeletons combined with higher energetic costs associated with calcification (Wittmann & Pörtner, 2013), and effects mediated by demography and species interactions such as changes in competition, predation and habitat structure (Connell *et al.*, 2013; Kroeker *et al.*, 2013c; Linares *et al.*, 2015; Sunday *et al.*, 2017). Although community-level outcomes of ocean acidification (and the species interactions that underlie them) have been documented for a wide range of marine communities, the effects of CO_2 on recruitment, succession and development have been mainly investigated in algal communities (Kroeker *et al.*, 2011; 2012; 2013b) and similar studies are lacking for marine invertebrates (although see Brown *et al.*, 2016). Furthermore, the effects of the timing and duration of acidification events during succession have seldom been addressed, although the response of marine communities to acidification has been shown to change with seasonality (e.g., Baggini *et al.*, 2014) and with timing and length of upwelling events (Iles *et al.*, 2012).

In this study, I observed how benthic marine invertebrate communities recruited and developed along a natural CO_2 gradient. I used reciprocal transplant experiments to determine if fouling communities would reflect a lasting response to short but discrete exposure to elevated CO_2 (e.g., mimicking an upwelling event) in early succession or reflect only most recent exposure to elevated CO_2 regardless of prior conditions. Based on succession theory, I expected that CO_2 would disrupt succession by altering recruitment of primary vs. secondary colonizers and alter

the interactions among these taxa via inhibition or facilitation (Connell & Slatyer, 1977). Further, I predicted that the relative importance of discrete exposure early on in succession, continuous exposure throughout succession, or exposure later in succession on abundance would depend on species' life history traits. For example, if timing of recruitment coincides with timing of acidification, a discrete exposure to CO_2 that occurs early on in succession may influence recruiting primary colonizers more than secondary colonizers that are not reproducing and/or recruiting at the time of acidification. Other factors, like environmental tolerances, growth rate, ability to induce a resting stage, and concentration of propagules may determine how a given taxa responds to and/or rebounds from a short and discrete CO_2 event. At the community level, I hypothesized that acidification would result in homogenized, low-diversity communities of biofouling organisms, dominated by a few weedy species with notable reductions in calcified taxa.

3.2. Materials and Methods

3.2.1. Site Description and Experimental Design

The study was conducted in Levante Bay on Vulcano Island (38°25′08″N, 14°57′40″E) in the Aeolian archipelago in Northeastern Sicily (Italy) from March to June, 2013 (12 weeks). At this site, shallow volcanic seeps emit carbon dioxide bubbles that create a gradient in seawater carbonate chemistry that has been characterized by a number of recent studies (Arnold *et al.*, 2012; Johnson *et al.*, 2012; Lidbury *et al.*, 2012; Boatta *et al.*, 2013; Calosi *et al.*, 2013a; Milazzo *et al.*, 2014). The biogeochemistry of the bay has been assessed to identify the most suitable areas for ocean acidification research (see Boatta *et al.*, 2013; Vizzini *et al.*, 2013) and the sites chosen for this study were outside of any measured metal contamination. Variability in

the pH gradient at the site is predominantly driven by currents influenced by westerly winds, and as such, the acidified water masses mostly run parallel to the northern shoreline of the bay (Boatta *et al.*, 2013). When winds are high, e.g., during storms that are common in winter and early spring, low pH waters are more restricted to the immediate vicinity of the seeps and do not extend as far along the shoreline (Boatta *et al.*, 2013). Two such storms occurred during the study period. I used two sites, ~70 m apart, along the CO_2 gradient; the first had low mean pH (7.78), and the second had ambient mean pH (8.10) (here I use high CO_2 and low pH interchangeably). my sites correspond to sites 40-60 and 120-130 in Boatta *et al.* (2013) for low pH and ambient pH respectively. I monitored temperature, salinity, and pH at each site at least once every two weeks throughout the observation period but daily for the first week and the last six weeks of the experiment. Samples for total alkalinity were taken every two weeks. I used these parameters to calculated dissolved inorganic Carbon (DIC), CO_2, HCO_3^-, and carbonate and aragonite saturation states using the CO2-SYS program (Pierrot *et al.*, 2006).

At each site, I suspended 70.5 × 70.5 cm semi-flexible PVC panels from buoys 1 m from the surface and 1 m from the bottom (n=3 panels per site). I deployed the panels on similar substrata at each site, which comprised of subtidal boulders and patches of seagrass. Each panel was oriented horizontally (Fig. 3.1), and 15 PVC tiles (14.5 cm x 14.5 cm) were secured to the underside of each panel using cable ties (n=15 tiles nested within each of 3 panels per site, therefore n=45 tiles per site). The panels were secured to the buoys and anchors using ropes to avoid horizontal spinning. Storms damaged some tiles and panels, so only undamaged tiles (n=20 per site, from across the three panels) were used in the analyses. After 8 weeks, I

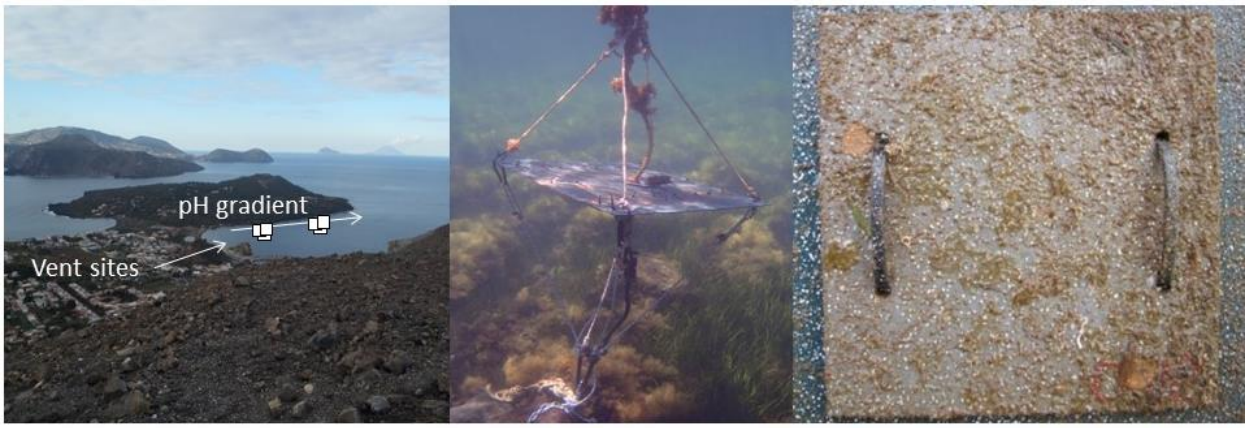

Fig. 3.1. Photographs depicting sites (white symbols) along the pH gradient (left) and the panel and tile system. Panels (centre), with downward facing PVC tiles (right) attached to the underside, were suspended ~1 m from both surface and bottom using a buoy and anchor.

reciprocally transplanted a subset of 10 tiles (selected randomly from the 20 total) from each CO_2 regime, while the other 10 tiles were not transplanted, to determine if CO_2 effects on recruitment occur early and persist or arise later during succession. Tiles from transplanted and non-transplanted groups were distributed randomly between the two panels in each site. At the time of this transplantation, I formed new panels (2 per site) with ten tiles each.

3.2.2. Species- and Community-level Measures

Photographs (one per tile) were taken every two weeks to determine changes in community composition, structure, Shannon's diversity, richness, stability over time and percent cover (point count) of primary and secondary colonizing fouling species for eight weeks. After transplantation, the tiles were left for one additional month before being photographed and retrieved for preservation. The tiles and panels were brought to the surface for photography. I

conducted all photographic analyses in ImageJ (Schneider *et al.*, 2012) and identified species to lowest taxonomic level possible in the laboratory. Primary colonizers were defined as those that recruited in the ambient site in the first four weeks, before the community reached 100% cover. Primary colonizers included two serpulid polychaete taxa (Serpulidae, Spirorbidae) and two algal guilds, a turf-forming green filamentous alga *Cladophora* sp., and a biofilm which was ubiquitous and has been described at this site as primarily a mix of diatoms and cyanobacteria, the composition of which changes along gradients of CO_2 (Johnson *et al.*, 2015). Secondary colonizers (i.e., those that recruited only after space occupancy reached 100% at week four) included bryozoans (e.g., *Schizomavella* sp. and *Patinella radiata*), ascidians (*Botryllus* sp. and *Diplosoma* sp.), and *Sphacelaria* sp., which is a branched brown alga. These secondary colonizers may require a layer of biofilm in order to recruit, or their propagules may have only been in the water column during the latter half of the experiment due to seasonal or episodic reproductive patterns. Natural succession in marine communities often coincides with a disturbance regime (e.g., winter storms, sedimentation) that creates space (Sousa, 1979a). The disturbance regime in Vulcano Bay may coincide with seasonality, as summer time systems recover from winter storms, and I expect that the succession observed from bare surfaces reflected both directional community development and response to warming temperatures over time.

3.2.3. Statistical Analyses

All statistical analyses were performed using open-source R (R Development Core Team, 2009). I used the *adonis* and *Betadisper* functions in the Vegan package (Oksanen *et al.*, 2015a) to analyze multivariate community structure (PERMANOVA test) and homogeneity of dispersions,

respectively. Community structure analyses were conducted on species abundance data (percent cover) of a given tile, standardized by total abundance of that species across all tiles, putting abundances of ecologically different species on the same scale. This community structure metric was calculated twice: pre-transplant using data from week 8, and post-transplant using data from week 12. I then calculated a Bray Curtis dissimilarity matrix on the standardized data. In all tests, for a given week, I used site as a fixed factor and separately tested for the effect of panel within site, and if initial or final panel (nested within initial or final site, respectively) had a significant effect, I included the term in the full model. To test if acidification influenced community structure at week 12 I used the 10 non-transplanted replicates per site. Next, to analyze if, during the reciprocal transplant, initial or final site or their interaction influenced community structure, I used all 20 tiles (10 of which had been transplanted from the other site). I calculated pre- and post-transplant community stability, the temporal mean over temporal variability of species abundances in a given tile, using the *community.stability* function in the *codyn* package (Hallett *et al.*, 2016).

I used both linear mixed effects models (LMEs, lme4 package, Bates *et al.*, 2015) and generalized liner mixed-effects models (GLMMs, glmmADMB package, Fournier *et al.*, 2012; Skaug *et al.*, 2013) to analyze percent cover and count data (from photographs), community stability, and proportion of secondary colonizers. For the first 8 weeks (pre-transplantation), I used site as a fixed effect (n=20 per site), after which (post-transplantation) I used initial site, final site, and their interaction as fixed effects to incorporate tiles that were reciprocally transplanted (n=10 per site) and those that were not (n=10 per site). For a given point in time, tile was the level of replication and I treated both initial and final panels nested within site as random factors and all models included a random intercept. Numerical response variables (species

richness, cumulative counts) were considered either normally distributed and analyzed with LMEs or Poisson or negative binomially distributed and analyzed with GLMMs, to assess effect of site, depending on distribution fit to the data. I assumed either a Beta or Binomial distribution (based on fit of distribution to data) for percent cover data and analyzed the effect of site with GLMMs. I used a GLMM with assumed Gamma distribution to analyze the effect of acidification on Shannon's diversity.

3.3. Results

3.3.1. Seawater Parameters

Seawater pH fluctuated with time of day and wind direction. However, pH was consistently lower in the low pH site compared to the ambient site (ΔpH_{NBS} = - 0.32 ± 0.19, mean ± SE, daily differences between sites averaged across experimental period, Table 3.1, LM, F=141.7, P<0.0001). Total alkalinity was similar across sites at a given time point (ΔTA = 3.45 ± 1.38, Table 3.1, LM, F=3.17 P=0.08). During the experimental period, temperatures ranged from 14.4°C to 20.8°C and salinity from 37.8 to 38.6. Differences in temperature (Δtemperature =0.01°C ± 0.15°C, LM, F=0.0019, P=0.97) and salinity (Δsalinity = 0.00 ppt ± 0.01 ppt, LM, F=0, P=1) were negligible between sites during the survey.

Table 3.1. Carbonate chemistry of seawater from ambient and low pH sites. Temperature, salinity, $pH_{NBS,}$ and total alkalinity were collected from March to June 2013 (mean ± SE, n = 98). Asterisks indicate calculated values in the CO2-SYS program (Pierrot *et al.*, 2006).

Seawater parameter	Control	Low pH
Temperature (°C)	18.96±0.15	18.97±0.15
Salinity	38.19±0.011	38.19±0.010
pH_{NBS}	8.10±0.13	7.78±0.24
Alkalinity (μmol kg^{-1})	2523.52±1.34	2526.97±1.41
pCO_2 (μatm)*	557.69±26.48	1499.91±151.71
DIC (μmol kg^{-1})*	2271.16±6.42	2424.09±10.38
HCO_3^- (μmol kg^{-1})*	2066.59±9.62	2270.29±10.15
Ω Calcite*	4.33±0.096	2.43±0.093
Ω Aragonite*	2.82±0.063	1.59±0.060

3.3.2. Primary Colonization Pre- and Post-transplant

All tiles were colonized initially by biofilm. The biofilm grew more rapidly, and peaked and declined in cover earlier on tiles at the acidified site relative to the ambient site. After eight weeks, biofilm cover was higher on tiles at the acidified site than the ambient site (Fig. 3.2a; statistics are summarized in Table 3.2). Filamentous alga, *Cladophora* sp., had higher cover at the acidified site at its peak, although by week 8 there was no difference in cover between sites (Table 3.2, Fig. 3.2c). Calcified polychaetes – serpulid and spirorbid worms – had at least a two-week delay in recruitment at the acidified site, and, by week 8, covered significantly less space (Table 3.2, Appendix B, Fig. S3.1a, c) and had less than a third the density at the acidified site relative to the ambient site (Table 3.2, Fig. 3.2e, g). Both serpulids and spirorbids would be

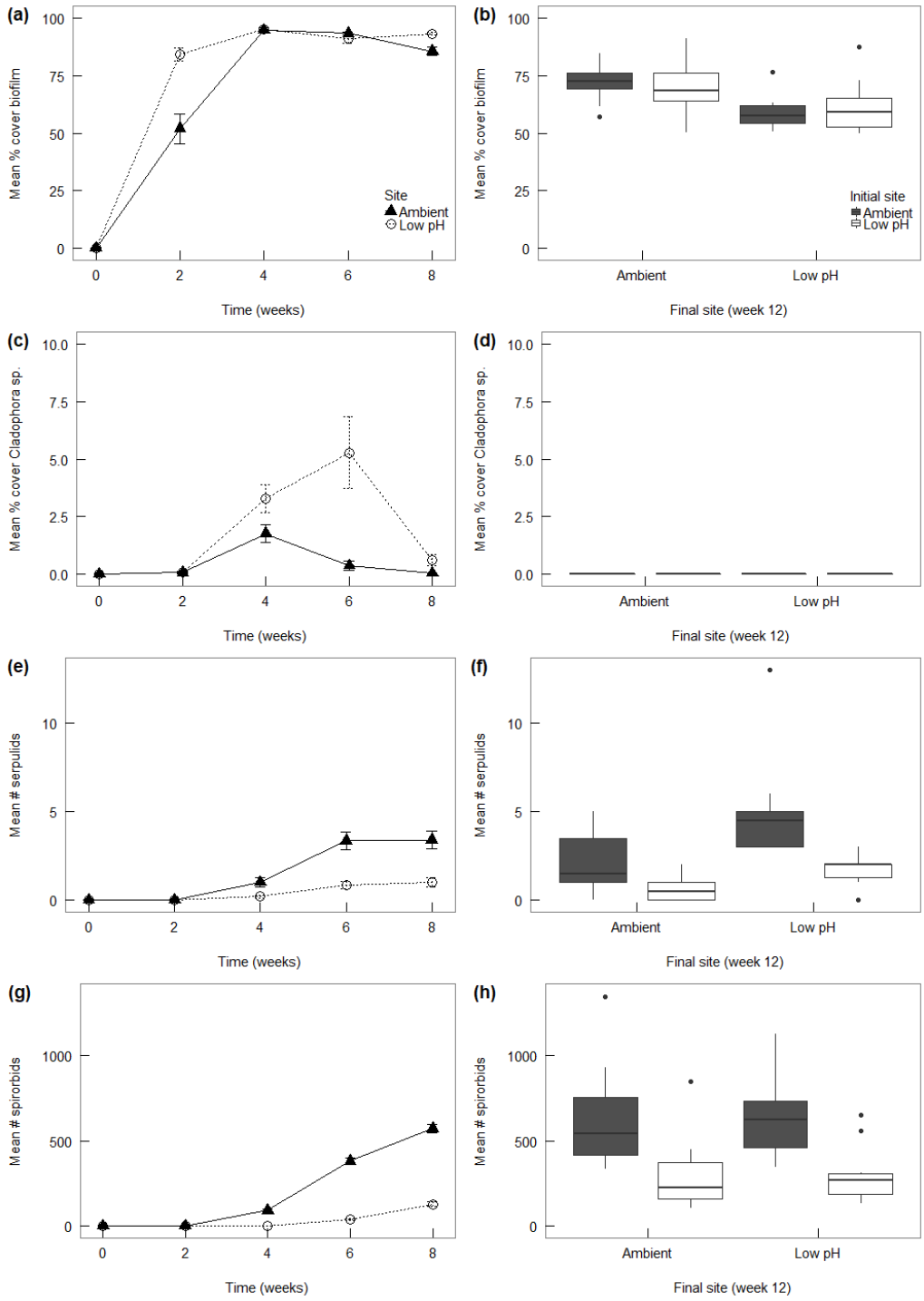

Fig. 3.2. Abundance of selected primary colonizers in ambient and low pH sites over time. Left-hand panels are trends through week 8 (i.e., pre-transplantation; n=20) and right-hand panels are patterns at week 12 (post-transplantation; n=10) of both transplanted and non-transplanted tiles. For the right-hand panels, shading indicates initial site and position on x-axis indicates final site. Species are: (a, b) biofilm (% cover), (c, d) *Cladophora* sp. (% cover), (e, f) serpulids (# individuals) and, (g, h) spirorbids (# individuals). Error bars indicate standard error.

Table 3.2. Statistical results from both GLMM (using z statistic) and LME (using X^2) from analysis of percent cover of a given species. [p] indicates week in which peak % cover of this species occurred.

Group	Species	Abundance measure	Week 8 ([p]=peak)	Mean abundance ambient site	Mean abundance low pH site	*z or* X^2*	*P* ***<0.05**
Primary colonizers	Biofilm complex	% cover	8	85.6	93.1	-4.43	**<0.0001***
	Cladophora sp.	% cover	8	0.050	0.62	-1.44	0.15
	Serpulids	% cover # individuals	8 [p]	0.52 3.4	0.30 1.0	3.33 4.81	**<0.001*** **<0.001***
	Spirorbids	% cover # individuals	8 [p]	7.06 572.3	2.05 129.1	3.05 14.4*	**<0.001*** **<0.001***
Secondary colonizers	*Diplosoma* sp.	% cover # colonies	8	5.0 2.0	2.0 0.4	1.79 5.22*	0.074 **0.022***
	Botryllus sp.	% cover # colonies	8	0.44 1.9	0.69 2.8	-1.95 -1.30	0.051 0.19
	Thin ramified bryozoan	% cover	8	0.69	0.07	2.57	**0.01***
	Patinella radiata	% cover # colonies	8	0.050 2.2	0.074 2.8	-0.19 -0.30	0.85 0.76
	Schizomavella sp.	% cover # colonies	8	0.15 1.2	0.025 0.2	0.98 2.35	0.33 **0.0019***

considered primary colonizers under ambient conditions but classified as secondary colonizers (arriving after 100% space occupancy had been reached) under acidified conditions.

Eight weeks into the experiment, a subset of tiles was reciprocally transplanted among sites. One month after transplantation, biofilm cover was related to only the most recent exposure to CO_2 (i.e. tiles maintained in or transplanted to low pH) as coverage was lower on tiles that ended up at the acidified site than those in the ambient site, (GLMM, Final site P=0.050, Table 3.3, Fig. 3.2b), regardless of origin, and there was no evidence of the pre-transplant effects of CO_2

Table 3.3. Statistical results of GLMMs using percent cover of a given species and initial site, final site and their interaction as fixed effects (n=20).

Group	Species	Initial site		Final site		Initial site * Final site	
		z	*P* **<0.05*	*z*	*P* ****<0.05***	*z*	*P* **<0.05*
Primary colonizers	Biofilm complex (% cover)	-0.40	0.69	-1.96	**0.050***	0.49	0.62
	Cladophora sp. (% cover)	0.0	1.0	0.0	1.0	0.0	1.0
	Serpulids (% cover)	-1.83	0.067	3.06	**0.0022***	-0.65	0.52
	(# individuals)	-2.51	**0.012***	3.19	**0.0014***	0.22	0.83
	Spirorbids (% cover)	-0.79	0.43	0.81	0.42	-0.47	0.64
	(# individuals)	-2.33	**0.02***	0.46	0.65	-0.17	0.87
Secondary colonizers	*Diplosoma* sp. (% cover)	0.13	0.89	0.80	0.43	1.68	0.093
	(# colonies)	-1.62	0.11	-0.80	0.42	0.82	0.41
	Botryllus sp. (% cover)	0.49	0.63	1.19	0.26	-0.48	0.63
	(# colonies)	-0.02	0.99	0.27	0.79	0.46	0.4
	Thin ramified bryozoan (% cover)	0.30	0.77	3.41	**<0.001***	-2.21	**0.027***
	Patinella radiata (% cover)	-0.20	0.84	1.38	0.17	-0.49	0.62
	(# colonies)	-0.04	0.97	-0.53	0.60	0.58	0.56
	Schizomavella sp. (% cover)	-2.59	**0.0096***	-0.70	0.49	0.29	0.77
	(# colonies)	-0.34	0.73	0.20	0.84	-0.31	0.76

carrying over (GLMM, Initial site P=0.69, Table 3.3, Fig. 3.2b). *Cladophora* sp. cover was reduced to zero after 8 weeks, therefore I was unable to determine if CO_2 was more important in early or late succession for this taxon. Transplant results suggested that serpulid recruitment was influenced by CO_2 early on and persisted, although there were also CO_2 effects present during late succession. Overall, tiles that originated at the ambient site recruited more serpulid individuals than those that originated at the acidified site, regardless of final site (GLMM, Initial site P=0.012, Table 3.3, Fig. 3.2f) and cover of this species showed a non-significant trend in the same direction (Initial site P=0.067, Table 3.3). However, by the end of the experiment, there

were more individuals and higher cover of serpulids on tiles that had final exposure to high CO_2, rather than ambient conditions (# individuals: GLMM, Final site P=0.0014, Table 3.3, Fig. 3.2f, Appendix B, Fig. S3.1b). Initial site alone influenced the number of individuals, but not cover, of spirorbids, such that tiles that originated in the ambient site had more spirorbids, regardless of their final locations (# individuals: GLMM, Initial site P=0.02, Table 3.3, Fig. 3.2h, Appendix B, Fig. S3.1d).

3.3.3. Secondary Colonization Pre- and Post-transplant

Fewer colonies of the colonial ascidian, *Diplosoma* sp., recruited by week 8 at the acidified site (Table 3.2, Appendix B, Fig. S3.1e) but these colonies covered a similar amount of space in both sites (Table 3.2, Fig. 3.3a). At the same time point, another colonial ascidian, *Botryllus* sp., had similar cover between sites (Table 3.2, Fig. 3.3c) and no difference in number of recruiting colonies between sites (Table 3.2, Appendix B, Fig. S3.1g). Bryozoans, a phylum with a broad range of morphologies, yielded mixed responses to acidification. At week 8, an erect calcitic bryozoan, *Patinella radiata*, had both similar cover (Table 3.2) and number of colonies at the acidified site compared to the ambient site (Table 3.2, Appendix B, Fig. S3.1i). Thin ramified bryozoans had higher cover under ambient conditions and were essentially absent from the acidified site (Table 3.2, Fig. 3.3e), while the encrusting aragonitic bryozoan, *Schizomavella* sp. recruited fewer colonies on tiles at the acidified site compared to the ambient site (Table 3.2, Fig. 3.3g) but had similar cover (Table 3.2) between sites at week 8.

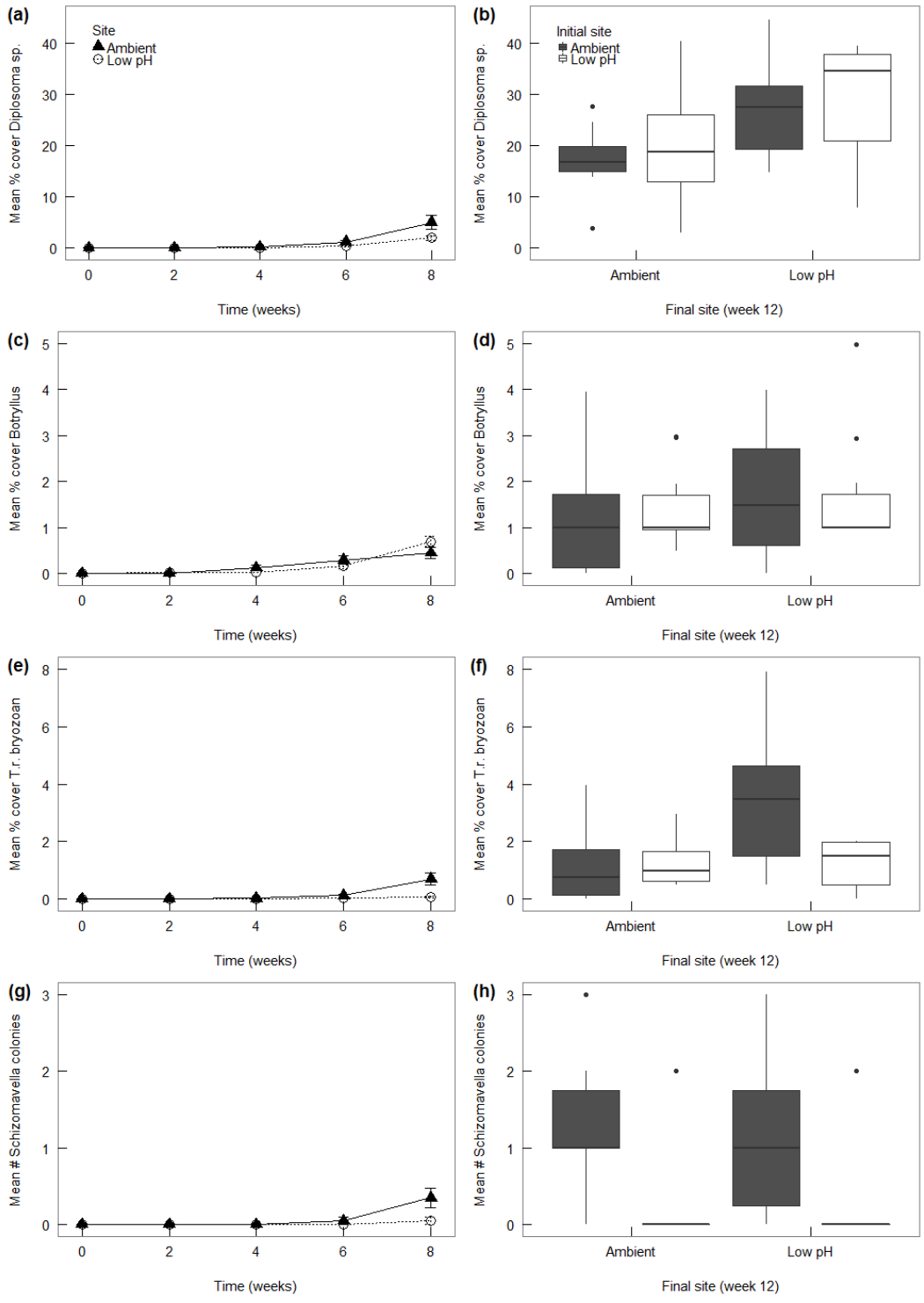

Fig. 3.3. Abundance of selected secondary colonizers in ambient and low pH sites over time. Left- and right-hand panels as in Figure 2. Species are: (a, b) *Diplosoma* sp. (% cover), (c, d) *Botryllus* sp. (% cover), (e, f) Thin ramified bryozoan (% cover) and, (g, h) *Schizomavella* sp. (# colonies). Error bars indicate standard error.

After the transplant experiment, soft-bodied ascidians appeared to be largely resistant to changes in acidification. Neither initial site nor final site influenced cover or number of *Diplosoma* sp. or *Botryllus* sp. (Fig. 3.3b,d, Appendix B, Fig. S3.1f,h Table 3.3). The bryozoan *P. radiata* remained resistant to acidification after transplantation, and there was neither an effect of initial nor final site on numbers of colonies and cover of this species (Table 3.3). Post-transplant thin ramified bryozoan cover was influenced by final site, as overall cover was higher on tiles that ended the experiment at the acidified site (Final site $P<0.001$), and this effect was especially strong for tiles transplanted from the ambient site (Initial*final site $P=0.027$, Table 3.3, Fig. 3.3f). Early successional CO_2 effects were apparent in post-transplant recruitment of *Schizomavella* sp., such that tiles that originated in the ambient site had more colonies than those from the low pH site (Initial site $P=0.0096$, Table 3.3, Fig. 3.3h).

3.3.4. Community-level Results Pre- and Post-transplant

The above changes in species recruitment and succession culminated in significant shifts in community structure (relative abundances of recruited species) on tiles at the acidified site at week 8 (PERMANOVA, $R^2=0.16$, $P=0.0001$, Fig. 3.4a). Although community structure differed, there was no difference in community variance between sites (Betadisper, $F=0.63$, $P=0.80$, Fig. 3.4a). Tiles were colonized more quickly under acidified conditions than those at the ambient site but this difference was only apparent for the first four weeks, and the tiles had similar cover at the 8^{th} week (LME, $X^2=2.16$, $P=0.14$). Pre- transplant community stability was higher for tiles at the acidified site than the ambient site (LME, $X^2=19.5$, $P<0.0001$, Fig. 3.5a), indicating that over this time period, communities at the ambient site changed more than those at the acidified site.

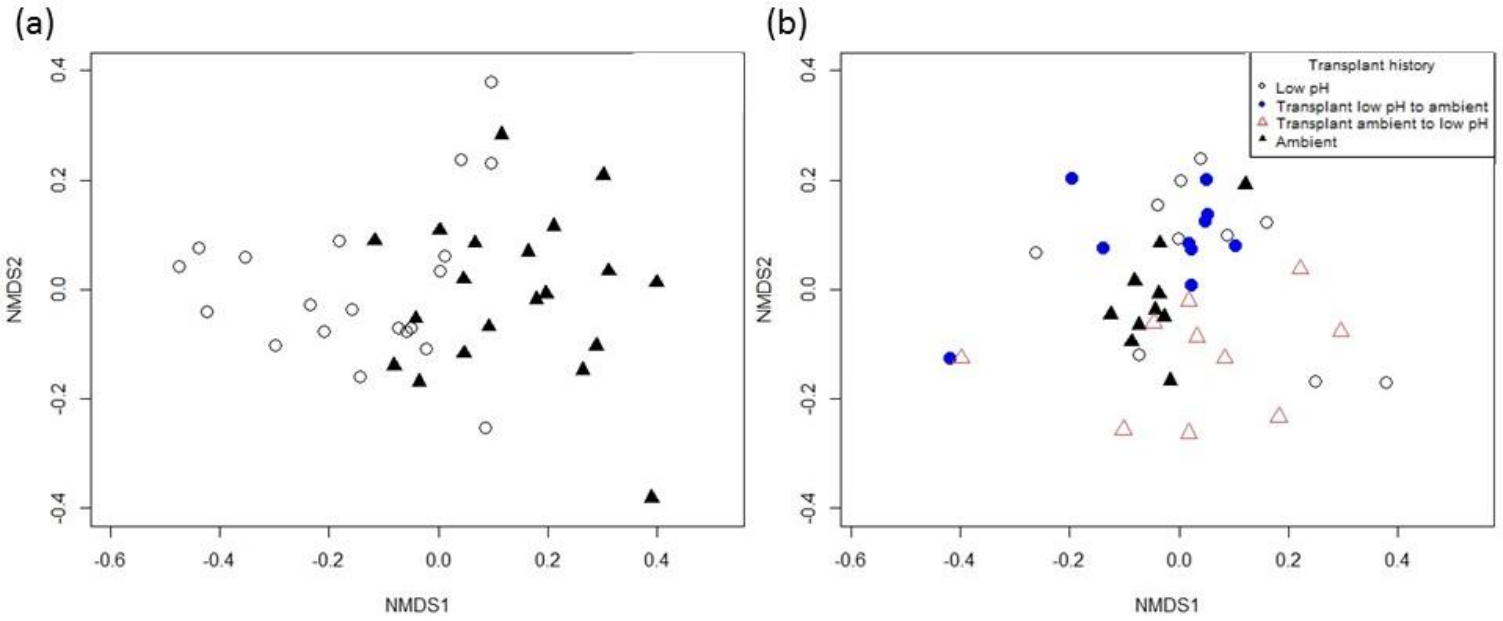

Fig. 3.4. nMDS ordination plot showing the relationship between communities after (a) 8 weeks on tiles from low pH (open circles) vs. the ambient site (solid black triangles), n = 20 tiles, and (b) 12 week on tiles that either remained in low pH (open circles), were transplanted from low pH to the ambient site (solid blue circles), were transplanted from ambient site to low pH site (red open triangles), or remained in the ambient site (solid black triangles), n = 10 tiles.

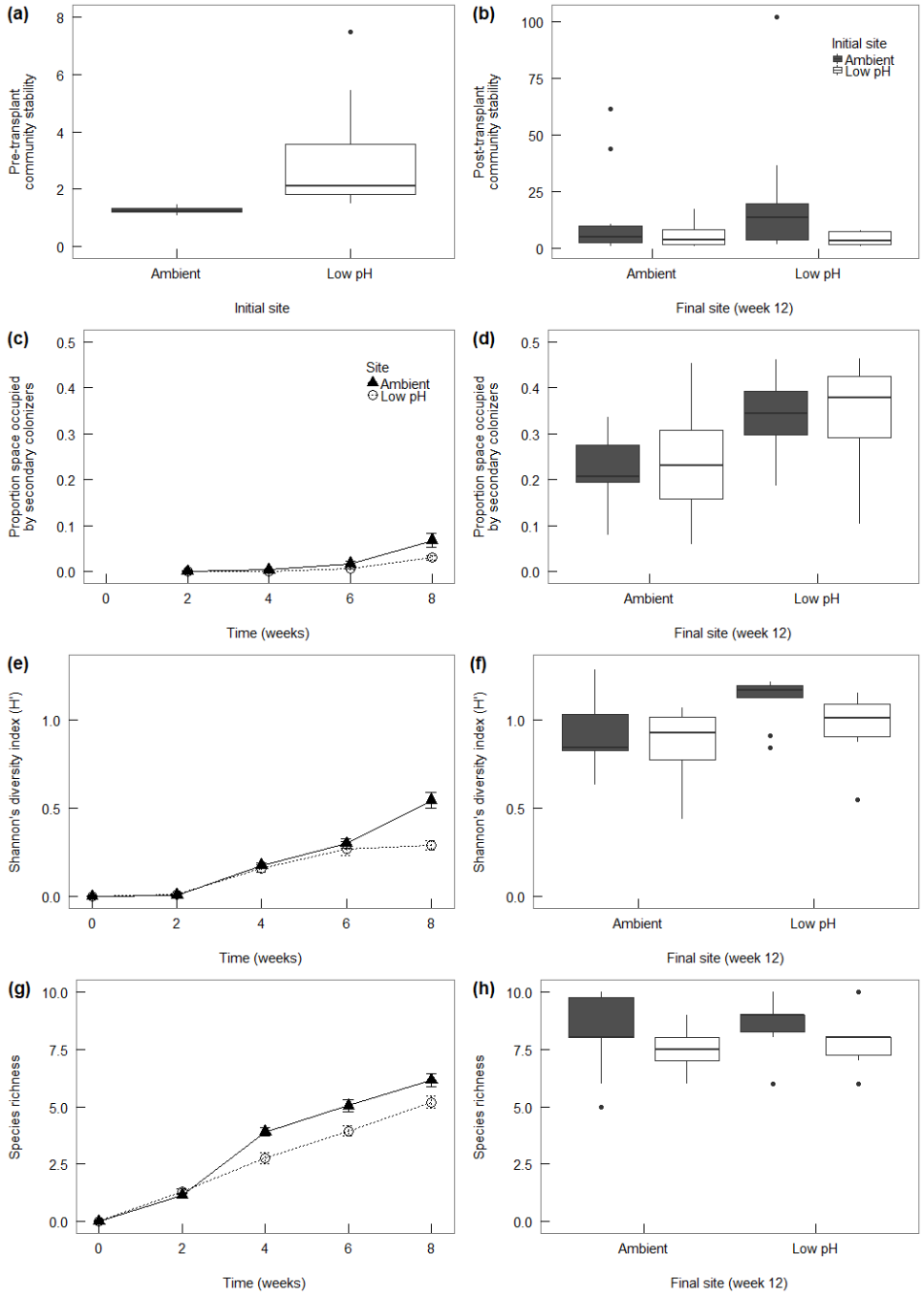

Fig. 3.5. Community-wide measures in ambient and low pH sites over time, left- and right-hand panels as in Figure 3.2, except for panel (a) which represents a cumulative measure over the first 8 weeks. Measures are: (a, b) community stability, (c, d) secondary colonizers space occupation, (e, f) Shannon's diversity, and (g, h) species richness. Error bars indicate standard error.

Secondary colonizers, which arrived after the 4th week, initially gained cover at the same rate in both sites but by the 8th week took up more space at the ambient site than at the acidified site (LME, X^2=4.04, *P*=0.044, Fig. 3.5c), indicating that succession from primary to secondary species occurred earlier at the ambient site. I observed negative effects of acidification on diversity (GLMM, z=3.41, *P*=0.00065, Fig. 3.5e), but species richness was similar between sites (GLMM, *z*=1.25, *P*=0.21, Fig. 3.5g) after 8 weeks.

After transplantation, both initial site and final site influenced community structure, i.e. communities that originated at the ambient site were different overall than those that originated at the acidified site (PERMANOVA, initial site, R^2=0.10, *P*=0.0001, Fig 3.4b: triangles vs. circles) and communities that ended at the acidified site differed from those that ended at the ambient site (PERMANOVA, final site, R^2=0.053, *P*=0.013, Fig. 3.4b: open vs. solid symbols). There was no evidence of an interaction between the effects of CO_2 on early and late succession (PERMANOVA, initial*final site, R^2=0.027, *P*=0.26, Fig. 3.4). In addition, although there was no evidence that CO_2 affected community variability early in succession (Betadisper, initial site, F=0.14, *P*=0.71, Fig 3.4: triangles vs. circles), there was an influence of final site on variability – as tiles that were transplanted to the high CO_2 site were significantly less variable than those that ended at the ambient site (Betadisper, final site, F=8.04, *P*=0.0073, Fig. 3.4: open symbols are more dispersed than solid symbols). Community stability between the 8th and 12th week was similar between sites, regardless of transplantation history (LME, final site, X^2=0.0001, *P*=0.99, initial site: X^2=2.32, *P*=0.13, initial*final site: X^2=0.94, *P*=0.33, Fig. 3.5b).

However, the proportion of secondary colonization was higher on tiles that ended under low pH conditions, regardless of origin (GLMM, final site, z=2.63, *P*=0.020, initial site: z=0.33, *P*=0.75, initial*final site: z=-0.26, *P*=0.80, Fig. 3.5d). After transplantation, Shannon diversity was

significantly higher on tiles that ended at the elevated CO_2 site (GLMM, final site: z=2.23, *P*=0.026, Fig. 3.5f), while the negative effects of CO_2 observed during early succession appeared to have no persisting influence on diversity by the end of the experiment (GLMM, initial site: z=-0.69, *P*=0.49, initial*final site: z=-0.58, *P*=0.56). Species richness appeared resistant to acidification after the transplantation experiment, as neither early nor late CO_2 effects influenced the number of recruiting species (GLMM, initial site: z=-0.47, P=0.64, final site: z=0.31, P=0.76, initial*final site: z=-0.10, P=0.92, Fig. 3.5h).

3.4. Discussion

Timing and abundance of species recruiting from plankton are important determinants of long-term community composition and structure (Sutherland, 1974; Sams & Keough, 2012) and, depending on the mechanism of succession, can determine long-term community stability (Connell & Slatyer, 1977). Environmental heterogeneity and disturbance regimes during recruitment can influence successional outcomes by promoting coexistence or dominance of early or late recruiting species (Platt & Connell, 2003; Cifuentes *et al.*, 2010). Global change impacts on communities and ecosystems may therefore stem from the particular way in which environmental drivers interact with life-history trade-offs (e.g., competitive ability vs. dispersal / colonization ability) of early and late successional species, and how these species inhibit or promote one another. This is reasonably well understood for drivers like temperature in terrestrial ecosystems (e.g., Gounand *et al.*, 2016; Lancaster *et al.*, 2016), but in marine environments and for emerging drivers like ocean acidification, changes in succession – even when observed (e.g., Kroeker *et al.*, 2013b) – are rarely explicitly examined in terms of the underlying mechanisms and time-history contingency.

Here, I used transplant experiments to elucidate the relative importance of CO_2 effects early vs. later in succession on species abundance, diversity and composition. This is important for understanding how acidification might affect communities as they progress through successional and seasonal development. It is also key for determining the effects of discrete acidification events on marine communities. Upwelling regions experience intermittent acidification events that can span weeks (e.g., up to six week periods, Chan *et al.*, 2017) or months (e.g., from early spring to late summer, Feely *et al.*, 2008) and these events are expected to become less frequent but longer-lasting and stronger (Iles *et al.*, 2012). Acidification events also occur in areas with high organic loading via terrestrial run-off, where discrete events can last for days to weeks but multiple cumulative events can occur over several months (Guadayol *et al.*, 2009). Eutrophication-driven acidification may intensify in the future as eutrophication will likely increase with increased human development (Rabalais *et al.*, 2009).

On my experimental tiles, soft-bodied, weedy taxa, algae and ascidians, had an advantage in acidified conditions and outcompeted calcified taxa that were more vulnerable to the effects of acidification, as has been widely reported (Wittmann & Pörtner, 2013). Developing communities responded quickly to acidification (effects on some species were apparent after two weeks) and some of these effects were not reversed one month after transplantation. In sum, I found that succession was substantially altered by acidification, even when acidified conditions were not maintained for the full duration of the experiment. Future work should carefully consider temporal variation in acidification over longer experimental periods spanning important dynamics in community succession.

3.4.1. Recruitment and Development

Early successional stages in many shallow benthic habitats – including downward-facing experimental surfaces – are dominated by photosynthetic microalgae and weedy macroalgae. Many photosynthetic marine taxa can take advantage of elevated CO_2 (Connell *et al.*, 2013; Cornwall *et al.*, 2017). At Vulcano Island and other seep sites, biofilms are higher in percent cover and productivity and have altered composition relative to reference sites (Lidbury *et al.*, 2012; Johnson *et al.*, 2013, 2015; Baggini *et al.*, 2015). I observed the same boost in biofilm under acidified conditions during the early phase of succession, although it is unclear if composition changed. It is possible that the biofilm at low pH sites altered subsequent invertebrate recruitment by changing settlement cues (see Doropoulos *et al.*, 2012), as succession was delayed in this site, despite early biofilm abundance. Filamentous green *Cladophora* sp. had a higher and slightly later peak in abundance on tiles at the acidified site. Such shifts in primary producer assemblages alter biomass of resources available for grazers (Russell *et al.*, 2013) and may alter settlement patterns of recruiting invertebrates (Hadfield, 2011).

I found that calcified primary colonizers had lasting responses to short but discrete exposure to increased CO_2, whereas only one of the calcified secondary colonizers exhibited this response. This pattern could be caused by the differential duration of exposure experienced by primary (four weeks longer) vs. secondary species or traits of the species in each of the categories (e.g., primary colonizers were heavily calcified polychaetes, compared to relatively lightly calcified bryozoans). The recruitment of two types of calcified tube-forming polychaetes was both reduced and delayed under acidification. Delayed recruitment under acidification causes individuals of these species to arrive after the community has reached 100% cover and could imply that these organisms face stiffer competition for space than their counterparts that

recruited earlier into the ambient site. Tile site of origin influenced the recruitment of both spirorbids and serpulids and these effects persisted through time, regardless of transplantation into or out of the acidified site. My results align with observations of reduced abundances of both serpulids and spirorbids near CO_2 seeps off Ischia (Cigliano *et al.*, 2010; Donnarumma *et al.*, 2014), but suggest that this effect emerges very early in the establishment of these taxa. The two-week delay in recruitment could be due to a combination of direct effects on adult reproduction, larval and juvenile recruitment and/or indirect effects such as inhibition by settled species. Acidification has been shown to impair serpulid larval calcification and juvenile growth (Lane *et al.*, 2013) and compromise tube ultrastructure (Li *et al.*, 2014) in the laboratory. Spirorbid growth could have been influenced by early exposure to CO_2, as there were fewer spirorbids on tiles that originated in acidified sites, but similar space coverage, suggesting that spirorbids under acidification were larger. This could be a consequence of accelerated growth under acidification or differential mortality of smaller individuals within the population. Negative effects of acidification on these polychaetes may have higher level consequences as these ecosystem engineers form complex reefs that have high associated biodiversity (Smith *et al.*, 2013; Fabricius *et al.*, 2014).

At various points of succession, colonial ascidians (*Diplosoma* sp. and *Botryllus* sp.) appeared either to tolerate or respond positively to acidification, which may reflect increased growth rate, increased facilitation, and/or reduced competition under increased CO_2 conditions. It is difficult to disentangle these effects as growth rate in this context is undoubtedly influenced by other species, and facilitation and competition are difficult to infer without experimental manipulation. These ascidians, although native to the Mediterranean, are among a suite of globally invasive taxa that overgrow other filter feeders and can cause economic damage to the aquaculture

industry (Zhan *et al.*, 2015). my results add to growing evidence that some ascidians respond positively to both natural (Donnarumma *et al.*, 2014) and experimental (Peck *et al.*, 2015) acidification (but see Fabricius *et al.*, 2014) for an example of reduced ascidian cover at tropical seep sites). Overall, fast-growing nuisance species like ascidians are expected to benefit from future acidification (Hall-Spencer & Allen, 2015) and reduced competition with calcifying native taxa might increase relative dominance of invasive ascidians in an acidified ocean.

I observed mixed effects of natural acidification on lightly calcified bryozoans, which may be related to differences in their carbonate mineralogy. The cyclostome bryozoan *Patinella radiata*, with a primarily calcitic skeleton, did not change in abundance near seep sites. Studies off Ischia have shown that this species can grow and reproduce at low pH (Donnarumma *et al.*, 2014; Taylor *et al.*, 2015). However, a thin ramified bryozoan appeared earlier at the ambient than the acidified site and an encrusting bryozoan *Schizomavella* sp., with a mainly aragonitic or bimineralic skeleton (Smith *et al.*, 2006), had reduced recruitment at the acidified site. Carbonate mineralogy of bryozoans can help predict vulnerability to acidification for a given species - aragonite skeletons are more soluble than those composed of mainly calcite, and calcite solubility increases with proportion of Mg (Fortunato, 2015; Pickett & Andersson, 2015; Taylor *et al.*, 2015). However, mineralogy is not the sole determinant of dissolution rate. Other factors such as surface area, porosity, surface complexity, organic matrix material, and ambient pH at the calcification surface, may also play a role in response to acidification (Ries *et al.*, 2009; Smith & Garden, 2013; Taylor *et al.*, 2015). Morphological differences between related species highlight the importance of examining species-specific responses to acidification. However, the relevance of these morphological differences among species may be outweighed by relative competitive ability if and when these bryozoans are at risk of being overgrown by other species.

At CO_2 seeps, within-seep vs. outside-seep recruitment is difficult to disentangle and life history strategy may determine the extent of direct effects of acidification on species-specific recruitment. If recruits are coming from within-seep source populations, which is most likely for species with short pelagic larval phases, observed recruitment effects (both positive and negative) could represent a culmination of both direct effects of acidification on larvae (e.g., physiological, Kurihara *et al.*, 2008; Ross *et al.*, 2011; Przeslawski *et al.*, 2015; and/or behavioural Doropoulos *et al.*, 2012; Doropoulos & Diaz-Pulido, 2013; Webster *et al.*, 2013), transgenerational effects of acidification on nearby adult populations (Calosi *et al.*, 2013b; Harvey *et al.*, 2016; Ross *et al.*, 2016), and multigenerational adaptation to chronic acidified conditions (Calosi *et al.*, 2013b). In contrast, propagules arriving from outside the seeps will not experience generational effects of acidification.

3.4.2. Species Interactions and Community-level Results

Acidification first delayed succession of primary colonizers, then accelerated secondary succession. First, communities in acidified sites developed more slowly (i.e. were more stable) than ambient communities and had a smaller proportion of space used by secondary colonizers. After 8 weeks, however, the pace of change eventually recovered to levels similar to the control sites. At this point, the proportion of secondary colonizers increased on tiles at the high CO_2 site, independent of colonization history. This mismatch in timing indicates that ocean acidification may alter species interactions between and within primary and secondary colonizer guilds. For example, the biofilm trajectory, likely a diatom bloom, was altered at the acidified site, resulting in reduced biofilm coverage at the acidified site by the end of the experiment. This likely contributed to increased cover of serpulids and bryozoans on tiles that experienced higher CO_2 at the acidified site since, at ambient levels of CO_2, the abundant biofilm overgrew and/or pre-

empted space occupation by these invertebrates compared to the acidified site where there were fewer calcified invertebrates and lower biofilm cover during this time period. The benefits to the calcifying organisms transplanted to the acidified site may be short lived however, as some of these species were negatively affected by acidification overall.

Accelerated secondary succession (despite an initial delay) and competition-mediated reductions in invertebrates at the ambient site likely contributed to higher species diversity on tiles that were maintained in or transferred to the acidified site. This pattern was not observed for species richness however, indicating that evenness or abundance of species was driving the diversity result. This unexpected result further underscores the importance of understanding shifting species interactions under acidification (Gaylord *et al.,* 2015). Responses to CO_2 could also be modulated at seep sites by seasonal effects on both calcifying invertebrates and algal competitors (Baggini *et al.*, 2014), as seawater temperature increased from 14°C to 20°C during my experiment. Competition between serpulids and algae has been documented in benthic communities near Ischia CO_2 seeps, although the pattern described there (Kroeker *et al.,* 2013c) is opposite to what I have described here in shaded fouling communities. Thus, microhabitat could play an important role in competitive outcomes under acidification, and shaded areas may provide a refuge from algal competition for those calcified filter feeders that are able to recruit under acidification, although see Celis-Plá *et al.* (2015) for examples of positive combined effects of shade and acidification on macroalgae.

The observed species-level changes at the acidified site, likely driven by direct effects and mediated by interspecific interactions, culminated in community-level shifts in structure. my results conformed to the general expectation that communities experiencing high CO_2 may shift from calcified to mainly non-calcified consumers (Christen *et al.*, 2013) likely due to energetic

trade-offs which result in less energy available for calcification (Gaylord *et al.*, 2015). My results complement a growing number of studies documenting changes in community structure and diversity with increased CO_2 in a range of habitat types (Kroeker *et al.*, 2013b; Campbell & Fourqurean, 2014; Meadows *et al.*, 2015; Raulf *et al.*, 2015; Sarmento *et al.*, 2015; Brown *et al.*, 2016). The significant trend towards homogeneity among invertebrate communities under acidified conditions by the end of my experiment is similar to that described for fouling communities in western Canada (Brown *et al.*, 2016), and for algal communities close to CO_2 seeps (Porzio *et al.*, 2011; Kroeker *et al.*, 2013c).

3.4.3. Conclusions

I found that elevated CO_2 conditions in the Mediterranean stimulated the initial colonization of settlement panels by biofilm. Despite the promotion of biofilm, succession was delayed at the acidified site and secondary colonization was lower. After eight weeks, however, subsequent secondary succession accelerated quickly, resulting in higher secondary colonization, altered community structure, and a more homogeneous biofouling community than that found at ambient levels of CO_2. Life history strategies, such as larval dispersal ability, environmental tolerances, growth rate and competitive ability, influence species-specific responses of organisms as they colonize new substrata, and are important to consider, even in closely related species (Gambi *et al.*, 2016). I found marked shifts in recruitment patterns which may alter routes of energy flow between trophic levels; later settlers may arrive out of sync with food sources, predators, or competitors (Dupont & Thorndyke, 2009; Nagelkerken & Connell, 2015). I also found that acidification altered community structure and these changes were driven both by past exposure (colonization history) and recent exposure to high CO_2. Accelerated secondary succession, homogenization, and changes to diversity under acidification occurred independently

of colonization history. These processes might be driven more by proximate environmental conditions and small-scale within-site recruitment. The observed community-level shifts are therefore likely a result of not only persistent and transient effects of interspecific variability in response to increased CO_2 but also, importantly, shifting interactions between and within primary and secondary colonizer guilds (Connell & Slatyer, 1977; Gaylord *et al.*, 2015). Overall, these short and longer-term acidification-driven changes in community succession may represent an as-of-yet undocumented feature of many marine communities, and could have important implications for ecosystem function and food web dynamics.

Chapter 4. Increased Food Supply Mitigates Ocean Acidification Effects on Calcification but Exacerbates Effects on Growth

4.1. Introduction

Ocean acidification (OA) has been recognized as one of the most significant threats to marine life (Wittmann & Pörtner, 2013), with demonstrated negative effects across many taxa in terms of growth and calcification (Kroeker *et al.*, 2013a). Although the negative effects of acidification are not universal (Harley *et al.*, 2012; Wittmann & Pörtner, 2013; Hall-Spencer & Allen, 2015), they are more common than positive effects and have been demonstrated in a wide variety of taxa (Kroeker *et al.*, 2010, 2013a). In many cases, impairment by acidification reflects an underlying energetics problem, as organisms may need to redirect energy from growth and reproduction towards acid-base regulation, especially at sites of calcification (Sokolova *et al.*, 2012; Stumpp *et al.*, 2012b). Given that sub-lethal negative effects of OA can result from this energetic trade off, a number of studies have proposed the hypothesis that negative responses to OA could be minimized in situations where energy (e.g., food) is more readily available (Ramajo *et al.*, 2016). If true, this has far-reaching implications for how we both understand and study OA (Rossoll *et al.*, 2012; Gaylord *et al.*, 2015).

Although the energy limitation hypothesis is ecologically relevant and physiologically plausible, many of the recent tests of the idea suffer from a lack of adequately framed and precisely stated expectations regarding the combined effects of OA and food supply, or from inappropriate statistical comparisons among different experimental groups. Perhaps as a result of the loose framing of expectations, the most appropriate meta-analytical techniques have not yet been employed to seek generalities across studies. Here, I 1) clarify the hypothesis put forward by a recent meta-analysis (Ramajo *et al.*, 2016) and others (Edmunds, 2011; Comeau *et al.*, 2013;

Thomsen *et al.*, 2013; Crook *et al.*, 2013; Drenkard *et al.*, 2013; Hettinger *et al.*, 2013; Pansch *et al.*, 2014; Towle *et al.*, 2015; Pan *et al.*, 2015; Ramajo *et al.*, 2015; Maier *et al.*, 2016; Büscher *et al.*, 2017), and 2) test the hypothesis using available data in a more appropriate meta-analytical framework.

Meta-analysis can be used as a *post-hoc* tool to assess generality or consistency of a well-defined, *a priori* hypothesis (Lortie & Callaway, 2006). I consider the following hypothesis: the negative response to acidification by calcifiers is reduced by high food availability (or, alternatively, magnified by food limitation) (see Ramajo *et al.*, 2016 and studies there within). Ideally, each independent study should test the same hypothesis by setting controls at naturally occurring food levels and then either enhancing or restricting food availability. The inconsistency among studies in Ramajo *et al.* (2016), both in setting controls (i.e. either "low" or "high" food was considered the control, depending on the study, so the direction of applied treatment also differed) and baselines (i.e. the magnitude of the control: some studies consider naturally occurring food levels, while others consider starvation as a baseline), make inferences more difficult. In this meta-analysis, I test the hypothesis by comparing patterns to both predictions, recognizing that relating control conditions to natural environments is essential for interpreting the results; this limitation is discussed in further detail below.

Here, the crux of the hypothesis that a negative response to OA by calcifiers could be modified by food availability lies in the idea that increased food supply does not simply offset some of the negative consequences of ocean acidification; rather, it implies a positive *interactive* effect of food and OA (*sensu* Morris *et al.*, 2007). For this type of statistical interaction, the term 'positive' refers to the fact that the observed response to simultaneously elevated food and CO_2 is more positive, or less negative, than the response predicted from the single-factor effects by a

null, non-interactive model. The proper statistical test of the food limitation hypothesis would therefore be to estimate the *statistical* interaction between food supply and ocean acidification where the effect of OA on performance is stronger with limited food and weaker with abundant food. The distinction between this positive interactive scenario and two alternatives (non-interactive and negative interactive scenarios) is outlined in Figure 4.1. The non-interactive scenario is one in which the effect of OA does not depend on food availability (Fig. 4.1a, slopes are equal). Here, I use the term 'non-interactive' to imply a null additive model in multilevel meta-analysis and a null multiplicative model in factorial meta-analysis. In the positive interactive scenario, the response of an organism to acidification is altered by food addition such that the negative effect of OA apparent under low food conditions is reduced or disappears when food supply is high (Fig. 4.1b, low food slope is more negative). Finally, in the negative interactive scenario, food addition actually promotes a stronger negative response to CO_2 (Fig. 4.1c, high food slope is more negative). This last case is not predicted directly from an energetic model of effects of OA, but thus far the only example of a significant interaction between factorially manipulated CO_2 and food supply is of this type (see Cole *et al.*, 2016). These three hypotheses lead to how food supply alters the effect of OA on performance (Fig. 4.1d-f) and, equivalently, how OA affects an animal's performance response to food supplementation (Fig. 4.1g-i). Here, I regard an organism's performance as any measure that can be metabolically linked to energetic supply (Sokolova *et al.*, 2012) and expect that performance metrics that relate more directly to energy use and allocation will be more influenced by food addition. Using a factorial meta-analysis, I can further test that a negative effect of CO_2 and a positive effect of food supply can combine in a multiplicative way (i.e. no interaction, Fig. 4.1j) or in an

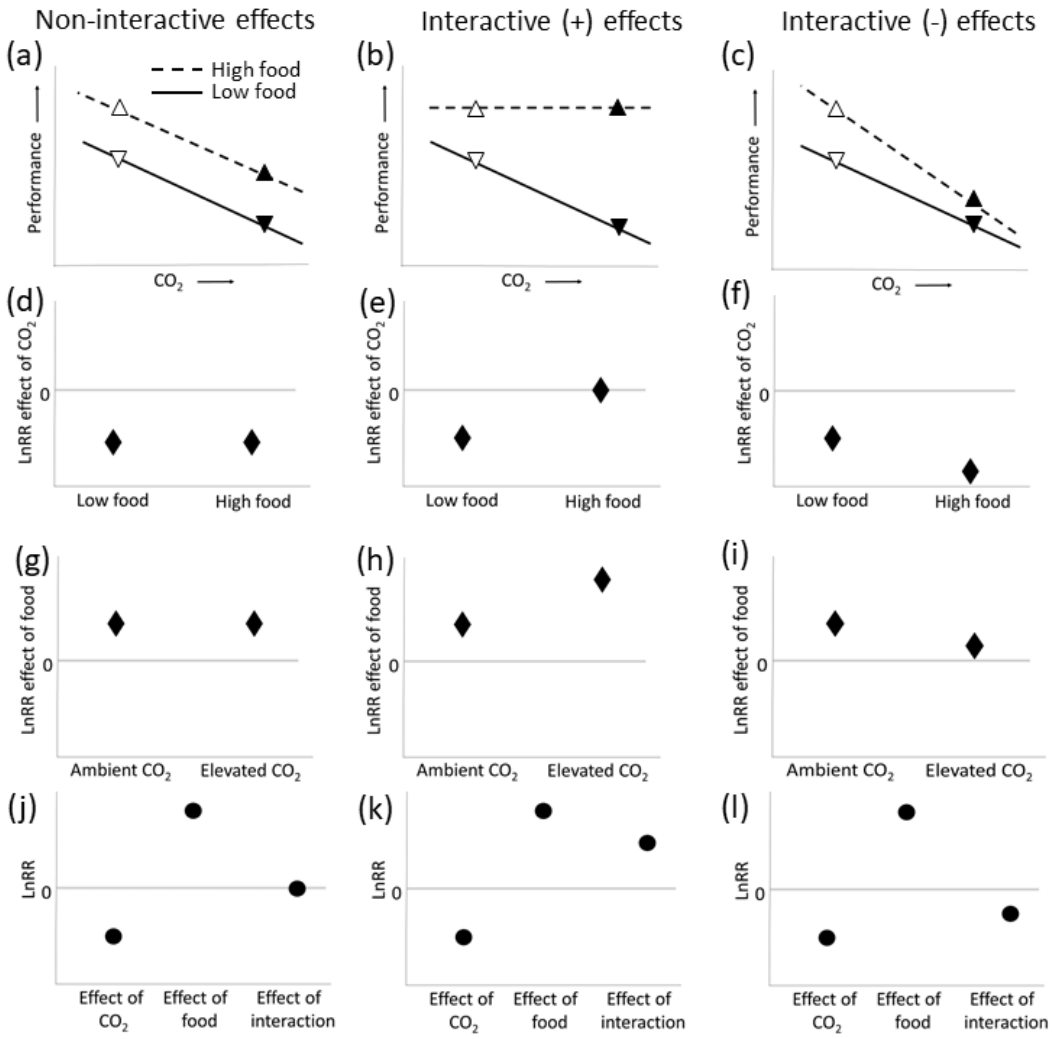

Fig. 4.1. Schematic demonstrating hypothetical responses of organisms where high CO_2 has a negative effect and high food supply has a positive effect on performance. Left-hand panels show a scenario where organismal response to high CO_2 is not modified by food supply, demonstrating a non-interactive effect of food and OA on performance (a). Middle and right-hand panels depict interactive effects of food and ocean acidification on performance, where organismal response to high CO_2 is modified by food supply in a positive (b) or negative (c) way. The outcomes of (a), (b), and (c) could be realized across many studies in a meta-analysis, where the mean effect sizes (in Ln response ratio, LnRR) of OA may remain the same, regardless of food addition (d) or differ under food supplementation (e, f). In (d), while higher food may be beneficial and OA may be detrimental, a change in food supply does not alter the magnitude of the OA effect. Similarly, the effect of food supply could have no relationship with CO_2 (g) or be influenced by change in CO_2 (h, i). Finally, in a factorial meta-analysis, the interaction between food supply and OA could be non-significant (a simple multiplicative outcome, in this case (j), significantly positive (k), where the interaction food and OA on average improves the performance of organisms relative to the non-interactive case (compare b to a), or significantly negative (l), where the interaction between food and OA on average decreases performance of organisms relative to the non-interactive case (compare c to a).

interactive way, either where the interaction on average improves (Fig. 4.1k) or decreases performance relative to the non-interactive null expectation (Fig. 4.1l).

Ramajo *et al.* (2016) set out to explore these possibilities using 12 studies that factorially manipulated OA and food supply to calcifying invertebrates from a range of taxa (corals, crustaceans, molluscs and echinoderms) and life history stages (larvae, juveniles, and adults) found in different environments (both temperate and tropical). However, rather than comparing OA effects at high and low food supply (i.e. slopes of lines, Fig. 4.1a-c), Ramajo and colleagues compared both the high food acidified treatment (solid upward-pointing triangles, Fig. 4.1a&b) and the low food acidified treatment (solid downward-pointing triangles, Fig. 4.1a-c) to a single reference treatment: high food, non-acidified (open upward-pointing triangles, Fig. 4.1a-c). This comparison using a common denominator is atypical in the meta-analysis literature, where, in subgroup analyses, independent effect sizes are computed within subgroups (Borenstein *et al.*, 2009a). Because the effect size of acidification was not calculated independently for each subgroup (level of food supply) by Ramajo *et al.* (2016), the response ratios (lnRR) presented for low and intermediate food supply cannot be considered true effect sizes and thus cannot be used to compare if the effect of CO_2 changes under food supply levels. Therefore, the authors inadvertently tested a substantially different hypothesis, namely that when food is abundant, increasing CO_2 has a small effect, but that *simultaneous* exposure to OA *and* food deprivation has a large negative impact relative to well-fed, non-acidified controls. Because the analysis did not include any data from organisms under ambient CO_2 conditions and low or intermediate food supply (open down-facing triangles, Fig. 4.1a-c), the analysis cannot determine if or how the response to OA changes with food supply (i.e. cannot distinguish between the interactive vs. non-interactive scenarios outlined in Figure 1).

To clarify the relationship between OA effects and food supply, I re-examined the available literature using two different types of meta-analysis: multilevel meta-analysis (employed by Ramajo *et al.* (2016) and others (Konstantopoulos, 2011) and factorial meta-analysis (Gurevitch *et al.*, 2000; Morris *et al.*, 2007; LaJeunesse, 2011). Within each of these types of meta-analysis I used three different datasets to test the hypothesis that the response to OA is modified by food supply. I first created an updated dataset by extracting data from the eleven published studies in Ramajo *et al.* (2016) and six additional studies (Taylor *et al.*, 2014; Cole *et al.*, 2016; Maier *et al.*, 2016; Büscher *et al.*, 2017; Hurst *et al.*, 2017; Swezey *et al.*, 2017a) (see Appendix C, Table S4.1). In order to compare these results to findings in Ramajo *et al.* (2016), I next replicated their dataset twice, first including the incorrect log response ratio (LnRR) calculation along with other errors in the dataset, and secondly by correcting the LnRR calculation in order to demonstrate the impact that this miscalculation makes on the results and inferences (see Appendix C).

4.2. Methods

Two meta-analytic techniques were explored to test the hypotheses posed above. The first, multilevel meta-analysis, uses ln response ratios of the effect of CO_2 (comparing across food levels) and the effect of food (comparing across CO_2 levels). The second technique, factorial meta-analysis, is suitable for papers that employ fully-factorial experiments and is considered a more powerful method for this type of dataset. The analysis used by Ramajo *et al*. (2016), specifically their calculation of effect size, was not appropriate for the stated hypothesis and, this mismatch calls their conclusion into question. I have therefore replicated results from Ramajo *et al.* (2016), using both types of meta-analysis. These methods can be found in the Appendix C.

4.2.1. Multilevel meta-analysis (Fig. 4.2)

I extracted data on growth and calcification responses to CO_2 from 17 articles in which both food supply and CO_2 were manipulated. All data (68 observations for calcification measures, 144 for growth measures) were extracted from figures using WebPlotDigitizer (Rohatgi, 2015). The papers used in the multilevel analysis were all the published articles from Ramajo *et al.* (2016) and six additional articles found during an ISI Web of Knowledge search using similar search terms (Appendix C, Table S4.1). Here, I used the width variable from Taylor *et al.* (2014) because this is the most commonly used measure of urchin growth in both fisheries and population-level literature. Furthermore, Taylor *et al.* (2014) was only used for the LnRR effect of CO_2 analysis but not for the effect of food analysis since negative and positive values cannot be compared using LnRR (for example, a high food value of + 20% change in growth and low food value of – 20% change will result in ln (-1) which is undefined, therefore change in weight can only be used in this analysis if all values are positive). I only used the aggregate ambient CO_2 level described in Maier *et al.* (2016) and only one elevated CO_2 level (intermediate) because, as above, LnRR cannot evaluate dissolution at the same time as calcification. I added a constant (0.5%) to all percentage growth measurements in Büscher *et al.* (2017) since values close to zero approach infinity when taking ln(0).

Meta-analytic calculations and statistics were performed in R (Version 3.3.1, R Development Core Team, 2009), using the *metafor* package (Viechtbauer, 2010). I calculated a weighted ln Response Ratio (lnRR) using OA/Control within each food supply level (*escalc* function, *metafor* package in R, Viechtbauer, 2010). I constructed multilevel models with food supply as a fixed effect and aspects of non-independence as random effects using the *rma.mv* function in R (Viechtbauer, 2010) and checked these models for publication bias (using a contour-enhanced

funnel plot, Peters *et al.*, 2008) and sensitivity to outliers (following methods in Habeck & Schultz, 2015). I used restricted maximum likelihood (REML) approaches to test if effect size estimates were significantly different than zero and significantly different between food supply and CO_2 levels. Some levels of CO_2 were different between food levels within an experimental treatment, therefore I averaged between food levels to obtain a single CO_2 level for each low food-high food pair to regress against the LnRR effect of food. I excluded intermediate food levels because the sample size was too small to make meaningful comparisons (the minimum number of studies included in meta-analysis generally depends on the sample sizes of the studies themselves, but Higgins & Green (2008) suggest a minimum of 10 studies, and here only 3 studies included intermediate food levels). The data used included several non-independent measures: common control for multiple levels of CO_2, multiple variables for same response (growth responses only), and multiple independent studies within the same article. I accounted for dependent sampling errors caused by using a common control for multiple levels of CO_2 by constructing a variance-covariance matrix of the effect size estimates (Gleser & Olkin, 2009). I accounted for multiple variables for the same response and multiple independent studies within an article by including an unstructured random effect of response variable unit (of the form ~Unit|Paper_no) and food supply (of the form ~Food.supply|Paper_no) which allows the random effects to have different variances for each outcome while also allowing random effects to be correlated (Berkey *et al.*, 1998). I plotted estimates for the mean and confidence intervals generated from the rma.mv models. For the calculated LnRR of food supply (high food/control, low food), I used data from all CO_2 levels, therefore there was an unequal number of observations in each category. To account for this, I included an unstructured random effect for CO_2 level (of the form ~ CO_2_level|Paper_no) in addition to an unstructured random effect of

response variable unit (of the form ~Unit|Paper_no). I tested all models first for an overall effect of the moderator (Q_M), food supply or CO_2, and then tested between levels of the moderator – i.e. testing for the interaction between food and CO_2 (Q_{Mcoef}), e.g., High vs. Low food or Ambient vs. Elevated CO_2. These tests of heterogeneity described by the moderators (Q_M tests) are akin to a Wald-type Chi-squared test (Viechtbauer, 2010). When there was only a single moderator (as in Fig. 4.2e,f), the effect of the moderator (e.g., CO_2) is a test of the slope vs. zero (Q_{Mslope}) and the overall response (regardless of moderator) is a test of the intercept vs. zero ($Q_{Mintercept}$).

4.2.2. Factorial Meta-analysis (Fig. 4.3)

For this analysis, I used the same data as the multilevel analysis (Fig. 4.2), with one exception: Taylor *et al.* (2014) was excluded because we cannot take the ln of negative values (as above). In order to account for multiple CO_2 levels per paper, I averaged effect sizes across a single paper, as factorial LnRR are not appropriate for constructing a variance-covariance matrix to account for multiple comparisons to the same CO_2 control. I accounted for multiple responses per paper in this analysis in the same way. Multiple study sites within papers were considered to be independent enough to consider separately.

I calculated ln response ratios for overall and interactive effects of food limitation and high CO_2, using factorial meta-analysis methods (Gurevitch *et al.*, 2000; Morris *et al.*, 2007; LaJeunesse, 2011). Overall effects incorporate the effects of the interacting factor at all levels. In this analysis, I assume effects (food supply and high CO_2) are multiplicative, which is a more biologically realistic null model than simply additive (i.e. "you can't die twice", Sih *et al.*, 1998). I calculated overall and interactive effect sizes and sampling variance from equations

Appendix B.7 to B.12 in Morris *et al.* (2007). I weighted effect sizes by inverse sampling variance using a random effects model where weights were assigned using within study variance plus between studies variance (Borenstein *et al.*, 2009b) and corrected for the small number of studies using equation 7 in Hedges *et al.* (1999). I constructed 95% confidence intervals using equation 8 in Hedges *et al.* (1999) and inferred significance from these confidence intervals. If the interaction effect is not different than zero (CIs overlap zero), then I can infer multiplicative effects (Morris *et al.*, 2007). If otherwise, I infer significant interactive effects: if the interaction effect is positive (above zero), then the interaction between the two factors has a positive effect on performance relative to the simple multiplicative case; if negative (below zero), I infer the interaction of the two factors has a negative effect on performance relative to the multiplicative scenario (Morris *et al.*, 2007).

4.3. Results and Discussion

Using the updated dataset, I calculated log-response ratio (LnRR) of the effect of CO_2 using response to OA / control (solid vs. open symbols, Fig. 4.1a&b) where control was low CO_2 conditions under the same food supply level as the OA treatment. I found non-interactive effects of OA and food supply for both growth and calcification. In other words, the response to OA did not depend on food supply (calcification $Q_{M(coef)}$=1.84, P=0.17, Fig. 4.2a; growth $Q_{M(coef)}$=1.31, P=0.25, Fig. 4.2b). Furthermore, restricting this analysis to the data and methods used by Ramajo *et al.* (2016) did not alter my conclusions (calcification $Q_{M(coef)}$= 0.60, P= 0.44, Fig. 4.3b, and growth $Q_{M(coef)}$= 0.28, P= 0.61, Fig. S4.2d). With regard to the data plotted in Figures 4.2a and 4.3b, I remind readers that a statistical difference cannot be inferred between a significant result and a non-significant result (Gelman & Stern, 2006), although it is a common

mistake to draw this conclusion when the confidence interval crosses zero in one case but not the other. In other words, a difference in statistics (e.g., whether the confidence interval includes zero or not) cannot be used to infer a statistical difference between sampled populations.

Across food treatments, I detected an overall significant negative response to high CO_2 for calcification-related responses (Q_M=6.56, P=0.037, Fig. 4.2a). For calcification, the effect size of OA was positively related to the experimental change in CO_2 (Q_M=10.79, P=0.0045, Fig. S4.1a). In contrast, overall growth responses to OA were less variable, did not differ from zero (Q_M=1.33, P=0.52, Fig. 4.2b), and did not change with increasing experimental change in CO_2 (Q_M = 2.37, P=0.30 Fig. S4.1b). These results concur with a recent meta-analysis showing that calcification responses to OA across many taxa were stronger than growth responses (Kroeker *et al.*, 2013a). The non-significant growth response to OA found here suggest either that my analysis represents an insufficient sample size to detect a true effect or that the species tested were relatively resistant to acidification compared to responses reported elsewhere (Kroeker *et al.*, 2013a).

Across CO_2 levels, food supply had no effect (but trending positive) on calcification (OA: Q_M=5.33, P=0.069, Fig. 4.2c; continuous CO_2: $Q_{M(intercept)}$=3.19, P=0.074, Fig. 4.2e) and a strong positive effect on growth (OA: Q_M=20.59, P<0.0001, Fig. 4.2d; continuous CO_2: $Q_{M(intercept)}$= 15.74, P<0.0001, Fig. 4.2f). The response to high food supply did not depend on OA

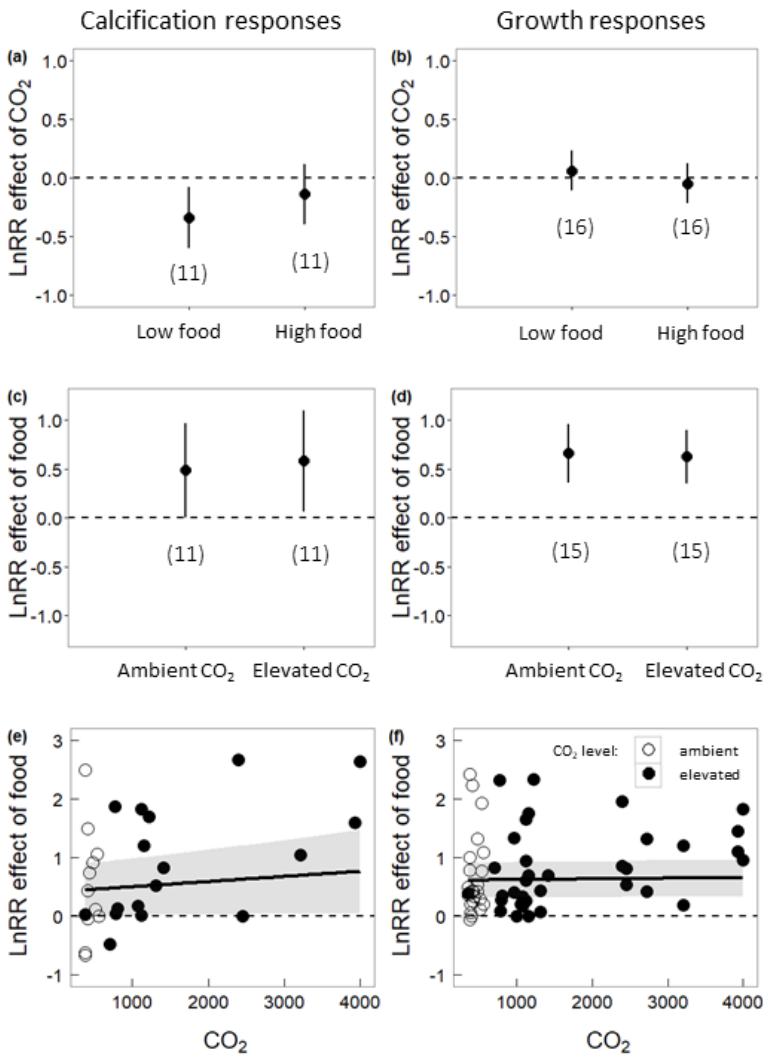

Fig. 4.2. Mean effect sizes (LnRR) for the responses of calcification (left hand panels) and growth (right hand panels) to food supply and elevated CO_2. Mean responses to elevated CO_2 did not depend on food supply for (a) calcification ($Q_{M(coef)}$=1.84, P=0.17) nor (b) growth ($Q_{M(coef)}$=1.31, P=0.25). Across food treatments, there was a significant negative response to high CO_2 for (a) calcification (Q_M=6.56, P=0.037) but not (b) growth (Q_M=1.33, P=0.52). Response to high food was not different between elevated and ambient CO_2 levels for (c) calcification ($Q_{M(coef)}$=2.62, P=0.11) or (d) growth ($Q_{M(coef)}$=1.64, P=0.20) responses, nor did response to high food supply depend on continuous CO_2 for (e) calcification (Q_{Mslope}=1.72, P=0.19, slope=0.0001) or (f) growth (Q_{Mslope}=0.48, P=0.49, slope=0.00). Across OA levels, food had no effect (but trending positive) on (c) calcification (Q_M=5.33, P=0.069) and a significant positive effect on (d) growth (Q_M=20.59, P<0.0001). Across a continuous CO_2 gradient, food had a significant positive effect on (f) growth ($Q_{M(intercept)}$=15.74, P<0.0001), but not (e) calcification ($Q_{M(intercept)}$= 3.19, P=0.074) responses. Parameter estimates and confidence intervals from fitted REML models. Symbols represent the treatment in each study from which the LnRR was calculated (open vs. closed circles for ambient and elevated CO_2, respectively). Numbers in brackets indicate the number of studies contributing to the LnRR; for (e) and (f), n=11 and n=15, respectively.

manipulation or continuous CO_2 level for growth (OA: $Q_{M(coef)}$=1.64, *P*=0.20, Fig. 4.2d; continuous CO_2: Q_M=0.48, *P*=0.49, Fig. 4.2f) or calcification responses (OA: $Q_{M(coef)}$=2.62, *P*=0.11, Fig. 4.2c; continuous CO_2: Q_M=1.73, *P*=0.19, Fig. 4.2e). These results suggest that growth may be more tightly linked to food supply than OA, while OA may be relatively more important for calcification.

As an alternative approach, I next employed a factorial meta-analysis on the same data to calculate overall effects of CO_2 and food addition and their interaction. This analysis is a powerful tool when using fully factorial datasets (i.e. each experiment manipulated both OA and food supply) (Gurevitch *et al.*, 2000; Morris *et al.*, 2007; LaJeunesse, 2011). Here, I confirm that CO_2 has a significant negative effect on calcification (LnRR=-0.26, lower=-0.29, upper=-0.23, Fig. 4.3a), as found in Fig. 4.2a. For growth in this analysis I was able to detect a small, significant negative effect of CO_2 (LnRR=-0.020, lower=-0.026, upper=-0.013, Fig. 4.3), whereas I was unable to detect this effect using multilevel meta-analysis (Fig. 4.2b). In this analysis, I find strong significant overall effects of food on both calcification (LnRR=1.07, lower=1.03, upper=1.11, Fig. 4.3a) and growth (LnRR=0.57, lower=0.56, upper=0.59, Fig. S4.2b), whereas previously I found a significant effect on growth only and a positive trend for calcification (Fig. 4.2c-f). When analyzed factorially, I found that the interaction between OA and food supply had a small and significant positive effect on calcification (LnRR=0.13, lower=0.07, upper=0.19, Fig. 4.3a). This result provides support for the positive interaction hypothesis that food addition can ameliorate response to CO_2 (Fig. 4.1b). For growth, I find support for the opposite hypothesis, where the interaction between food supply and CO_2 had a

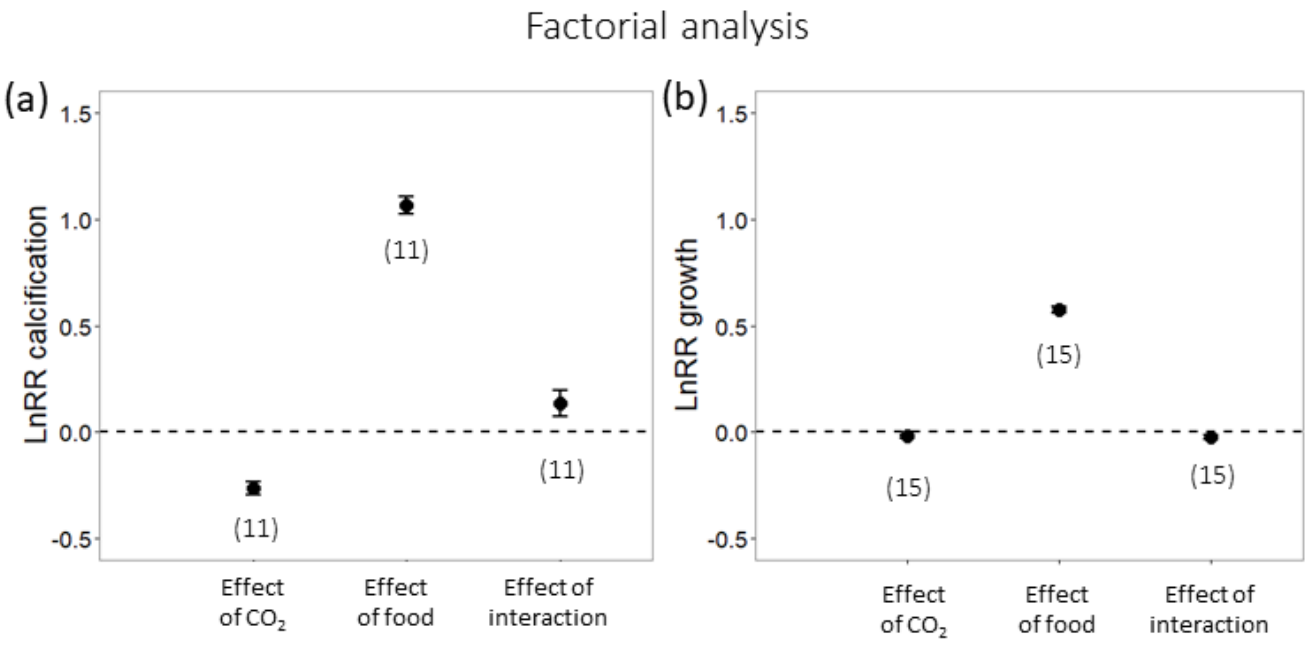

Fig. 4.3. Factorial analysis. Overall and interactive effect sizes for CO_2 and food supply for (a) calcification and (b) growth responses. (a) Here, I find that overall, CO_2 had a significant negative effect on calcification (LnRR=-0.26, lower=-0.29, upper=-0.23), whereas food addition had an overall significant positive effect on calcification (LnRR=1.07, lower=1.03, upper=1.11). The interaction between CO_2 and food supply was significantly positive for calcification (LnRR=0.13, lower=0.07, upper=0.19), meaning that OA effects were weaker (less negative) at high food. (b) For growth responses, CO_2 had a small but significant negative effect (LnRR=-0.020, lower=-0.026, upper=-0.013), food had a positive effect (LnRR=0.57, lower=0.56, upper=0.59). The interaction between CO_2 and food supply was small but significantly negative for growth (LnRR=-0.024, lower=-0.035, upper=-0.012), meaning that OA effects were stronger (more negative) at high food. Error bars are 95% confidence intervals calculated from standard error for small sample sizes. If the error bars do not overlap zero then a significant response is inferred. Numbers in brackets indicate the number of studies contributing to the LnRR.

small but significant negative interactive effect on growth (LnRR=-0.024, lower=-0.035, upper=-0.012, Fig. 4.3b). This result implies that negative CO_2 effects are actually more severe at high food concentrations, perhaps because high food conditions support a larger scope for the effect of CO_2 to act on (Fig. 4.1c).

When I restrict the factorial analysis to the data used by Ramajo *et al.* (2016), I also find negative effects of CO_2 and positive effects of food on both calcification and growth responses. In addition, the interactive effect of food supply and OA on calcification is similar to the previous result with more studies (LnRR=0.10, lower=0.043, upper=0.15, Fig. S4.3b). However, using only data from Ramajo *et al.* (2016), I find that the interactive effect of CO_2 and food supply on growth is slightly positive (LnRR=0.03, lower=0.015, upper=0.048, Fig. S4.3d), which is in the opposite direction to the same analysis using more data (Fig. 4.3b). However, a change in sign of the interactive effect is an indication of extraction errors in the original Ramajo *et al.* (2016) dataset (Table S4.1), as re-analysis without extraction errors yields a non-interactive effect. Therefore, the negative interaction effect on growth is only detectable with a larger sample size.

Overall, there was no consistent tendency for the effects of OA on growth and calcification to be modified in the same way by food supply. I find that for calcification there is evidence that positive effects of food supply do mediate negative response to acidification. For growth responses, in contrast, I found a significant negative interactive effect using factorial analysis, and no response using multilevel meta-analysis. Although both statistically sound methods, factorial analysis likely has more power to accurately estimate the effect size of the interaction, as it directly calculates an interactive effect for each study (Gurevitch *et al.*, 2000), whereas in multilevel analysis, interactive effects (across studies) are inferred from the difference between levels of one main effect on the other main effect, after the main effects are calculated (Viechtbauer, 2010). In particular, the multilevel inference method may be less powerful than factorial meta-analysis for detecting if the effect of CO_2 is different between high and low food levels given that each study defines food levels differently. There is currently no statistical comparison of the robustness of each meta-analytic techniques for this kind of data, but factorial

meta-analysis has been described as particularly useful for understanding the growing list of multiple stressor studies (Crain *et al.*, 2008; Piggott *et al.*, 2015). The findings of the multilevel analyses align more with the findings of the individual studies making up the meta-analysis, where the interaction between food and OA was non-significant in 21 of 22 cases, although, it should be noted that the single significant interactive case revealed an increased OA effect on growth at higher food rather than a decreased one (Cole *et al.*, 2016) (consistent with Fig. 4.1c and findings of factorial meta-analysis on growth). The number of studies considered here and by Ramajo *et al.* (2016) is relatively low, although neither sensitivity nor bias analyses indicated cause for concern (see methods for details). It is also worth noting that some of the included studies did find interactive effects for other response variables where negative responses to OA were ameliorated under high food, including metabolic rate (Pan *et al.*, 2015), aragonite crystal length (Crook *et al.*, 2013) and, frequency of normal development (Cole *et al.*, 2016), suggesting food supply could be important in mediating other physiological and developmental responses to OA beyond calcification and growth responses.

There may be physiological mechanisms, yet to be explored, for the various hypotheses described above. Mechanistically, for added food to reduce the negative impact of OA (i.e., a positive interactive outcome), either feeding rates would need to increase to compensate for higher energetic demands, or energy acquisition from ingested food would need to be more efficient. The reverse would need to be true to support the negative interactive outcome, where OA impacts are most severe at high food supply. Currently, there is little evidence to support compensatory feeding under acidification. In fact, feeding and clearance rates have been shown to decline under acidification across a wide range of taxa, both herbivorous and carnivorous, encompassing a variety of feeding appendages and behaviours (Clements, 2016), which may

help to explain the negative interactive effect I found for growth. Similarly, available evidence does not suggest that increased assimilation efficiency is likely to be common under high CO_2 (Wang *et al.*, 2015; Sui *et al.*, 2016; Xu *et al.*, 2016). From a physiological standpoint, therefore, the mechanisms underlying the significant positive interactive effects on calcification described here are yet to be pinpointed. It should be noted, however, that reductions in search and handling times and increases in palatability under acidification (Cripps *et al.*, 2016; Duarte *et al.*, 2016) could influence an organism's energetic balance in such a way that OA costs are minimized by higher food availability, but most published experiments – including those in my meta-analysis – do not allow for these types of longer-term effects to emerge. Finally, it is important to recognize that food supply could mediate OA effects in a non-interactive scenario for one response variable (i.e. maximum metabolic rate), but appear interactive for another variable if the combined effects of OA and food limitation crosses some important physiological threshold (e.g., metabolic supply becomes insufficient to meet demand and the animal dies) or ecological threshold (e.g., population intrinsic growth rate, λ, drops below 1) (Kroeker *et al.*, 2017). To the best of my knowledge, these scenarios have not been explicitly tested.

Moving forward, I recommend that researchers take great care when framing and testing hypotheses related to the effects of food availability on responses to OA. The individual studies in this meta-analysis overwhelmingly found non-interactive effects, yet several authors use phrasing such as "food supply reduces the impacts of experimental OA" (Ramajo *et al.*, 2016), "failure to provide food can increase vulnerability to OA in experimental assessments" (Ramajo *et al.*, 2016), "food supply… can mitigate the negative impacts of future OA" (Pansch *et al.*, 2014), "feeding and energy availability can mediate reductions in growth due to OA stress" (Towle *et al.*, 2015), and "Zooplanktivory ameliorates the effects of ocean acidification"

(Edmunds, 2011). Others have pointed out this discrepancy, for example Drenkard *et al.* (2013) comment on the misclassification of non-interactive results as interactive by Edmunds (2011) and underscore that, despite misinterpretation, both of their datasets confirm that energetic status does not alter calcification sensitivity to ocean acidification. The authors of the above papers may have intended to convey that the negative effects of OA at low food availability can be *offset* by supplying more food. I believe the language used above could equally be interpreted as a modification of the effect of OA (i.e. an interactive effect) or an offsetting of the effect of OA (i.e. a non-interactive effect). Careful and deliberate consideration of language used to describe experimental results can help guide further mechanistic experiments to enhance our understanding of ocean acidification responses.

Many of the studies cited here were driven by observations in the field where ecosystems are observed to be healthy in the presence of high food despite acidic conditions (Melzner *et al.*, 2011; Thomsen *et al.*, 2013; Pansch *et al.*, 2014). In the absence of a non-acidic, high food 'control', however, it isn't clear if acidic conditions are truly having no effect, or if there is simply no difference between non-acidified, low food situations and acidified, high food situations. Of course, this does not rule out the broader contention that adding more food will allow organisms to maintain performance under increasing acidification; indeed, that outcome would be expected from a non-interactive scenario (compare the open downward-facing triangle to the filled upward-facing triangle in Fig. 4.1a). However, if this argument is applied to global change scenarios generally, it would be akin to comparing a present-day ocean with little food to a future ocean with an abundance of food. While it is certainly possible that OA can increase food availability (e.g., for some herbivores; Gaylord *et al.*, 2015), this will not be the case for all species, nor in all habitats, and the opposite can also be true (Polovina *et al.*, 2008). Unless

changes in food supply through time are known in advance, the proper comparison is the effect of OA (elevated vs. ambient CO_2) at low food supply compared to the effect of OA (elevated vs. ambient CO_2) at high food supply (Figure 4.1a-c solid and dashed lines, respectively). Overall, my results suggest that the ultimate role of food as a modifying influence on ecological responses to ocean acidification will depend on the relative importance of calcification and growth to fitness and population, community, and ecosystem dynamics. Further, I suggest that future changes in ocean acidity could affect systems of both high and low productivity and food availability, but the ecological effects may be difficult to predict without knowledge of the ways in which acidification indirectly affects food supply.

Understanding the distinction between interactive and non-interactive effects is important for furthering our mechanistic understanding of ocean acidification. If these effects are indeed non-interactive (as suggested by multilevel meta-analysis), we can make specific predictions about the effect of OA separately from the effect food, allowing us to have somewhat more confidence in cautiously predicting OA effects for food supply conditions not directly considered in the original studies. However, the negative interactive effect on growth (found in the factorial analysis) suggests that ecological contexts in which food is super-abundant may be more susceptible to OA effects than more food-limited systems, and the converse is true in terms of calcification. The presence of mixed interactive effects prevents us from generalizing broadly from a limited set of results as the outcome of one effect cannot be predicted independently from the other, and suggests that future OA impacts may vary meaningfully from one context to another.

Overall, I provide evidence that food supply both mitigates and worsens the negative effects of OA, depending on the response variable. Clearly, there remains much work to be done on this

topic. I agree with Ramajo *et al*. (2016) that researchers should, where possible, explicitly relate food supply levels to natural conditions (e.g., Drenkard *et al.*, 2013; Thomsen *et al.*, 2013) and organismal requirements, as interactive effects of food supply and OA could emerge when crossing ecological thresholds. Furthermore, if and when "low food" treatment levels equate to starvation of the organism, researchers should consider that other energetic processes, such as metabolic depression, are likely to become important and this could change the response to OA. More studies are needed to determine the full extent to which responses to OA change across a wide range of food supply levels and feeding modes. The careful selection of natural field-relevant food supply levels can help identify when responses to OA are likely to cross ecological thresholds, which can generate interactive outcomes at higher levels of biological organization even when the underlying effects are non-interactive. Given that both food supply and marine carbonate chemistry are changing simultaneously through time, a more detailed understanding of the inter-relationship of their effects, both physiological and ecological, remains a research priority.

Chapter 5: Energetic Context and Competition for Limiting Resources Determine Species and Community Responses to Ocean Acidification

5.1. Introduction

As atmospheric and oceanic carbon content continue to rise and track worst-case projections, ocean pH is declining at rates unprecedented in the geological record (Hönisch *et al.*, 2012; IPCC, 2013; Henson *et al.*, 2017). This rapid ocean acidification (OA) will likely have severe consequences for marine organisms (Kroeker *et al.*, 2013a; Wittmann & Pörtner, 2013). The majority of the recent deluge of acidification studies (Kroeker *et al.*, 2013a) have been conducted without consideration of food supply to experimental organisms. This is an important oversight because sub-lethal physiological responses to OA are likely the result of energetic (or ATP) trade-offs, where maintenance costs increase under stress at the expense of other processes like growth, reproduction and calcification (Wood *et al.*, 2008; Sokolova *et al.*, 2012). Acidification-driven ATP trade-offs towards acid-base regulation processes like protein synthesis and ion regulation, necessary for maintaining homeostasis, have been documented in both laboratory and field settings (Stumpp *et al.*, 2012b; Calosi *et al.*, 2013a; Navarro *et al.*, 2013; Pan *et al.*, 2015). Energetic supply, in the form of ATP, comes from the breakdown of carbohydrates, amino acids, and lipids; and food limitation is therefore a key constraint of total energy available for allocation (Sokolova *et al.*, 2012). Therefore, it is important to understand how food supply might combine with acidification, not only to put published work in perspective but also to better understand mechanisms underlying the observed energetic trade-offs.

Experiments that simultaneously manipulate food supply and CO_2 can help determine if higher energetic supply can modify the outcome of acidification-driven energetic tradeoffs. Energetic tradeoffs in response to stress can be inferred by identifying decreases in non-maintenance

organismal performance metrics (e.g., growth, activity, reproduction, storage) or increases in maintenance costs (e.g., metabolic rate) or direct changes in ATP allocation (Kooijman, 2010; Sokolova *et al.*, 2012). Most studies that address the relationship between food supply and ocean acidification measure organismal performance metrics like calcification and growth (Ramajo *et al.*, 2016, Chapter 4), although changes in ATP allocation have also been directly measured (Pan *et al.*, 2015). The expectation for these experiments is that increases in total energy supply should mitigate negative responses to OA at the organismal level (Ramajo *et al.*, 2016)(Chapter 4, Prediction Fig. 4.1b – positive interaction between food and OA). However, for growth responses, food supply has been shown to combine additively with acidification at the organism level (Fig. 4.1a, no interaction) across individual studies (Edmunds, 2011; Melzner *et al.*, 2011; Comeau *et al.*, 2013; Thomsen *et al.*, 2013; Crook *et al.*, 2013; Drenkard *et al.*, 2013; Hettinger *et al.*, 2013; Pansch *et al.*, 2014; Towle *et al.*, 2015; Pan *et al.*, 2015; Ramajo *et al.*, 2015; Cole *et al.*, 2016) or in fact slightly exacerbate the negative effects of acidification (Fig. 4.1.c, negative interaction, Fig. 4.3b), indicating that energy availability in the environment may not mediate bio-energetic mechanisms of OA at the individual level. This response could be in part due to food replete conditions supporting higher growth and therefore a larger scope for the effect of CO_2, but the underlying mechanisms driving this pattern of response at the individual level are difficult to determine, especially since food limitation is not explicit in every study's control.

Many acidification-driven energetic trade-offs at the organism level (e.g., growth rate, size, reproductive output) can influence population parameters like carrying capacity, population size, birth rate, and death rate (e.g., Dynamic Energy Budget (DEB) theory; Kooijman, 2010; Nisbet *et al.*, 2010; Muller & Nisbet, 2014). Some of the links between energetic trade-offs and a species' population dynamics require clear integration of species-interactions; for example,

decreased calcification under acidification could promote shell weakening that increases predation risk and therefore influences the calcifier's population death rate (Kroeker *et al.*, 2014). Integrating interacting populations of virtual species with a range of traits can lend insight into community properties like production, frequency distribution of species, and biodiversity (Maury & Poggiale, 2013; Enquist *et al.*, 2015). Processes like species interactions at small scales can produce patterns at large scales (e.g., intertidal zonation Wootton, 2001). Community patterns can also be maintained by energetic homeostasis of individuals under stress (e.g., consumption Ghedini & Connell, 2016). The extent to which cascading effects of a stress-induced energetic trade-off at the individual level have community level consequences have yet to be explored. Overall, there has been little consideration beyond the individual level in understanding the relationship between food supply and response to acidification (but see Pansch *et al.*, 2014). Here, I present results from a field-based mesocosm experiment at the community level manipulating phytoplankton food availability and quality and propose a conceptual framework for these results in competitively structured communities.

I examined species-level responses to OA and food availability and quality within the community context and how these responses align with theoretical predictions outlined in Chapter 4, modified for three food levels: natural phytoplankton from the water column (no food added) and naturally supplied phytoplankton supplemented with low- or high-quality phytoplankton diets. I examined three hypotheses about how increased food supply and elevated CO_2 could combine. First, I hypothesized (1) that some species' abundances will decline in response to acidification, especially those that use calcium carbonate to build and maintain their skeletons (Wittmann & Pörtner, 2013). Based on meta-analysis in Chapter 4, I next expected (2) that if individuals are food limited, adding any food beyond naturally occurring levels will

increase species abundance, and that a high-quality (high fatty acid phytoplankton) diet will have a stronger positive effect on abundance than a low-quality diet. Although, in a community setting, changes in food availability or quality could influence competition outcomes, and not all species may benefit from food supplementation (Chesson & Huntly, 1997). Using the simplified expectations of a positive effect of food addition and negative effect of acidification, these factors can combine (i) non-interactively, where these effects are independent (Fig. 5.1i), (ii) in a positive interactive way, where the combination of food addition and acidification has a positive (or less negative) effect on abundance when compared to the control response (Fig. 5.1ii), and (iii) in a negative interactive way, where the combination of diet and acidification has a negative effect on performance compared to the control response (Fig. 5.1iii). If individual growth influences abundance (% cover) in a community setting, following from the growth results of factorial meta-analysis in Chapter 4, I expected (3) that effects of food quality and acidification to combine interactively, such that negative effects of acidification on abundance are slightly greater under high food supply (prediction iii). Deviations from this response would indicate that there are other physiological or ecological mechanisms at play. Next, I observed spatial dominance patterns between the two most abundant species to test whether acidification or food supply or their combination alters the spatial landscape. I then determined whether community level properties, like species richness, community structure and biomass, tend to follow conventional expectations regarding increases in resource supply (i.e. that increases in food availability should increase productivity and species richness, Wright 1983; Cardinale *et al.*, 2009) and declines in pH (i.e. acidification) should decrease productivity and richness (Nagelkerken & Connell 2015).

5.2. Methods

5.2.1. Study System

This experiment was conducted on marine biofouling communities, sessile suspension feeding invertebrates. At my field site, these communities include colonial and solitary tunicates, barnacles, bryozoans, polychaetes, hydroids, and mussels. Mussels and hydroids at this site have been shown to be vulnerable to acidification (Brown *et al.*, 2016). A complete species list with abundance information can be found in Appendix D, Table S5.2. The two most dominant species were the invasive ascidian *Botryllus schlosseri* and the cryptogenic hydroid *Obelia dichotoma* (Gartner *et al.*, 2016). Marine fouling communities have long been used as model systems for studying patterns and processes in communities (e.g., stability, Sutherland, 1974). These quickly developing communities are ideally suited to studying recruitment and succession (Chalmer, 1982). Further, these communities typically have a high proportion of invasive to native species (Stachowicz & Byrnes, 2006), which make them well suited to studying species interactions.

5.2.2. Experimental Design

The twelve-week experiment was conducted at the Reed Point Marina in Port Moody, British Columbia (49°17′31″N, 122°53′25″W) from July to October 2015. The length of the experiment was considered enough time for fouling species at this site to colonize tiles and interact with each other (Brown *et al.*, 2016). I employed a field-deployed, flow-through mesocosm system, modified from Brown *et al.* (2016) by using more powerful pumps (to increase flow rate) and smaller mesocosms (to increase replication). Sixty semi-submerged translucent polyethylene mesocosms (25 L, N=60) were supplied continuously (~ 16 L per min) with unfiltered seawater pumped from 1 m depth to preserve natural fluctuations in temperature, salinity, and pH. One weighted PVC tile (14.5 cm x 14.5 cm, N=60) was suspended in each mesocosm and propagules

of fouling organisms settled naturally on shaded undersides of the tiles. Photographs of the tiles were taken once per week and percent cover of all species was analyzed by overlaying a grid of 100 points on each image. All photographic analyses were conducted in ImageJ (Schneider *et al.*, 2012). All tiles were preserved in 3% formalin for dry weight estimates and species ID. The undersides of the tiles were scraped of biomass, which was put in a 60 °C drying oven for 48 hours, then weighed.

Mesocosms were bubbled with air or a CO_2 mix, to create randomly assigned ambient (~545 ppm, n=30) or elevated (~1605 ppm, n=29) CO_2 conditions, respectively. One mesocosm was dropped from the analysis because the settlement tile was dropped and lost during measurement. Temperature, salinity, and pH (NBS scale) were measured twice per week at midday (using Yellowstone Scientific Instruments Model 85, Montana USA for temperature, salinity; Oakton 6 pH meter, Oakton Instruments, Illinois USA, for pH calibrated using National Bureau of Standards buffers). Water samples were taken once per week for dissolved inorganic carbon (DIC) analysis following Dickson (2007). I used these measurements to calculate the remaining carbonate parameters with the CO2SYS program (Pierrot *et al.*, 2006). Carbonate parameters are described in Appendix D, Table S5.1. While CO_2 is the unit of manipulation, CO_2 is difficult to measure in the field and pH is an accurate indirect measure of CO_2. Therefore, throughout the manuscript I refer to elevated CO_2 and low pH interchangeably. Elevated CO_2 mesocosms were intended to maintain consistently lower pH values than the controls, and while this was achieved, CO_2 addition and equilibration were not equal among mesocosms, which resulted in a range of average pH values in the elevated CO_2 treatment. This gave us the opportunity to view my results along a gradient of pH, as opposed to high vs. low pH.

Mesocosms were randomly assigned to be supplied with food either naturally from plankton and other suspended sources in the water that was pumped through them (no food added, n=20), or this natural food supply supplemented four times per week (~9.75 mg C mesocosm^{-1}week^{-1}) with concentrated dead algal cells of green flagellate *Tetraselmis* sp. (n=20) or diatom *Thalassiosira weissflogii* (n=19) (Brightwell Aquatics, USA). These two taxa are found worldwide in marine and brackish waters, are similar in size (between 8 and 20 μm), and are of a size class which many suspension feeders in fouling communities can consume (Mook, 1981). *Thalassiosira* spp. typically have higher fatty acid content (including triacylglycerols, sterols, and importantly Eicosapentaenoic acid - EPA, an omega-3 fatty acid, known also as 20:5(n-3)) than *Tetraselmis* spp. (Volkman *et al.*, 1989; Brown, 1991) and values reported by the manufacturer of the mixtures support this claim. In addition to phytoplankton, the mixtures contain purified water, proprietary amino acids, Ascorbic acid, and Citric acid. I used a FlowCAM (Fluid Imaging, USA) to estimate cell counts per mL of each concentrated diet. I then varied the amount of each diet going into the mesocosms by matching carbon content (~97500 pg C/mL once mixed in the 25 L mesocosms) but not cell counts of the two different phytoplankton species (Mullin *et al.*, 1966; Verity *et al.*, 1992; Montagnes *et al.*, 1994). Food was added to mesocosms in pulses four times a week (twice in one day, separated by 8 hours, every 3-4 days). During feeding, the pumps to all mesocosms (including the no food added treatment) were turned off to prevent the added food from being immediately flushed out. Each mesocosm (including those in the no food added treatment) was stirred after food addition then allowed to sit for 30 minutes, which should be enough time for a developed fouling community to consume phytoplankton at these concentrations (Lesser *et al.*, 1992). Note that this method was chosen for ease of application, and may not reflect natural phytoplankton bloom dynamics.

5.2.3. Statistical Analyses

I used generalized linear models (GLMs) to understand how the effect of low pH varied with food availability and among species by the end of the experiment (week 12) in R (Version 3.3.1)(R Development Core Team, 2009). Although I found 24 species overall, many were in low abundances, so, for this analysis, I only used species with a treatment mean of at least 1% cover or 1 individual (10 species total). When possible, I used counts as a more accurate representation of abundance. For all models, I graphically assessed the data distribution fit using probability plots using the *fitdistrplus* package (Delignette-muller & Dutang, 2015) and then used the most appropriate distribution in analysis using GLMs: numerical response variables were assumed to be Poisson distributed and percent cover (out of 100 points) or proportional data were treated as binomial responses. I fitted the models with orthogonal contrasts and tested with analyses of co-variance with likelihood-ratio chisquare and Type III Sum of Squares.

I regressed species abundance across concentration of hydrogen ions ($[H^+]$), instead of pH, in order to capture the log-scale of the measured pH in my statistical analyses. Although all calculations were made in $[H^+]$, the x-axes demonstrate how pH changes on a log scale and refer to this unit in pH units throughout the results for ease of interpretation. We chose this method of visualization to emphasize the log nature of pH, which is often ignored or considered linear. The values of $[H^+]$ used in the analysis came from the minimum tenth percentile of pH values across the entire experiment (12 weeks). I used this method because 1) it provides a higher resolution look at the effect of CO_2 when compared to treating CO_2 as a categorical variable, 2) it more appropriately captures variation in shallow coastal pH values (e.g., even where no CO_2 is added, 10% of the pH values are on average 7.8 pH), 3) the 10% value allows us to capture the lowest pH values of the experiment in each treatment in order to detect a lower limit of pH in biological

response (e.g., is there a lower limit to abundance for a given species, after which abundance drops off dramatically?). I also calculated response to average $[H^+]$ and presented these similar results in Appendix D (deviations from minimum $[H^+]$ are highlighted in the text).

To test my first hypothesis (1), I first looked at how $[H^+]$ influenced species abundance across all food treatments. Here, I adjusted p-values to control for a false discovery rate (Benjamini & Hochberg, 2000) to account for multiple comparisons when comparing across the ten most abundant species (De Cáceres *et al.*, 2010). Next, if food treatment or the $[H^+]$ * food treatment was significant (Hypotheses 2, 3), I conducted pairwise Wald tests comparing the "no food added" treatment to the "low-quality" and "high-quality" diets respectively and their interaction with $[H^+]$ and corrected for multiple comparisons. Similarly, to understand how species richness, evenness, space occupation, and biomass changed with acidification and food availability, I regressed these variables against $[H^+]$. I constructed a linear model (LM) for evenness and GLMs with binomial, Poisson, and Gamma distributions for space occupation, number of species and biomass respectively, with $[H^+]$ and food treatment as fixed crossed factors. The appropriate distribution for each model was chosen after visualizing the data fit to candidate distributions.

To understand how food availability, quality and their possible trade-off with physiological effects of OA influenced spatial dominance between the two most abundant species (the invasive ascidian *Botryllus schlosseri* and the cryptogenic hydroid *Obelia dichotoma*), I calculated a ratio of the relative abundance of these two species (% cover of each species + 0.5%, to avoid problems associated with dividing by zero). I took the ln ratio of these species to centre results around 0, where positive values indicate that the ascidian dominated and negative values indicate dominance by hydroids. I used a linear mixed effects model, including week as a fixed effect and mesocosm within week as a random effect (allowing for both an uncorrelated random intercept

and slope), using the *lme4* package (Bates *et al.*, 2010), to understand how this ratio changed over time and with treatments. I tested this multi-level model using an ANOVA with Type III sum of squares and a Kenward-Rogers approximation for degrees of freedom, which is appropriate for small sample repeated measures designs with complex covariance structures (Schaalje *et al.*, 2002).

To analyze multivariate community data to test whether food quality, quantity and OA affected community-level patterns, I used permutational routines on all 24 species over time and at the final time point. I first used principal response curves (Van Den Brink *et al.*, 1999) to evaluate multivariate community structure over time using the *prc* function in the *vegan* package (Oksanen *et al.*, 2015b). I next standardized by total percent cover of a given species across all tiles, within a given week, to better capture changes in rare species. Next, I performed a Hellinger transformation to this data, as the similarity index used in *prc* is Euclidean distance (Legendre & Gallagher, 2001). This method is a useful graphical tool but at this point, we are unable to statistically use this tool to analyze two interacting fixed effects. We can subset the data to test for overall effects of food availability and acidification by using pairwise permanovas (corrected for multiple comparisons) that permutate the data at each time point (Oksanen *et al.*, 2015b). To understand how community structure varied among treatments within mesocosms, I used constrained analysis of principal coordinates (*capscale*). I used week 12 of the experiment and standardized the data in the same way as above. I then calculated a Bray-Curtis (BC) resemblance matrix and used the *adonis* function, a permutational multivariate analysis of variance, to the effects of diet and acidification on community structure, and *Betadisper*, a test for homogeneity of multivariate dispersions, to understand community variability under acidification and diet.

5.3. Results

5.3.1. Species Responses Within a Community Context

Substantial variation in species responses to food addition and experimental acidification was found for both minimum 10th percentile of pH (Fig. 5.1) and average pH (Fig. S5.1). Inconsistencies between response to minimum 10th percentile of pH and average pH are noted in the text, statistical tests found in Appendix D, Tables S5.5 and S5.6. Of the ten most abundant species, eight responded significantly to acidification (Fig. 5.1; results of statistical tests can be found in Appendix D, Table S5.3). The barnacle *Balanus crenatus* and the nudibranch *Hermissenda crassicornis* had reduced abundance under low pH, regardless of food treatment (no food added, high-quality food added, or low-quality food added), and there was no interaction between pH and food treatment in either case (Fig. 5.1f, i, Table S5.3). For other taxa, however, any significant main effects of pH cannot be interpreted in isolation given a number of significant interactive effects between food treatment and pH (see below). Only a single species, a calcified bryozoan *Disporella* sp., had no response to acidification, food treatment, or their interaction (Fig. 5.1g).

Pairwise comparisons between each diet and the control (no food added but phytoplankton available from the water column) suggested that neither diet had a consistent effect in magnitude nor direction across all species and most species responded to one of the two diets only (results of these statistical tests, corrected for multiple comparisons, can be found in Appendix D, Table S5.4). Many of these responses had interactions with pH (discussed below). Two taxa experienced direct effects of a single diet with no main or interactive effect of pH (non-interactive outcomes, theoretical prediction i, Fig. 5.1): the invasive ascidian *Botryllus schlosseri* and the invasive caprellid, *Caprella mutica*. Relative to the control food treatment, *B. schlosseri*

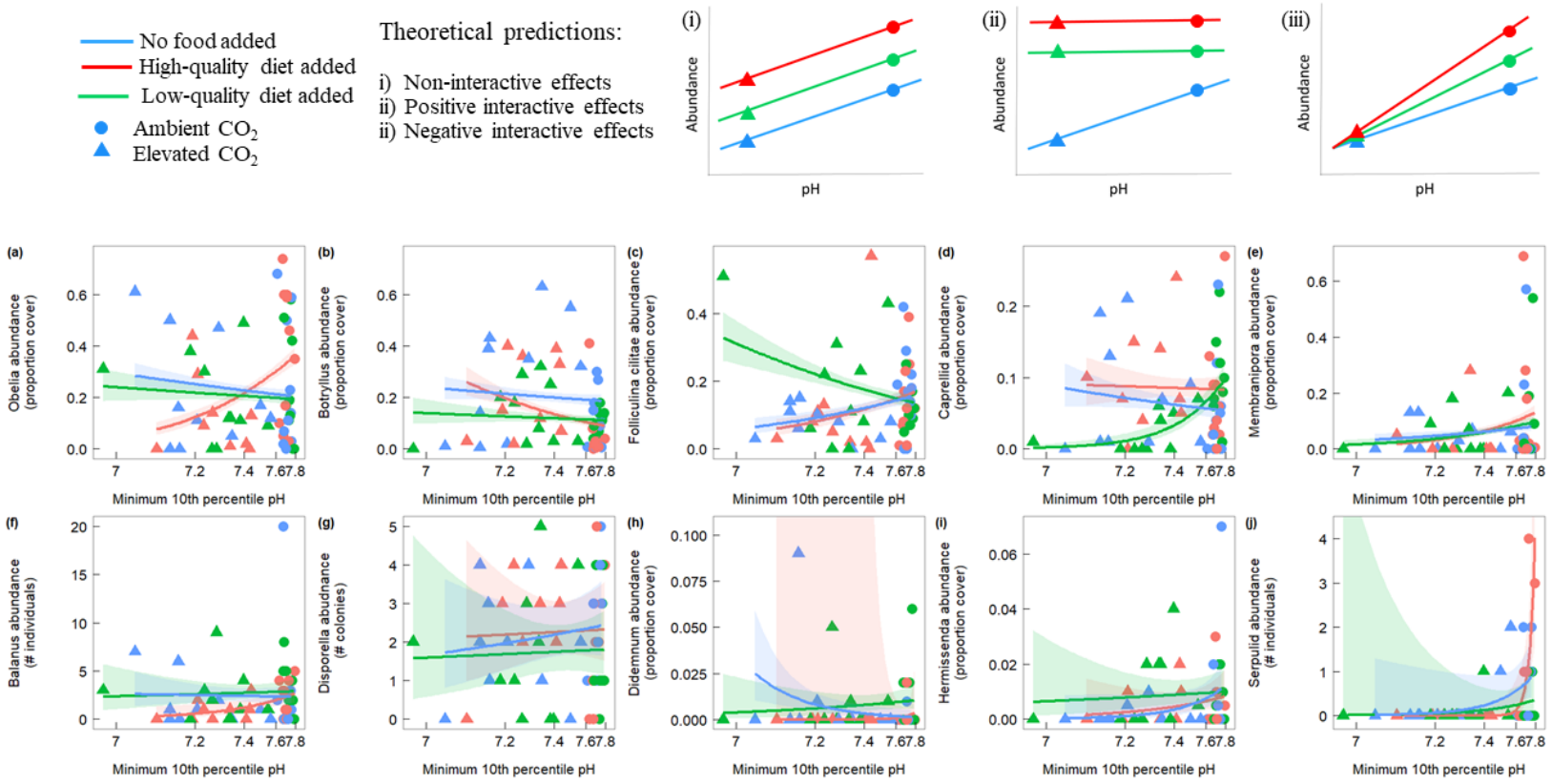

Fig. 5.1. (i-iii) Theoretical predictions for the combination of food supply and acidification (modified from Chapter 4, Fig. 4.1a-c with an additional food level) and (a-j) data from the last time point of the experiment for ten of the most abundant species. A positive effect of food addition and negative effect of acidification can combine (i) non-interactively, where these effects are independent (i.e. green or red slope = blue slope), (ii) in a positive interactive way, where the combination of food addition and acidification has a positive (or less negative) effect on abundance when compared to the control response (i.e. green or red slope > blue slope, reading slope from right to left), and (iii) negative interactive effects where the combination of diet and acidification has a negative effect on performance compared to the control response (i.e. green or red slope < blue slope, reading slope from right to left). Regressions of species abundance (% cover and # individuals or colonies) across minimum tenth percentile of hydrogen ion concentration values across all weeks (here represented as pH which is -log([H+]) for illustration purposes). Circles represent data from tiles in ambient CO_2 mesocosms and triangles represent those from high CO_2/low pH conditions. Blue symbols and regression lines are from mesocosms with ambient food supply (organisms fed on plankton and other suspended matter coming in from pump), whereas red and green lines and points represent data from added phytoplankton in a high-quality diet (*Thalassiosira* sp.) and low-quality diet (*Tetraselmis* sp.), respectively. Selected species had mean cover greater than 2%. Error bars represent 95% confidence intervals on a binomial model for % cover and Poisson model for counts (n=10 for all treatments, except 9 for high-quality food, low pH).

had an overall negative response to the *Tetraselmis* (low-quality) diet (Fig. 5.1b, Tables S5.3, S5.4), whereas *C. mutica* had an overall positive response to the *Thalassiosira* (high-quality) diet (Fig. 5.1d, Tables S5.3, S5.4).

Seven of the ten species exhibited significant interactive effects of acidification and food supply. There were no species that demonstrated interactive effects that met the commonly-stated expectation, where a positive overall effect of diet mitigated the negative overall effects of acidification, creating an interactive effect of diet and CO_2 that was positive (Ramajo *et al.* 2016, theoretical prediction ii Fig. 5.1). Two species did exhibit positive interactive effects, where beneficial effects of a diet emerged under acidification. The soft-bodied invasive ascidian, *B. schlosseri*, was the only species that benefitted significantly from acidification across food supply conditions and in particular, the high-quality diet. The tectinaceous tube-dwelling protist *Folliculina* sp. benefitted from the low-quality diet under acidification. The benefits for these two species only emerged under low pH conditions and, for *Botryllus*, abundance was reduced when fed *Thalassiosira* under control pH conditions (Fig. 5.1b,c, Tables S5.3, S5.4). Most interestingly, some species exhibited responses that do not match the explicit predictions made thus far (but see Chapter 4) – where the benefit of a given diet disappeared under low pH conditions (high-quality food: calcified *Membranipora membranacea* and serpulid worms, Fig. 5.1e,j; low-quality food: *Didemnum* sp., Fig. 5.1h, Tables S5.3, S5.4) or where additional food supply exacerbated or brought out the negative effects of CO_2 (high-quality food: dominant non-calcified hydroid *Obelia dichotoma* and trending for calcified barnacle *Balanus crenatus* Fig. 5.1a,f; low-quality food: *C. mutica* Fig. 5.1d, Tables S5.3, S5.4). I did find clear overall negative effects of low pH on the abundance of *M. membranacea* and serpulid worms despite the presence

of these negative interactions, but the main effects are not clearly interpretable for hydroids or barnacles.

It should be noted that when measured using average pH, some of the interactive responses were altered (Fig. S5.1, Statistical results found in Tables S5.5, S5.6). *M. membranacea* abundance declines in response to average pH and increases in response to high-quality food but there is no significant interaction between these factors (Fig. S5.1e). Similarly, serpulid recruitment was lower under average pH but there was no longer an interaction with high-quality food (Fig. S5.1j). Response by *C. mutica* is altered slightly when measured under average pH: a significant interaction emerges where under *Thalassiosira* diet there was no average response to acidification but under ambient food supply caprellid abundance increased with acidification (Fig. S5.1d).

5.3.2. Dominance Switching Between the Two Most Abundant Species

I found that the ratio between the two most abundant species, the hydroid *Obelia dichotoma* and the ascidian *Botryllus schlosseri*, over the entire experiment was not consistent through time (Fig. 5.2). Initially hydroids were far more dominant than ascidians and covered over 20 times more space, but, over time, ascidians increased in abundance relative to hydroids and, in some cases, came to occupy more space than hydroids (Table S2; GLM, Week: F=107.12, P<0.0001). Over time, an overall effect of acidification emerged, where tiles in elevated CO_2 (low pH) conditions had a higher proportion of ascidians, and, by the end of the experiment, tended to be dominated by ascidians, while ambient CO_2 conditions were still dominated by hydroids (GLM, CO_2*Week: F=107.12, P=0.0094). There was no overall effect of diet or an interaction between

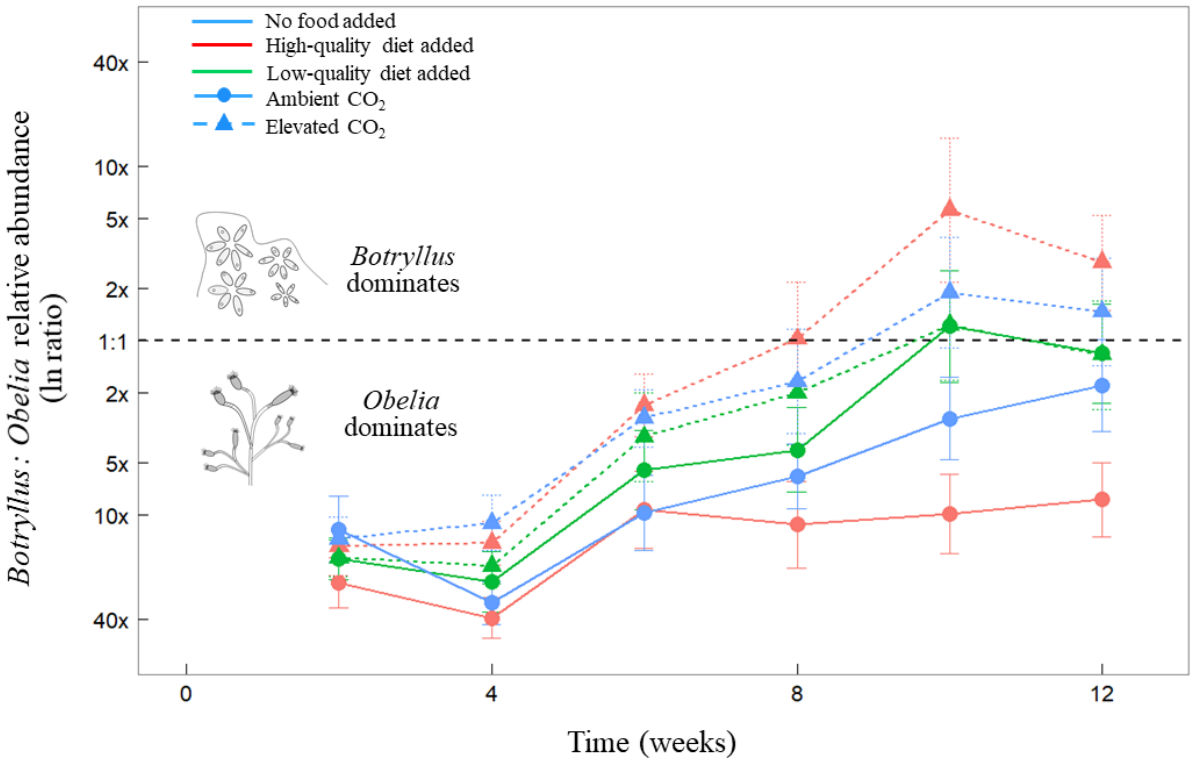

Fig. 5.2. Ln of the relative abundance of ascidian *Botryllus schlosseri* and hydroid *Obelia dichotoma*), with values on the y-axis transformed to represent the magnitude of differences in abundances over the course of the experiment. 1:1 ratio line indicates where the two species are in equal abundance. Colours represent food addition treatments, where blue is no food added where organisms only fed on natural phytoplankton, green is low-quality diet added and red is a high-quality diet added. Circles and solid lines represent data from ambient CO_2 conditions, while dashed lines and triangles represent data from elevated CO_2 conditions. Error bars represent standard error (n=10 per treatment, save 9 for high quality diet under elevated CO_2).

time and diet. However, an interaction between acidification and diet did emerge over time, where the effects of acidification described above became more extreme under a high-quality diet (GLM, CO2*Food quality*Week: F=107.10, P=0.039).

5.3.3. Community-level Response to CO_2 and Food Supply

Species richness within mesocosms by the end of the experiment decreased in low pH conditions but there was no overall effect of food quality or quantity, or an interaction between acidification and food availability (Fig. 5.3a, Table S5.4). The overall decline in richness reflects the aggregate species-level responses where a number of species declined in abundance in response to acidification for at least one of the diets. Evenness among species within mesocosms was not affected overall by acidification but was influenced by the interaction between diet and acidification such that communities fed high-quality diets had low evenness under ambient pH conditions (when dominated by hydroids) but evenness increased under low pH conditions (when communities were dominated by ascidians) (Fig. 5.3b, Table S5.4). This effect of high-quality food and its interaction with pH was only apparent when using 10^{th} percentile $[H^+]$ and disappears when regressed against mean $[H^+]$ (Fig. S5.2, Tables S5.5, S5.6).

The total amount of space occupied was significantly influenced by the interaction between diets and acidification, but the significant overall effects of acidification and each food quality treatment are difficult to interpret given this interaction (Fig. 5.3c, Tables S5.3, S5.4). Under ambient CO_2 conditions, the communities fed *Tetraselmis* covered significantly less space than those under control conditions, and those fed *Thalassiosira* covered significantly more space than the controls. Under acidification, these patterns were reversed: low-quality diet communities occupied more space than the control diet and high-quality diet communities took up the least amount of space on the tiles. Total biomass was not influenced by acidification, food supply, or their interaction (Fig. 5.3d, Table S5.4).

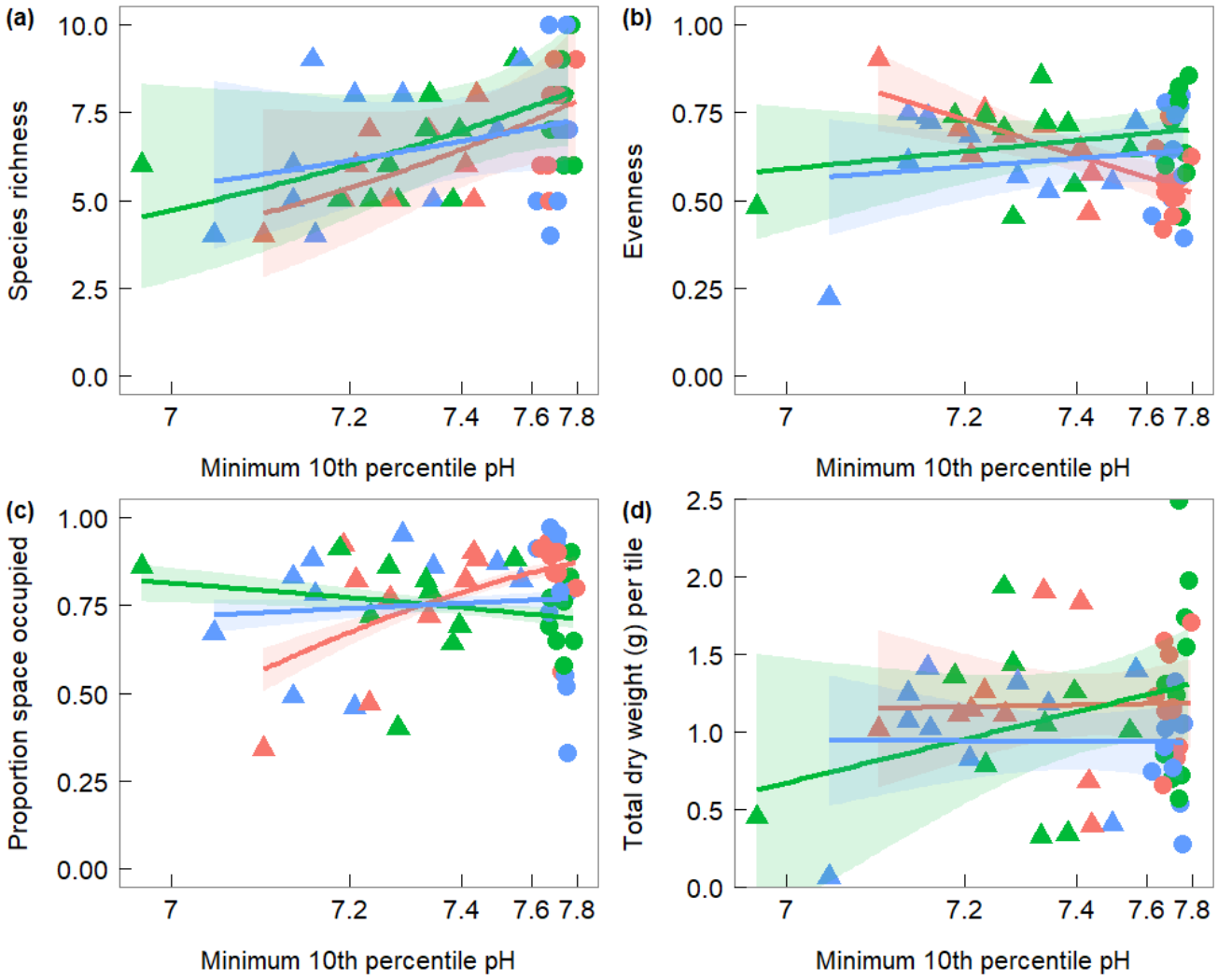

Fig. 5.3. Regression of end of experiment (a) species richness, (b) evenness, (c) proportion space occupied per tile, and (d) biomass per 1% cover of tiles across minimum tenth percentile of hydrogen ion concentration, represented as pH as in Fig. 5.1. Colours and shapes as in Fig. 5.1. Error bars represent 95% confidence intervals on a binomial model for % cover and Poisson model for counts (n=10 for all treatments, except 9 for high-quality food, low pH).

Principle response curve analysis over the course of the experiment revealed different trajectories for the various elevated CO_2 and food-addition treatments when compared to the no-food control (Fig. 5.4a). This analysis also plots species scores, which indicate relative contributions to the community level results. Overall, I found a significant effect of high CO_2 (dashed vs. solid lines, Permutational ANOVA, F=16.08, *P*=0.005) trajectories, but not an overall effect of food quality (red vs. green vs. blue lines, Permutational ANOVA, F=2.06, *P*=1.00). Pairwise tests (corrected for multiple comparisons) indicate that within the no food added and the low-quality food treatments, there was no significant difference between ambient and high CO_2 conditions (no food added: solid blue vs. dashed blue lines, Permutational ANOVA, F=4.56, *P*=0.885; low-quality food added: solid green vs. dashed green lines, Permutational ANOVA, F=2.01, *P*=1.00). However, within the high-quality food treatment, there was a significant difference between community level responses at ambient vs. elevated CO_2 (no food: solid red vs. dashed red lines, Permutational ANOVA, F=19.45, *P*=0.005).

At the final timepoint, acidification had a small but significant effect on community structure, but not food or the acidification x food interaction (Fig 5.4b; PERMANOVA, [H+]: F-model=1.91, R^2= 0.032, *P*=0.030; Food quality: F-model=1.31, R^2= 0.044, *P*=0.15, [H+]*Food quality: F-model=1.33, R^2= 0.044, *P*=0.13). The differences under acidified conditions were not driven by homogenization, as multivariate dispersion was similar between CO_2 levels (Betadisper, F =0.26, *P*=0.61) and Food supply levels (Betadisper, F =0.16, *P*=0.85).

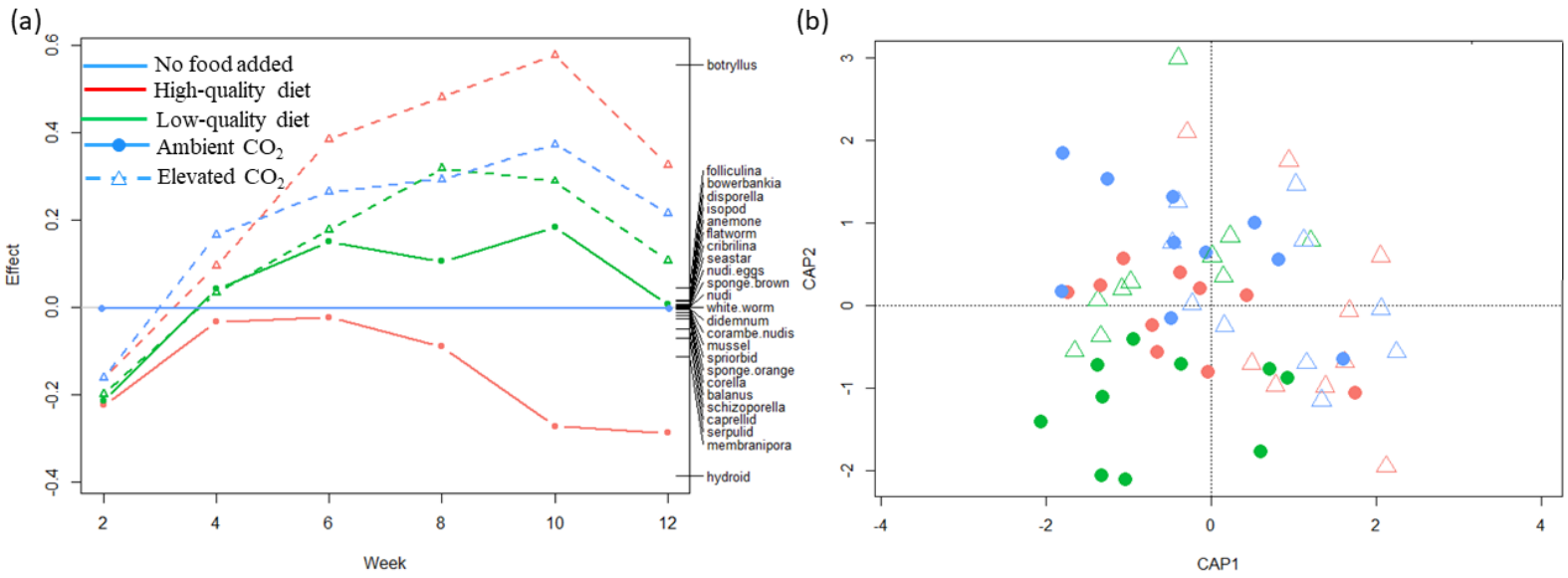

Fig. 5.4. Community structure relationships over time using principal response curves (a) and by the end of the experiment using constrained analysis of principle coordinates (b). Colours and symbols are as in Fig. 5.1, (n=10 for all treatments, expect 9 for high-quality food, low pH). (a) the solid blue line represents the control communities and the other treatments deviate from this line in terms of relative abundances of different species. The contribution of different species to the community effect axis is shown on the right. (b) ordination plot of constrained principal coordinates showing the relationship between communities in different treatments.

5.4. Discussion

Here I test the hypothesis that food supply can mitigate the negative effects of acidification in a community setting, by examining both species-specific and community-wide responses to increased CO_2 (measured as decreased pH) and increased food supply. There is some evidence for the direct effects of acidification and food supply on both species and community-level responses but these patterns are dwarfed by the interactive responses. For species abundance and community measures, I find little support for the most commonly stated hypothesis – that food addition interacts with acidification statistically and ameliorates its negative effects – and instead find evidence for the exacerbation of negative effects of acidification under food addition. I

hypothesize that some of these patterns, described below, could be driven by competitive interactions.

5.4.1. Species-level Direct Effects

A broad array of taxa in the fouling community were affected by experimental acidification, food supplementation, or both. To my knowledge, this is the first record of response to acidification by spirotrich ciliates (here *Folliculina* sp.), *Didemnum* sp. ascidians and nudibranchs. Zooplanktonic ciliates in large-scale mesocosms have been shown to be generally resistant to changes in abundance with increase CO_2 (Lischka *et al.*, 2017). The resistance to acidification under ambient food supply for *Didemnum* sp. is unsurprising, as ascidian abundance has been shown to be largely unaffected by natural acidification (Brown *et al.*, 2017). Declines in overall nudibranch abundance (combined data from egg casings and adult sightings) in response to acidification is concerning for this predator in fouling communities (Epelbaum *et al.*, 2009; Willis *et al.*, 2017). It should, however, be noted that nudibranchs are mobile and were perhaps actively avoiding low pH conditions. Caprellids, like other amphipods, have been found in high abundances under low pH conditions at natural vent sites (Cigliano *et al.*, 2010), while here I found acidification increased caprellid abundance but only under ambient food supply. Heavily calcified serpulids have been shown to decline in response to experimental and natural acidification (Donnarumma *et al.*, 2014; Li *et al.*, 2014; Lucey *et al.*, 2016; Brown *et al.*, 2017) and here I did not find any recruitment below a minimum pH level of 7.4; it is clear that this taxon will be a "loser" under ocean acidification. Declines in *Membranipora* abundance with acidification described here are in direct contrast to increases in abundances in response to similar experimental conditions in the same location (Brown *et al.*, 2016). This difference in response could be due to differences in environmental conditions between years or difference in

the competitive environment (the role of competition for dominant space-holders is considered in more detail below). Bryozoans have been shown to have a wide range of responses to acidification, and the resistance by *Disporella* across all food supply levels is one of a number of interesting demonstrations of resistance by calcifying species. While calcification is widely considered a strong predictor for sensitivity to acidification (Wittmann & Pörtner, 2013), the variability in response across and within calcified taxa found here and elsewhere (e.g., Calosi *et al.,* 2013b; Busch & McElhany 2017) highlights the need to identify mechanisms of acidification resistance in calcifiers (e.g., dolomite calcite, Nash *et al.,* 2012, although see limitations to using mineralogy, Busch & McElhany 2017), susceptibility in non-calcifiers (e.g., acid-base balance, Melzner *et al.,* 2009) or other traits that may influence response to environmental stressors (e.g., coloniality, Swezey *et al.,* 2017).

Contrary to theoretical predictions for species in isolation, food supplementation did not increase abundance in all species. This finding could be consistent with satiation, if food provided to the mesocosms from phytoplankton in the water column coming in via the pump was abundant. However, it is likely that pump flow does limit food intake as the species within mesocosms grow more slowly and reach a lower biomass than those grown outside of mesocosms (N. Brown *personal observation*). Another consideration is that not all suspension feeders consume the same partition of the plankton and the chosen diet provided to these communities may have been outside the size range of some species or some species may be selective in consumption. Only in one species (*Botryllus schlosseri*) did abundance decline with food addition (of *Tetraselmis* diet) at all pH levels. This could be a direct result of either toxicity of this particular diet or a physical deterrent to feeding (e.g., filtration blocking) to this species. But given that this diet has similar

size range and added chemical components as the *Thalassiosira* diet, these possibilities do not appear likely.

5.4.2. Species Interactions Could Generate Unexpected Species-level Responses to Low pH and Food Supply

I found that for some species, food can increase abundance at one end of the pH spectrum – relative to the ambient food supply conditions – while depressing abundance at the other. I hypothesize that this pattern (here observed as a negative interaction for hydroids, caprellids and serpulids, and a positive interaction for ascidians and folliculinids) could be generated by inter-specific interactions. Using an inferred interaction between the two most abundant species in my system, I develop a simplified conceptual model that describes potential responses to acidification under different food conditions in isolation and in competition (i.e. in a community setting). This conceptual idea is a first attempt to understand how species interactions could modify an underlying energetic model in response to acidification stress.

I assume that direct response to food addition is positive in both species in isolation. In my simplified model, the hydroid *Obelia dichotoma* decreases in abundance in response to low pH at all food supply levels (Fig. 5.5, left-hand panels), while *Botryllus schlosseri*¸ the invasive ascidian, increases in abundance in response to low pH at all food supply levels (Fig. 5.5, right-hand panels). First, I might expect that, in isolation, the positive effects of food supply are largest when environmental conditions are favourable, based on growth results from the meta-analysis in Chapter 4 (Fig. 5.1 prediction iii, Fig. 5.5a,b). Note that negative interaction is in reference to the overall effect of the interaction on the organism. Next, I expect that in a multi-species

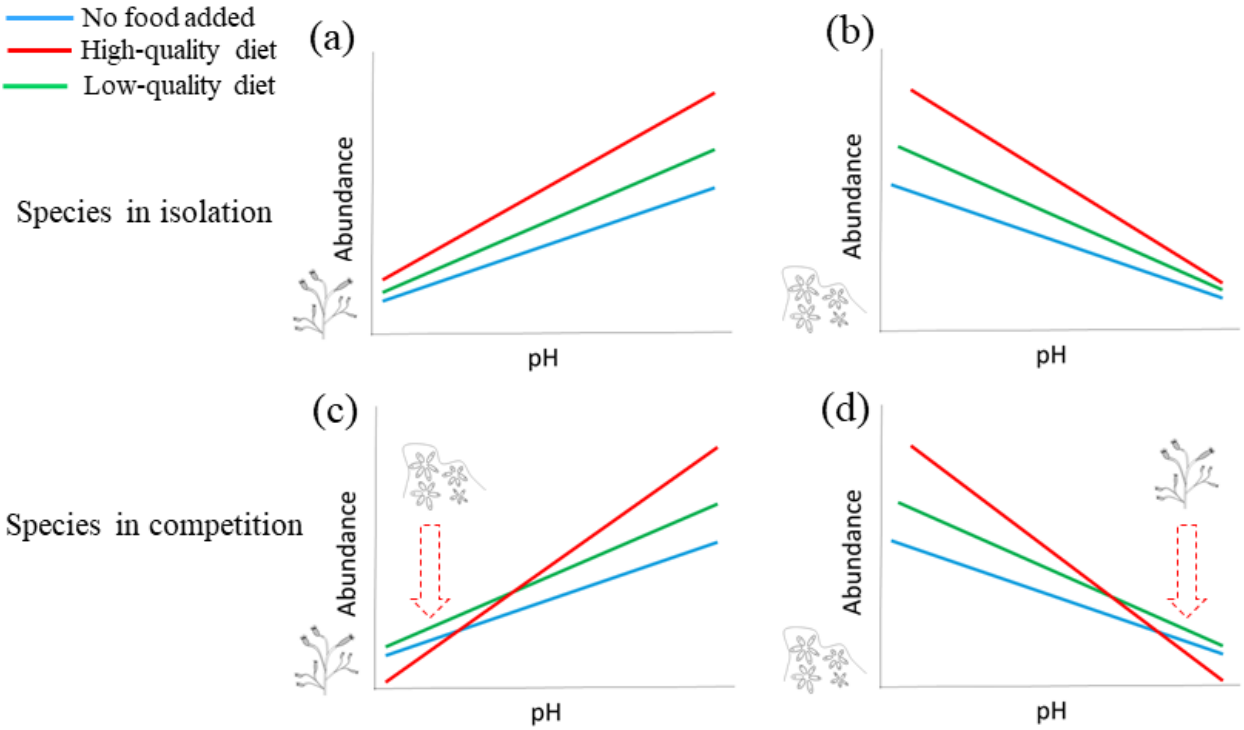

Fig. 5.5. Theoretical predictions for how the combination of acidification and food addition might change depending on if the species under consideration is in isolation vs. in a competitive environment. I expect that food supply will have a larger effect when environmental conditions are favourable, both for species that respond overall (a) negatively (e.g., hydroid) or (b) positively (e.g., ascidian) to acidification and overall positively to food addition. I then hypothesize that presence of a competitor in a given pH range would depress the abundance of the focal species in that pH range, below ambient food supply (i.e. red lines cross blue lines). For example, if the ascidian can take up the high-quality diet (red lines) under low pH conditions, the abundance of the hydroid in this treatment is reduced under those pH conditions (c). Similarly, if hydroids that take up high-quality diet under high pH conditions could reduce abundance of ascidians under high pH conditions (d). Compare panels c and d to Figure 1 a and b, respectively.

Assemblage, shifting competitive balances under acidification (Kroeker *et al.*, 2013c) could outweigh any benefits of food addition and depress the abundance of the inferior competitor below ambient food treatments (i.e. lines crossing). In my example, ascidians that do well under acidification displace hydroids in that treatment (Fig. 5.5c), whereas under control pH conditions, hydroids are able to take advantage of high-quality food under control pH conditions and displace ascidians (Fig. 5.5d). Therefore, here I might understand interactive effects of low pH and food availability as a result not of the organisms' direct responses to these two factors but as a result of indirect effects (species interactions).

My conceptual example is based on the pattern I see in the data, where I found a significant interactive effect of food and low pH on hydroid abundance (Fig. 5.1a, Tables S5.3, S5.4), which could be a result of interaction with ascidians that responded positively to acidification under all food conditions and especially under high-quality-food (Fig. 5.1b, Tables S5.3, S5.4). Both of these species exhibited depressed abundances below ambient food supply conditions at one end of the pH spectrum. The outcome of competition has been shown to be influenced by acidification in a number of systems (Diaz-Pulido *et al.*, 2011; Connell *et al.*, 2013; McCormick *et al.*, 2013b; Short *et al.*, 2014; McCoy *et al.*, 2016). Indeed, it is possible that temporal variation in competitive effects at my study site may explain the change in sign of acidification effects on the bryozoan *Membranipora membranacea* from positive in an earlier experiment, where acidification reduced the abundance of competitively dominant mussels (Brown *et al.*, 2016), to negative in the present experiment, where acidification may have reduced *Membranipora* cover directly or perhaps indirectly via enhanced abundance of competitively dominant ascidians. In all of these examples, it is unclear if these changes in dominance were due to direct effects of acidification on abundance (i.e. population size of given species N_1 or N_2

in a Lotka-Volterra competition model) or effects on the competition coefficient (i.e. effect of species 1 on species 2, α_{12}, and vice versa, α_{21}) (Macarthur & Levins, 1967). Further experiments are necessary to disentangle the mechanism: growing each species in isolation (i.e., identifying the patterns in Fig. 5.5a,b) and combination (both species, Fig. 5c,d) would allow us to fully understand if response to acidification and food availability are changing with the presence of an interspecific competitor and conversely how the per capita effects of each species on the other changes with resource availability and acidification (Gaylord *et al.*, 2015).

Competition is the most likely candidate for this pattern, as there is not strong evidence for other explanations. For example, toxicity of the algal species could increase under acidification from increases in disease load (Williams *et al.*, 2014) or production of toxic compounds (Jin *et al.*, 2015), but this is unlikely as phytoplankton cells were dead and only in acidified conditions for a short time. Increased predation pressure under acidification combined with a desirable high-quality resource (i.e. hydroids fed high-quality food) could be responsible for depression in abundance of hydroids, however the sole candidate predators in the system, the nudibranch *Hermissenda crassicornis*, did poorly under acidification (Fig. 5.1i). These nudibranchs can also feed on ascidians so reduced ascidian abundance under high-quality food and ambient pH conditions could be a result of predation by this species, although *H. crassicornis* has been shown to prefer feeding on hydroids over *B. schlosseri* (Epelbaum *et al.*, 2009). Furthermore, nudibranchs have been shown to preferentially feed on hydroids that are themselves feeding (kleptopredation, Willis *et al.*, 2017), but whether this mechanism extends to ascidians or if nudibranchs can detect high-quality prey required further investigation.

5.4.3. Shifting Competitive Outcomes as a Result of Changes in Resource Supplies and Environmental Conditions

Over the course of the experiment, in most treatments, the ratio between hydroids and ascidians appeared to move away from hydroid dominance towards invasive ascidian dominance. However, hydroids fed high-quality food were able to maintain dominance, but only under ambient CO_2 conditions. These spatial dominance patterns could be driven by direct effects of acidification or food supply on each species, changes in competition between dominant species under acidification and/or increased resource supply, or a combination of these effects.

Although hydroids have previously been reported to be sensitive to acidification (Brown *et al.*, 2016), here I found no direct effect of acidification under control or low-quality diets, and the negative effect of acidification only emerged under high-quality food. Ocean acidification is expected to increase the abundance of nuisance species like invasive species and typically weedy organisms (Hall-Spencer & Allen, 2015) although there is no empirical evidence to support changes in invasion processes, impact or biotic resistance under acidification. Ascidians are particularly successful invaders (Zhan *et al.*, 2015) and some species have been shown to be resistant to acidification (Brown *et al.*, 2017). Since tiles were not at 100% cover, I expect that succession here had yet to reach equilibrium, and potentially the ascidians might eventually outcompete hydroids in all treatments.

Subtidal sessile invertebrate communities typically compete for both food (exploitation) and space (interference) and it can be hard to disentangle these effects (Buss & Jackson, 1981). Here, resource type likely matters, as I found maintenance of hydroid dominance only under high-quality diet and not low-quality diet or control diet. Complicating matters further are differences in morphology (ascidians are generally prostrate, whereas hydroids are erect and branching) and

feeding apparatus (hydroids are tentacles feeders whereas ascidians are filter-feeders) between species. The *Thalassiosira* (high-quality) diet in particular could be providing a novel resource axis that only a few species can take advantage of, although many of the species described in this paper, and certainly hydroids and ascidians, are generally opportunistic and non-selective feeders (Lesser *et al.*, 1992; Petersen, 2007; Orejas *et al.*, 2013). Regardless, the diet of concentrated dead *Thalassiosira* cells was evidently beneficial for some species, and although this species of plankton can be found in the study area, it is unclear if the communities would respond the same way to a natural increase in *Thalassiosira* population.

OA has been shown to reverse spatial dominance in fish (McCormick *et al.*, 2013b), fleshy algal communities (Connell *et al.*, 2013; Kroeker *et al.*, 2013c), cyanobacterial communities (Van de Waal *et al.*, 2011) and in simulations of coralline algal communities using historical data (McCoy *et al.*, 2016). Acidification stress could decrease feeding efficiency (Clements, 2016) or increase the limiting resource level (R*, Tilman, 1990) needed to sustain a population for hydroids but not ascidians, allowing the latter species to dominate and change the outcome of competition. Manipulative competition-OA experiments have thus far shown that elevated CO_2 and presence of a seaweed competitor can combine both additively (Diaz-Pulido *et al.*, 2011) or interactively (Short *et al.*, 2014). Further experiments paying close attention to limiting resources will be necessary to establish generalities.

Increased food availability is expected to cause declines in competition intensity between species (MacArthur, 1970; Gaylord *et al.*, 2015), however, this theory makes the assumption that communities are at equilibrium and that food is actually available to all species. Seed-addition experiments in rodent communities have found a similar pattern where food addition enhanced the dominance of one species at the expense others (Brown & Munger, 1985). Theory predicts

that habitats with higher resource levels, or less competition for resources, should be more susceptible to invasion (Fluctuating Resources Availability Theory, Davis & Pelsor, 2001; Olyarnik *et al.*, 2009). This is supported by experiments that show increasing concentrations of a limiting resource supports invasion success and decreases competitive ability of native species (e.g., Romanuk & Kolasa, 2005; Burns, 2013). However, others find that the negative effects of invasion are less pronounced at high resource levels (Riley & Dybdahl, 2015) and invasion success can be inhibited by other dominant species that benefit from resource addition (Lennon *et al.*, 2003). Furthermore, all invasive species are not alike, and traits of the invader (e.g., *r*- vs. *k*-selected) might determine outcome of competition under differing resource levels (Mata *et al.*, 2013). Importantly, I found that acidification stress can tip the balance of community composition towards invasion, even under the resource conditions that enabled the native community to resist the ascidian invasion under ambient conditions.

5.4.4. Community-level Outcomes and Consequences of Dominance Switching

Theories involving resource availability at the community level are nuanced with spectrums of resource limitation for each species, the balance among resources in a community, and the exploitation of a given fraction of the available resources (Cardinale *et al.*, 2009). With the assumption that increased overall resources are beneficial to all species, at least in terms of direct effects, increases in food availability should support (1) a higher number of species (via reduced extinction probability, species-energy theory, Wright 1983), (2) species that are more evenly distributed across space (via decreased competitive intensity), (3) communities that occupy more space (space becomes limiting resource, resource ratio theory, Braakhekke & Hooftman 1999) and (4) communities with higher biomass (Cardinale *et al.*, 2009). Further, I expected that

acidification would decrease richness and secondary production for both calcified and non-calcified species (Nagelkerken & Connell, 2015). However, I did not find these patterns to be widely supported here. The expectations from my test of resource addition to potentially selective suspension feeders may not be as straightforward a test of adding energy to a system as, for example, adding nitrogen to N-limited plant communities. We do not yet have a set of expectations for how acidification might influence the response to food addition for this kind of complex community (e.g., cascading from energetic limitation of individuals, Kooijman, 2010), but I could expect that community properties reflect the sum of species responses that may cancel each other out (expectation = non-interactive, Fig. 5.1i), or the most dominant species (expectation = interactive, as exhibited by abundant hydroids and ascidians, Fig. 5.1iii).

Species richness declines have been found in response to experimental manipulations of CO_2 and natural CO_2 from vent sites (Nagelkerken & Connell, 2015). These species-poor communities could potentially be more susceptible to invasion (Stachowicz *et al.*, 2002b), and this may be responsible for the increased abundance of ascidians under acidification. Conversely, communities dominated by ascidians that do well under acidification might drive low diversity. Disentangling the diversity-invasibility response to acidification would require manipulation of ascidian presence. Dominant species can be important for driving species interactions and diversity, especially if they provide habitat (Hillebrand *et al.*, 2008). Here, hydroids are a more complex habitat than ascidians and their branches often host other species (e.g., mussels, caprellids, bryozoans, N. Brown *personal observation*). Declines in complexity of habitat-forming species due to acidification has been shown to be a driver of biodiversity loss (Sunday *et al.*, 2017). Importantly, the reductions in species richness show that food addition, at least for the diets tested, cannot compensate for the negative effects of acidification.

Evenness is inextricably linked to the abundance of the most dominant species. Responses to experimental resource enrichment have largely resulted in decreases in evenness via the rise of a dominant species (Hillebrand *et al.*, 2008; Liess *et al.*, 2009). Since I saw increases in percent cover by the two most dominant species under food addition (at different ends of the pH spectrum), I might then expect evenness to be overall lower in the high-quality diet treatment, but this was only true when hydroids were dominant (under ambient pH conditions). Likely this was a combination of differences in magnitude of dominance (Fig. 5.2) and traits of the dominant species which can affect aggregate properties (Norberg *et al.*, 2001). Regardless, it was surprising that the high-quality diet, low pH communities were the most even, since this community type had the highest proportion of ascidians (i.e. where ascidians were most dominant) and acidification has been shown to decrease evenness (Kroeker *et al.*, 2011). Insight into how invasibility might be influenced by community evenness and how invasive species transform evenness (Hillebrand *et al.*, 2008) is needed to elucidate the mechanism.

Occupied space was never 100% in any of the communities, but most communities still had high enough cover to suggest that space could also be a limiting resource and that competition is important (Menge, 2000). Increases in space occupation under ambient CO_2 conditions and the *Thalassiosira* diet could have be driven by hydroid dominance in this treatment. Similarly, species that benefitted from *Tetraselmis* diet and acidification (*Folliculina* sp.) could be contributing to the increases in space occupation in that treatment. Decreased space occupation under food addition (described for both diets) is a surprising result. This pattern could be a result of (1) competition for the food source from species recruiting on the inner walls of the tanks that may compete for food but are not represented in the tile community (in particular mussels that strongly benefit from *Tetraselmis* diet – N. Brown *pers. comm.*) or (2) the presence of colonial

ascidians (*Botryllus* in high-quality diet under high CO_2 and *Didemnum* in low-quality diets under ambient CO_2) that use chemical inhibition to deter other species from recruiting and free up space once they seasonally senesce (Teo & Ryland, 1995; Joullié *et al.*, 2003). Again, cause and effect are difficult to disentangle here since native communities that have temporal gaps in space occupation may be more susceptible to invasion (Stachowicz *et al.*, 1999, 2002b). Overall, the competition dynamics for space may depend on food availability at different points in succession and decreased space occupation under high food supply at the final time point may only represent a temporary transition or be a result of lag time of the effect of food supply.

Some studies have found that limiting resources can constrain community structure along spatial (Ballance *et al.*, 1997) and temporal (Coma & Ribes, 2003) productivity gradients. Community structure responses do show evidence of distinct communities under acidification and diet and their interaction. In principal response curves, the high-quality food addition treatment under ambient CO_2 conditions were, as expected from the species-level results, dominated by hydroids, while the same food level under high CO_2 conditions were dominated by ascidians. This supports the species-level results, an interactive effect of food supply level and CO_2, for the high-quality food. Again, this is contrary to the hypothesis that food addition might collapse differences found between CO_2 levels under no food addition. In fact, within a community context I found the opposite, that the largest differences between ambient and elevated CO_2 emerged when food supply was high. However, in a constrained principal components analysis on the final time point, I didn't find support for the interaction. This is a similar analysis to principle response curves, which used percent cover of most species but counts for some rare species and a Bray-Curtis similarity matrix on a set of standardized species abundances designed to down-weight the effects of the most abundant species. Overall, this could indicate that acidification had stronger

community-wide effects than food addition at the level achieved here, especially for rare species, that masked any distinct communities based on repeatable single species responses across tiles.

5.4.5. Conclusions

Food addition treatments did not perfectly mimic addition of a limited resource or overall increase in energy to the system (Cardinale *et al.*, 2009) and likely competition for food and space are confounded here (Buss & Jackson, 1981). However, the artificial increase in concentrations of a given algal species may reflect expected changes in phytoplankton community under climate change where some species or taxa will benefit at the expense of others (Dutkiewicz *et al.*, 2015) and timing of production peaks may be altered (Poloczanska *et al.*, 2016). I further expect that the quality of phytoplankton could decline under acidification (Rossoll *et al.*, 2012), restricting transfer of essential fatty acids to upper trophic levels (Bermudez *et al.*, 2016). The species used here have both been shown to be resistant to acidification (high-quality *Thalassiosira weissflogii*, Goldman *et al.*, 2017, King *et al.*, 2015; low-quality *Tetraselmis* spp., Cripps *et al.*, 2016) but these effects could change under multivariate climate scenarios (Li *et al.*, 2017) or in multi-species assemblages (Liu *et al.*, 2017). Finally, whole-ecosystem phytoplankton production has been shown to be declining in the open ocean (Polovina *et al.*, 2008; Capuzzo *et al.*, 2017), but eutrophication has caused increases in production in coastal waters (Rabalais *et al.*, 2009). The ideal experimental test would be of a longer duration and include both a living phytoplankton community and suspension feeders that are subject to the same climate conditions. It is important to understand how bottom-up processes might influence integrative processes and I have shown that change in the concentration of a single phytoplankton species can have significant downstream consequences on competition and community level metrics.

Overall, the community level responses reflected both a summation of the species-level responses within the community context and traits of the dominant species. Species richness and community structure demonstrated the negative effects of acidification that are not overcome by diet alone. Space occupation and evenness in contrast were likely influenced by the traits of the dominant species. There are some community function metrics that might act on important rare species, (e.g., N-fixation in plants) but these were not explored here. It is clear that, both at the species and whole-community level, acidification is an important driving force. The degree to which effects at species level might stabilize or destabilize effects at community level is yet to be determined, but my results suggest that interspecific interactions between dominant species may be a key mechanism that drives ecological responses to acidification. I demonstrated that food availability, at least for the diets provided, does not generally mitigate the negative effects of acidification and can, in fact, exacerbate these effects. This exacerbation, i.e. larger differences between ambient and elevated CO_2 when food supply is high, is particularly important as reported responses to acidification could be substantially altered with consideration of diet and indirect effects (Kroeker *et al.*, 2013a; Ramajo *et al.*, 2016). Finally, the proposed hypothesis regarding the ability for food addition to mitigate the negative effects of acidification is thus far not widely supported at species or community levels, and there is need for a new set of predictions for mechanics of community energetic response to acidification. It is becoming apparent that understanding the underlying energetic and competitive context is essential to predicting climate change responses.

Chapter 6: Concluding Remarks

In this dissertation, I examined the responses of marine fouling communities to natural and experimental CO_2 enrichment. Understanding the mechanisms underlying community-level responses to ocean acidification is essential to improving predictions involved in forecasting the effects of climate change (Gaylord *et al.*, 2015). Here, I investigated whether CO_2-based acidification could influence the dynamics of recruitment and succession to shape community structure and diversity. I manipulated timing and duration of acidification events to examine if the effects of CO_2 are persistent or transient. I noted if there were consistent traits among species that responded positively or negatively to acidification. I explored the role of indirect effects through which CO_2 can act on communities - namely species interactions and food supply. I manipulated food supply in order to elucidate predictions about how this bottom-up process alters or is altered by acidification.

In this chapter, I first review the major findings from Chapters 2 through 5. Throughout, I refer to and answer specific questions posed in the introduction (question number in brackets) and interpret the findings in light of the main hypotheses posed in the introductions of each chapter. Next, I synthesize findings across chapters and identify commonalities, differences, and potential context dependencies in my results and compare these to findings in the literature. Following from the overall results of my dissertation, I consider next steps for the field of the ecology of ocean acidification. Finally, I highlight the challenges my dissertation has addressed and summarize my contribution to this field of research.

6.1. Major Findings

In Chapter 2, I found, consistent with my predictions, that ocean acidification, at levels predicted by the end of this century (experimentally added ~800 ppm CO_2), significantly modified community structure by altering the relative abundance of recruiting species (Q2.1). Furthermore, community variability was reduced both among and within the acidified mesocosms, which resulted in more homogenous biofouling communities from one experimental tile to the next. By the end of the experiment, Shannon diversity was 41% lower in the acidified treatment relative to ambient conditions (Q2.3). Since species richness and total use of free space were similar between treatments, the observed community structural changes were primarily attributable to differences in the relative abundance of recruited species under acidification. Some calcified species were vulnerable to acidification, consistent with my prediction, however, other calcifying species were resistant or increased in response to CO_2 and some non-calcified species were vulnerable, both surprising results (Q2.2). By the end of the experiment, recruitment of the habitat-forming mussel (*Mytilus trossulus*) was reduced by over 30% in the elevated CO_2 treatment compared to the ambient treatment. This reduction may in part be due to early mortality of juvenile mussels, as differences between CO_2 treatments were not apparent until partway through the experiment, and after peak mussel recruitment. Acidification did not appear to affect mussel growth, as average mussel sizes were similar between treatments at the end of the experiment. Hydroid (*Obelia dichotoma*) cover was significantly reduced in the elevated CO_2 treatment after eight weeks. Conversely, the percent cover of bryozoan colonies (*Mebranipora membranacea*) was higher under acidified conditions with differences becoming apparent after six weeks. Neither recruitment nor final size of barnacles (*Balanus crenatus*) was affected by acidification.

In the following chapter (Chapter 3), I used reciprocal transplant experiments along a shallow-water, volcanic CO_2 gradient to assess the importance of the timing and duration of high CO_2 exposure on patterns of colonization and succession in a natural benthic fouling community. As expected, the relationship between primary and secondary colonizers was key to alterations of succession. I showed that succession at the acidified site was initially delayed (less community change by eight weeks), despite initial promotion of biofilm under acidification (Q3.1). However, subsequent (over the next four weeks) secondary succession accelerated quickly at the acidified site, resulting in higher secondary colonization, altered community structure, and a more homogeneous biofouling community. The changes in community structure reflected both short- and longer-term acidification history (Q3.2). These community shifts were likely a result of interspecific variability in response to increased CO_2, as there were marked shifts in recruitment, and changes in species interactions. For example, high CO_2 altered biofilm development. This allowed serpulids to have the highest cover at the acidified site by the end of the experiment, even though early (pre-transplant) negative effects of CO_2 on recruitment of these worms was still detectable. As hypothesized, weedy species grew well under low pH conditions. The ascidians *Diplosoma* sp. and *Botryllus* sp., settled later and were more tolerant to acidification than their calcified competitors. *Botryllus schlosseri* is native to the Mediterranean but introduced in British Columbia, but difficulty of identifying juveniles here to species-level made it difficult to confirm the widespread *Botryllus schlosseri* over related *Botryllus lechii* that is not present in B.C. Overall, communities demonstrated substantial shifts in recruitment and succession from both discrete acidification events at different stages of successional development and continuous exposure to acidification.

In Chapter 4, I examine a key hypothesis of context dependency in ocean acidification research: that negative responses to high CO_2 could be reduced by higher energy input. This hypothesis is the basis for a general consensus in the literature that food availability can mitigate negative effects of acidification. I first expand this hypothesis by adding two alternative predictions about how acidification and food supply could combine and visualize prediction outcomes using both multi-level and factorial meta-analyses. Using factorial meta-analysis, I confirmed that food addition has a positive effect and CO_2 a negative effect on both growth and calcification for a small subset of species (mostly calcifying invertebrates like corals and mussels, but the growth dataset also included a species of flatfish). However, while food addition reduced the impact of CO_2 on calcification for these species, I found that food addition exacerbated the effects of acidification on growth (Q4.1, Q4.2). This result may be due to organisms in food-replete situations having an increased scope upon which CO_2 effects can act. These interactive effects were undetectable using a multilevel meta-analytic approach, where I found only non-interactive effects of food supply and acidification. Ongoing changes in food supply and carbonate chemistry, coupled with under-described, poorly understood, and potentially surprising interactive outcomes for these two variables, suggest that the role of food should remain a priority in ocean acidification research.

After formulating predictions for how ocean acidification and food supply combine to affect individual species in Chapter 4, I next explored the consequences of the same hypotheses in a community setting (Chapter 5). The marine invertebrates in the studies used for meta-analyses included similar types of organisms (mussels, oysters, bryozoans) that are also found in the fouling communities here, although the identity of the species differed from my study systems. I conducted a study in field-deployed mesocosms to test the potential for food availability and diet

to modify the effects of acidification on developing marine fouling communities. I supplemented natural food supply with one of two species of phytoplankton (*Thalassiosira* sp. and *Tetraselmis* sp.) that had different concentrations of fatty acids. After twelve weeks, I found substantial variation in response to acidification between treatments. The only species that benefitted significantly from acidification across food supply conditions was the invasive ascidian *Botryllus schlosseri*. Pairwise comparisons between each diet treatment and the control suggested that neither diet had a consistent effect in magnitude or direction across all species, and most species responded only to one of the two diets. Seven of the ten most abundant species exhibited significant interactive effects of acidification and food supply. There were no species that demonstrated the interactive effects generally expected from the literature, where a positive overall effect of diet mitigated the negative overall effects of acidification (Q5.1). Rather, for some species, additional food supply exacerbated or brought out the negative effects of CO_2 (e.g., for the hydroid *Obelia dichotoma*). Community richness significantly declined with acidification but there was no direct or interactive effect of food supply (Q5.2). Community structure responded similarly, with only an overall response to acidification, while space occupation, evenness, and biomass reflected patterns of ascidian or hydroid domination. I hypothesize that in many cases the response to food addition and acidification is moderated by competition; for example, only under ambient CO_2 conditions could hydroids take advantage of high-quality food to resist ascidian dominance. Importantly, I found that acidification stress can tip the balance towards invasion, even under resource conditions that would otherwise have resisted invasions. Overall, the proposed hypothesis regarding the ability for food addition to mitigate the negative effects of acidification is not widely supported at species or community levels. It is clear that acidification is a strong driving force in these communities but

understanding the underlying energetic and competitive context is essential to predicting climate change responses.

6.2. Synthesis

Species that use calcium carbonate to build and maintain skeletons and external structures are predicted to be more sensitive to ocean acidification (Wittmann & Pörtner, 2013). In field experiments, I found broadly that soft-bodied, weedy taxa (e.g., algae and ascidians), had an advantage in acidified conditions and outcompeted heavily calcified taxa (e.g., mussels, serpulids) that were more vulnerable to the effects of acidification, although calcified bryozoans and barnacles exhibited mixed responses. I found that invasive ascidians, *Botryllus schlosseri*, exhibit a positive response to acidification in their introduced range (Northeast Pacific) but no change in abundance (likely this species, but could be a related species in the *Botryllus* genus) in their native range when exposed to acidification (Mediterranean). Further, I found opposing effects within the same species in the same location but in different years (e.g., the response to acidification by *Membranipora membranacea* was positive in Chapter 2 (2012) and negative in Chapter 5 (2015). This could be driven by a number of factors including measured and unmeasured environmental differences (e.g., higher salinity in 2015 than 2012), differences in species composition between experiments that led to differences in competitive hierarchies, or differences in experimental set-up. Finally, through factorial meta-analysis, I found that the negative effects of acidification on calcification of marine invertebrates can be ameliorated by food addition, but the negative effects on growth are in fact slightly worsened by food addition (Q4.1, Q4.2). In field experiments, I found the interaction between acidification and food addition can result in substantial declines in abundance for some species that are otherwise

unaffected by changes in pH (Q5.1). These mixed responses call for examination of context dependency from interactions with other environmental factors (e.g., temperature and salinity, Harvey *et al.*, 2013), variability in pH regimes (Calosi *et al.*, 2017), habitat buffering (Wahl *et al.*, 2017), or food limitation (Thomsen *et al.*, 2013).

Overall, my findings support the expectation that ocean acidification will simplify marine communities by acting on important ecological processes that ultimately determine community structure and diversity (Vizzini *et al.*, 2017). This homogenization of variation (a constraint of community structure) with acidification occurred in two experiments (Chapter 2 and Chapter 3), where there were relatively fewer species observed overall (total of 6 and 12, respectively), but not in experiments in Chapter 5, with relatively more species in the pool (24 species). Perhaps communities with larger local species pools can resist homogenization of variation by having a larger number of functionally redundant species. In contrast to our results, perturbations of many kinds (habitat disturbance, introduction of a predator, or invasive species) have been found to increase variability in performance-related responses of species and ecosystems (Odum *et al.*, 1979), community compositional variability over time (Micheli *et al.*, 1999) and multivariate changes in abundances (Warwick & Clarke, 1993). Similarly, rising variance is considered an indicator for regime shifts (Carpenter & Brock, 2006). The spatial extent, frequency and intensity of a given disturbance might determine its influence on variability by altering heterogeneity within and among patches of a given size (Fraterrigo & Rusak, 2008). The mechanisms underlying increases or decreases in abundance and compositional community variability in response to stress have yet to be elucidated but they likely come about by changing species interactions (Fraterrigo & Rusak, 2008). We can perhaps extend the intermediate disturbance hypothesis for diversity (Sousa, 1979a, 1979b) to multivariate community variability (of which

beta-diversity is a component), and expect that variability is highest at intermediate levels of environmental stress.

Beyond homogenization of variance, I found consistent acidification-driven changes in the biofouling community structure and diversity, regardless of temporal/environmental context (e.g., higher salinity at the same site in Chapter 5 vs. Chapter 2) or location (i.e. Northeast Pacific Chapters 2 and 5, Mediterranean Chapter 3). These changes occurred as a result of both past and more recent exposure and these effects could be either transient or persistent, even after the removal from acidified conditions. In addition, I show that changes in structure and diversity with acidification can come about either as changes in relative species abundances with the same number and suite of species, or as changes in composition and number of recruiting species. I show that species interactions, especially competition with invasive species, are key moderators of these community responses to acidification. These interactions are especially important to consider when interaction outcomes or intensity are modified by stress (e.g., acidification) or resource supply (Gaylord *et al.*, 2015).

There are multiple consequences of the interaction between climate change and biological invasion, including new introductions via increased transport, propagule pressure, and biotic resistance and exacerbation of already established invasions via changing reproductive phenology, shifting community composition, and geographic range shifts (Dukes & Mooney, 1999; Occhipinti-Ambrogi, 2007; Rahel & Olden, 2008; Walther *et al.*, 2009; Mainka & Howard, 2010). Here, I found that the invasive ascidian, *Botryllus schlosseri*, increased in abundance and dominance in response to experimental acidification. This result adds to mounting evidence that under climate change, communities could undergo a shift in species composition from communities dominated by native species to communities dominated by

invasive species, as has been found in response to experimental heat waves (Sorte *et al.*, 2010c) and is already documented in long term studies (Stachowicz *et al.*, 2002b; Dijkstra *et al.*, 2010; Sorte & Stachowicz, 2011) that correlate with changes in temperature, salinity, and pH (Dijkstra *et al.*, 2010; Needles & Wendt, 2013). These shifts in dominance of invasive species could influence long term persistence at a given site or promote population expansion to other sites. It is imperative to play close attention to the abundance and spread of invasive species in the context of climate change as they pose a large threat to native communities and ecosystems (Lodge, 1993; Grosholz, 2002; Mainka & Howard, 2010; Sorte *et al.*, 2010).

6.3. Next Steps

6.3.1. Multiple Stressors

It is important to recognize that ocean acidification is not the only threat that marine ecosystems are facing: the effects of human impact are wide ranging and vary in scale from changes in climate variables like temperature and salinity to local eutrophication and spread of invasive species (Crain *et al.*, 2008; Russell & Connell, 2012; Harvey *et al.*, 2013; Sperling *et al.*, 2016). Multiple stressors can be viewed as context dependencies, as here I consider changes in phytoplankton availability to be an energetic context on which ocean acidification acts. Elsewhere, when added phytoplankton reaches extreme levels and, e.g., shades the benthos, this type of experiment would be considered a multi-stressor experiment of eutrophication and ocean acidification (e.g., Burnell *et al.*, 2013). The combination of multiple factors, regardless of the direction, need to be considered in climate change research, as they may interact non-additively and produce ecological surprises (Crain *et al.*, 2008, 2009; Darling & Côté, 2008). Frameworks that incorporate multiple stressors on individual organisms (Sokolova, 2013) and ecosystems

(Halpern *et al.*, 2015) are necessary steps forward to understanding the complexities in responses to multiple interacting stressors.

6.3.2. Ecosystem Function

My dissertation examined community responses to acidification and some of the possible mechanisms underlying these responses. There has been recent interest in the consequences of these observed community shifts in diversity and species composition on ecosystem functions: standing stocks and energy flow in ecosystems (Jax, 2005; Nagelkerken & Connell, 2015). For example, changes in nutrient cycling with acidification have been demonstrated in both benthic invertebrate (Widdicombe *et al.*, 2009; Bulling *et al.*, 2010) and phytoplankton (Schulz *et al.*, 2013) communities. Importantly for filter feeding communities, Navarro *et al.* (2013) found significant reductions in clearance rates of *Mytilus chilensis* under high CO_2 conditions. A few studies have assessed the effects of acidification on community respiration in a variety of systems; however, there seems to be no trend with CO_2 (e.g., coral reefs Leclercq *et al.*, 2002; phytoplankton Tanaka *et al.*, 2013; bacterial communities Motegi *et al.*, 2013). Increases in primary production with acidification have been shown in communities of phytoplankton (e.g., Engel *et al.*, 2013) and macroalgae (e.g. Pajusalu *et al.*, 2013); although these effects may change over time (Schulz *et al.*, 2013) and can be mediated by the presence of consumers (e.g., invertebrate mesograzers (Alsterberg *et al.*, 2013). Kroeker *et al.* (2011) found reduced biomass of benthic invertebrate communities under naturally acidified conditions. While this may be driven by changes in the weight of calcified structures and not by changes in soft tissue biomass (e.g., Christen *et al.*, 2013); I did not find similar declines in biomass here. Finally, functional diversity of assemblages in acidified conditions has been shown to be reduced (Kroeker *et al.*,

2013d), which will have consequences for overall ecosystem functioning. Many ecosystem functions are directly responsible for stability of services that humans rely on, and are therefore important to consider in climate change research (Jax, 2005; Doney *et al.*, 2012). Finally, although ecosystem functions have been examined under acidification, mechanistically linking shifts in community composition and diversity to cascading effects on ecosystem function, using experimental manipulation of diversity for example, will be important for understanding the pathway of CO_2 effects from organism to ecosystem.

6.3.3. Climate Change, Energetics, and Food Supply

There is a clear need for a conceptual framework to mechanistically support the hypotheses regarding how food supply and acidification interact at the organismal level. This could be achieved by first establishing a relationship between acidification and metabolic demand using stress tolerance frameworks (Sokolova *et al.*, 2012; Sokolova, 2013) and pH-specific regulation in energy acquisition and assimilation. Then we can explore the consequences of increasing demand and/or limiting supply on organismal performance. Next, organismal level responses (e.g., growth rate, survival, reproduction rate) could be linked to population level outcomes, as has been explored using dynamic energy budget modelling (Gaylord *et al.*, 2014; Monaco *et al.*, 2014; Muller & Nisbet, 2014). Finally, manipulative removal experiments (e.g., species removals, diversity manipulations) will be required to understand the relationship between food supply and acidification in a community setting.

6.4. Final Thoughts

My dissertation has addressed a number of challenges in the field of ocean acidification and climate change research more generally. First, my dissertation contributes to a recent push moving from organismal sensitivity to climate change in single-species studies to multi-species experiments at the community level (Harley *et al.*, 2006). Further, in my novel field-based mesocosms, I capture natural fluctuations in environmental parameters (especially pH) that have been shown to be important for determining realistic responses to acidification (Wernberg *et al.*, 2012; Shaw *et al.*, 2013a). I chose my study system in order to incorporate invasive species in my experiments, and contrasted the effects of a particular invasive species in its native and invaded ranges. Invasive species already pose a serious threat to native communities and are expected to benefit from climate change (Mainka & Howard, 2010), therefore close attention should be paid to these species. Finally, in my field-based experiments, I captured natural recruitment of organisms from plankton and focus on processes like community assembly, succession and species interactions to generate mechanistic hypotheses about the pathways of CO_2 effects. I test some of these predictions using meta-analysis and field-based manipulation of food supply. Mechanistic understanding of the effects of ocean acidification is critical to moving forward with accurate predictions (Gaylord *et al.*, 2015).

References Cited

Aberle N, Schulz KG, Stuhr A, Malzahn AM, Ludwig A, Riebesell U (2013) High tolerance of microzooplankton to ocean acidification in an Arctic coastal plankton community. *Biogeosciences*, **10**, 1471–1481.

Algueró-Muñiz M, Alvarez-Fernandez S, Thor P et al. (2017) Ocean acidification effects on mesozooplankton community development: Results from a long-term mesocosm experiment. *PLoS ONE*, **12**, 1–21.

Allen R, Foggo A, Fabricius K, Balistreri A, Hall-Spencer JM (2016) Tropical CO_2 seeps reveal the impact of ocean acidification on coral reef invertebrate recruitment. *Marine Pollution Bulletin*.

Alsterberg C, Eklöf JS, Gamfeldt L, Havenhand JN, Sundbäck K (2013) Consumers mediate the effects of experimental ocean acidification and warming on primary producers. *Proceedings of the National Academy of Sciences*, **110**, 8603–8608.

Arnold T, Mealey C, Leahey H, Miller A, Hall-Spencer JM, Milazzo M, Maers K (2012) Ocean acidification and the loss of phenolic substances in marine plants. *PLoS ONE*, **7**, e35107.

Baggini C, Salomidi M, Voutsinas E, Bray L, Krasakopoulou E, Hall-Spencer JM (2014) Seasonality affects macroalgal community response to increases in pCO_2. *PLoS ONE*, **9**, e106520.

Baggini C, Issaris Y, Salomidi M, Hall-Spencer J (2015) Herbivore diversity improves benthic community resilience to ocean acidification. *Journal of Experimental Marine Biology and Ecology*, **469**, 98–104.

Ballance LT, Pitman RL, Reilly SB (1997) Seabird community structure along a productivity gradient : Importance of competition and energetic constraint. *Ecology*, **78**, 1502–1518.

Bates D, Maechler M, Bolker B (2010) Lme4: linear mixed-effects models using S4 classes.

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, **67**, 1–48.

Bednaršek N, Feely RA, Reum JCP, Peterson B, Menkel J, Alin SR, Hales B (2014) *Limancina helicina* shell dissolution as an indicator of declining habitat suitability owing to ocean acidification in the California Current Ecosystem. *Proceedings of the Royal Society B: Biological Sciences*, **281**.

Benjamini Y, Hochberg Y (2000) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society*, **57**, 289–300.

Berkey CS, Hoaglin DC, Antczak-Bouckoms A, Mosteller F, Colditz GA (1998) Meta-analysis of multiple outcomes by regression with random effects. *Statistics in Medicine*, **17**, 2537–2550.

Bermudez JR, Riebesell U, Larsen A, Winder M (2016) Ocean acidification reduces transfer of essential biomolecules in a natural plankton community. *Scientific Reports*, **6**, 27749.

Boatta F, D'Alessandro W, Gagliano AL et al. (2013) Geochemical survey of Levante Bay, Vulcano Island (Italy), a natural laboratory for the study of ocean acidification. *Marine Pollution Bulletin*, **73**, 485–494.

Borenstein M, Hedges L V., Higgins JPT, Rothstein HR (2009a) Part Five: Complex Data Structures. In: *Introduction to Meta-Analysis*, pp. 239–242. John Wiley and Sons Ltd., New Jersey.

Borenstein M, Hedges L V., Higgins JPT, Rothstein HR (2009b) *Introduction to Meta-Analysis*. John Wiley & Sons, Chichester, U.K, 1-115 pp.

Boyd PW, Cornwall CE, Davison A et al. (2016) Biological responses to environmental heterogeneity under future ocean conditions. *Global Change Biology*, 1–18.

Braakhekke WG, Hooftman D a P (1999) The resource balance hypothesis of plant species diversity in grassland. *Journal of Vegetation Science*, **10**, 187–200.

Bradley MC, Perrin N, Calow P (1991) Energy allocation in the cladoceran *Daphnia magna* Straus, under starvation and refeeding. *Oecologia*, **86**, 414–418.

Breitburg DL, Salisbury J, Bernhard JM et al. (2015) And on top of all that… Coping with ocean acidification in the midst of many stressors. *Oceanography*, **28**, 48–61.

Van Den Brink PJ, Ter CJF, Dlo B (1999) Principal response curves: analysis of time-dependent multivariate responses of biological community to stress. *Environmental Toxicology and Chemistry*, **18**, 138–148.

Brook B, Sodhi N, Bradshaw C (2008) Synergies among extinction drivers under global change. *Trends in ecology & evolution*, **23**, 453–460.

Brown MR (1991) The amino-acid and sugar composition of 16 species of microalgae used in mariculture. *Journal of Experimental Marine Biology and Ecology*, **145**, 79–99.

Brown JH, Munger JC (1985) Experimental manipulation of a desert rodent community : food addition and species removal. *Ecology*, **66**, 1545–1563.

Brown NEM, Therriault TW, Harley CDG (2016) Field-based experimental acidification alters fouling community structure and reduces diversity. *Journal of Animal Ecology*, **85**, 1328–1339.

Brown N, Milazzo M, Rastrick S, Hall-Spencer J, Therriault TW, Harley CDG (2017) Natural acidification changes the timing and rate of succession, alerts community structure, and increases homogeneity in marine biofouling communities. *Global Change Biology*, 1–16.

Bulling M, Hicks N, Murray L, Paterson D, Raffaelli D, White P, Solan M (2010) Marine biodiversity-ecosystem functions under uncertain environmental futures. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**, 2107–2116.

Burnell OW, Russell BD, Irving AD, Connell SD (2013) Eutrophication offsets increased sea urchin grazing on seagrass caused by ocean warming and acidification. *Marine Ecology*

Progress Series, **485**, 37–46.

Burns CW (2013) Predictors of invasion success by Daphnia species: Influence of food, temperature and species identity. *Biological Invasions*, **15**, 859–869.

Busch S, McElhany P (2017) Using mineralogy and higher-level taxonomy as indicators of species sensitivity to pH: A case-study of Puget Sound. *Elem Sci Anth*, **5**, 53.

Buschbaum C, Dittmann S, Hong J-S et al. (2009) Mytilid mussels: global habitat engineers in coastal sediments. *Helgoland Marine Research*, **63**, 47–58.

Büscher J V., Form AU, Riebesell U (2017) Interactive effects of ocean acidification and warming on growth, fitness and survival of the cold-water coral *Lophelia pertusa* under different food availabilities. *Frontiers in Marine Science*, **4**, 101.

Buss LW, Jackson JBC (1981) Planktonic food availability and supension-feeder abundance: evidence of in situ depletion. *Journal of Experimental Marine Biology and Ecology*, **49**, 151–161.

De Cáceres M, Legendre P, Moretti M (2010) Improving indicator species analysis by combining groups of sites. *Oikos*, **119**, 1674–1684.

Cade B, Noon B (2003) A gentle introduction to quantile regression for ecologists. *Frontiers in Ecology and the Environment*, **1**, 412–420.

Cai W-J, Hu X, Huang W-J et al. (2011) Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience*, **4**, 766–770.

Calosi P, Rastrick SPS, Graziano M et al. (2013a) Distribution of sea urchins living near shallow water CO_2 vents is dependent upon species acid–base and ion-regulatory abilities. *Marine Pollution Bulletin*, **73**, 470–484.

Calosi P, Rastrick SPS, Lombardi C et al. (2013b) Adaptation and acclimatization to ocean acidification in marine ectotherms: an in situ transplant experiment with polychaetes at a shallow CO_2 vent system. *Proceedings of the Royal Society B: Biological Sciences*, **368**, 1–15.

Calosi P, Melatunan S, Turner LM et al. (2017) Regional adaptation defines sensitivity to future ocean acidification. *Nature Communications*, **8**, 13994.

Campbell JE, Fourqurean JW (2014) Ocean acidification outweighs nutrient effects in structuring seagrass epiphyte communities. *Journal of Ecology*, **102**, 730–737.

Capuzzo E, Lynam CP, Barry J et al. (2017) A decline in primary production in the North Sea over twenty-five years, associated with reductions in zooplankton abundance and fish stock recruitment. *Global Change Biology*.

Cardinale BJ, Hillebrand H, Harpole WS, Gross K, Ptacnik R (2009) Separating the influence of resource "availability" from resource "imbalance" on productivity-diversity relationships. *Ecology Letters*, **12**, 475–487.

Carpenter SR, Brock WA (2006) Rising variance: A leading indicator of ecological transition. *Ecology Letters*, **9**, 308–315.

Celis-Plá PSM, Hall-Spencer JM, Horta PA, Milazzo M, Korbee N, Cornwall CE, Figueroa FL (2015) Macroalgal responses to ocean acidification depend on nutrient and light levels. *Frontiers in Marine Science*, **2**.

Chalmer PN (1982) Settlement patterns of species in a marine fouling community and some mechanisms of succession. **58**, 73–85.

Chan F, Barth JA, Blanchette CA et al. (2017) Persistent spatial structuring of coastal ocean acidification in the California Current System. *Scientific Reports*, **7**, 2526.

Chesson P, Huntly N (1997) The roles of harsh and fluctuating conditions in the dynamics of ecological communities. *The American Naturalist*, **150**, 519–553.

Christen N, Calosi P, McNeill C, Widdicombe S (2013) Structural and functional vulnerability to elevated pCO_2 in marine benthic communities. *Marine Biology*, **160**, 2113–2128.

Cifuentes M, Krueger I, Dumont CP, Lenz M, Thiel M (2010) Does primary colonization or community structure determine the succession of fouling communities? *Journal of Experimental Marine Biology and Ecology*, **395**, 10–20.

Cigliano M, Gambi M-C, Rodolfo-Metalpa R, Patti FP, Hall-Spencer JM (2010) Effects of ocean acidification on invertebrate settlement at volcanic CO_2 vents. *Marine Biology*, **157**, 2489–2502.

Clark H, Gobler C (2016) Do diurnal fluctuations in CO_2 and dissolved oxygen concentrations provide a refuge from hypoxia and acidification for early life stage bivalves? *Marine Ecology Progress Series*, **558**, 1–14.

Clements JC (2016) Meta-analysis reveals taxon- and life stage-dependent effects of ocean acidification on marine calcifier feeding performance. *bioRxiv*, 1–18.

Cole VJ, Parker LM, O'Connor SJ, O'Connor WA, Scanes E, Byrne M, Ross PM (2016) Effects of multiple climate change stressors: ocean acidification interacts with warming, hyposalinity, and low food supply on the larvae of the brooding flat oyster *Ostrea angasi*. *Marine Biology*, **163**, 125.

Coma R, Ribes M (2003) Seasonal energetic constraints in Mediterranean benthic suspension feeders: effects at different levels of ecological organization. *Oikos*, **101**, 205–215.

Comeau S, Carpenter RC, Edmunds PJ (2013) Effects of feeding and light intensity on the response of the coral *Porites rus* to ocean acidification. *Marine Biology*, **160**, 1127–1134.

Connell JH, Slatyer R (1977) Mechanisms of succession in natural communities and their role in community stability and organization. *American Society of Naturalists*, **111**, 1119–1144.

Connell SD, Kroeker KJ, Fabricius KE, Kline DI, Russell BD (2013) The other ocean acidification problem: CO_2 as a resource among competitors for ecosystem dominance.

Proceedings of the Royal Society B: Biological Sciences, **368**, 1–9.

Cornwall CE, Revill AT, Hall-Spencer JM, Milazzo M, Raven JA, Hurd CL (2017) Inorganic carbon physiology underpins macroalgal responses to elevated CO_2. *Scientific Reports*, **7**, 46297.

Cox TE, Díaz-Castañeda V, Martin S et al. (2017) Effects of in situ CO2 enrichment on epibiont settlement on artificial substrata within a *Posidonia oceanica* meadow. *Journal of Experimental Marine Biology and Ecology*, **497**, 197–211.

Crain C, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters*, **11**, 1304–1315.

Crain C, Halpern BS, Beck M, Kappel C (2009) Understanding and managing human threats to the coastal marine environment. *Annals of the New York Academy of Sciences*, **1162**, 39–62.

Cripps G, Flynn KJ, Lindeque PK (2016) Ocean acidification affects the phyto-zoo plankton trophic transfer efficiency. *Plos One*, **11**, e0151739.

Crook ED, Cooper H, Potts DC, Lambert T, Paytan A (2013) Impacts of food availability and pCO_2 on planulation, juvenile survival, and calcification of the azooxanthellate scleractinian coral Balanophyllia elegans. *Biogeosciences*, **10**, 7599–7608.

Culler LE, McPeek MA, Ayres MP (2014) Predation risk shapes thermal physiology of a predaceous damselfly. *Oecologia*, **176**, 653–660.

Darling ES, Côté IM (2008) Quantifying the evidence for ecological synergies. *Ecology letters*, **11**, 1278–86.

Dashfield S, Somerfield P, Widdicombe S, Austen M, Nimmo M (2008) Impacts of ocean acidification and burrowing urchins on within-sediment pH profiles and subtidal nematode communities. *Journal of Experimental Marine Biology and Ecology*, **365**, 46–52.

Davis MA, Pelsor M (2001) Experimental support for a mechanistic resource-based model of invasibility. *Ecology Letters*, **4**, 421–428.

Delignette-muller ML, Dutang C (2015) fitdistrplus : An R Package for Fitting Distributions. *Journal of Statistical Software*, **64**, 1–34.

Diaz-Pulido G, Gouezo M, Tilbrook B, Dove S, Anthony KRN (2011) High CO_2 enhances the competitive strength of seaweeds over corals. *Ecology Letters*, **14**, 156–162.

Dickson AG (1990) Standard potential of the reaction – $AGCL_{(S)}+1/2H^{-2}_{(G)}=AG_{(S)}+HCL_{(AQ)}$ and the standard acidicity constant of the ion HSO4- in synthetic sea-water from 273.15-K to 318.15-K. *The Journal of Chemical Thermodynamics*, **22**, 113–127.

Dickson AG, Sabine CL, Christian JR (2007) *Guide to Best Practices for Ocean CO2 Measurements*. PICES Special Publication, 191 pp p.

Dijkstra JA, Harris LG (2009) Maintenance of diversity altered by a shift in dominant species: implications for species coexistence. *Marine Ecology Progress Series*, **387**, 71–80.

Dijkstra JA, Westman EL, Harris LG (2010) The effects of climate change on species composition, succession and phenology: a case study. *Global Change Biology*, **17**, 2360–2369.

Doney SC, Balch W, Fabry VJ, Feely RA (2009a) Ocean acidification: a critical emerging problem for the ocean sciences. *Oceanography*, **22**, 16–25.

Doney SC, Fabry VJ, Feely RA, Kleypas J (2009b) Ocean acidification: the other CO_2 problem. *Annual Review of Marine Science*, **1**, 169–192.

Doney SC, Ruckelshaus M, Duffy JE et al. (2012) Climate change impacts on marine ecosystems. *Annual Review of Marine Science*, **4**, 11–37.

Doney SC, Bopp L, Long MC (2014) Historical and future trends in ocean climate and biogeochemistry. *Oceanography*, **27**, 238–246.

Donnarumma L, Lombardi C, Cocito S, Gambi MC (2014) Settlement pattern of *Posidonia oceanica* epibionts along a gradient of ocean acidification : an approach with mimics. *Mediterranean Marine Science*, **15**, 498–509.

Doropoulos C, Diaz-Pulido G (2013) High CO_2 reduces the settlement of a spawning coral on three common species of crustose coralline algae. *Marine Ecology Progress Series*, **475**, 93–99.

Doropoulos C, Ward S, Diaz-Pulido G, Hoegh-Guldberg O, Mumby PJ (2012a) Ocean acidification reduces coral recruitment by disrupting intimate larval algal settlement interactions. *Ecology Letters*, **15**, 338–346.

Doropoulos C, Ward S, Diaz-Pulido G, Hoegh-Guldberg O, Mumby PJ (2012b) Ocean acidification reduces coral recruitment by disrupting intimate larval-algal settlement interactions. *Ecology Letters*, **15**, 338–346.

Drenkard EJ, Cohen AL, McCorkle DC, de Putron SJ, Starczak VR, Zicht AE (2013) Calcification by juvenile corals under heterotrophy and elevated CO_2. *Coral Reefs*, **32**, 727–735.

Duarte C, Navarro JM, Acuña K et al. (2014) Combined effects of temperature and ocean acidification on the juvenile individuals of the mussel *Mytilus chilensis*. *Journal of Sea Research*, **85**, 308–314.

Duarte C, López J, Benítez S et al. (2016) Ocean acidification induces changes in algal palatability and herbivore feeding behavior and performance. *Oecologia*, **180**, 453–462.

Dukes JS, Mooney HA (1999) Does global change increase the success of biological invaders? *Trends in ecology & evolution*, **14**, 135–139.

Dupont S, Thorndyke MC (2009) Impact of CO_2-driven ocean acidification on invertebrates early life-history – what we know, what we need to know and what we can do. *Biogeosciences Discussions*, **6**, 3109–3131.

Dutkiewicz S, Morris JJ, Follows MJ, Scott J, Levitan O, Dyhrman ST, Berman-Frank I (2015) Impact of ocean acidification on the structure of future phytoplankton communities. *Nature Climate Change*.

Edmunds PJ (2011) Zooplanktivory ameliorates the effects of ocean acidification on the reef coral *Porites* spp. *Limnology and Oceanography*, **56**, 2402–2410.

Engel A, Borchard C, Piontek J, Schulz KG, Riebesell U, Bellerby RGJ (2013) CO_2 increases ^{14}C primary production in an Arctic plankton community. *Biogeosciences*, **10**, 1291–1308.

Enquist BJ, Norberg J, Bonser SP et al. (2015) *Scaling from Traits to Ecosystems: Developing a General Trait Driver Theory via Integrating Trait-Based and Metabolic Scaling Theories*, 1st edn, Vol. 52. Elsevier Ltd., 249-318 pp.

Epelbaum A, Pearce C, Barker D, Paulson A, Therriault TW (2009) Susceptibility of non-indigenous ascidian species in British Columbia (Canada) to invertebrate predation. *Marine Biology*, **156**, 1311–1320.

Fabricius KE, Langdon C, Uthicke S et al. (2011) Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nature Climate Change*, **1**, 165–169.

Fabricius KE, De'ath G, Noonan S, Uthicke S (2014) Ecological effects of ocean acidification and habitat complexity on reef-associated macroinvertebrate communities. *Proceedings of the Royal Society B: Biological Sciences*, **281**.

Feely RA, Sabine CL, Lee K, Berelson W, Kleypas J, Fabry VJ, Millero FJ (2004) Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science*, **305**, 362–366.

Feely RA, Sabine CL, Hernandez-Ayon JM, Ianson D, Hales B (2008) Evidence for upwelling of corrosive "acidified" water onto the continental shelf. *Science*, **320**, 1490–1492.

Feely RA, Alin SR, Newton J et al. (2010) The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. *Estuarine, Coastal and Shelf Science*, **88**, 442–449.

Field A (2013) *Discovering statistics using IBM SPSS Statistics: and sex and drugs and rock "n" roll*, 4th edn. Sage publications, London, UK.

Findlay HS, Kendall M, Spicer JI, Widdicombe S (2010a) Relative influences of ocean acidification and temperature on intertidal barnacle post-larvae at the northern edge of their geographic distribution. *Estuarine, Coastal and Shelf Science*, **86**, 675–682.

Findlay HS, Kendall M, Spicer JI, Widdicombe S (2010b) Post-larval development of two intertidal barnacles at elevated CO_2 and temperature. *Marine Biology*, **157**, 725–735.

Fortunato H (2015) Bryozoans in climate and ocean acidification research: A reappraisal of an under-used tool. *Regional Studies in Marine Science*, **2**, 32–44.

Fournier DA, Skaug HJ, Ancheta J et al. (2012) AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models.

Optimization Methods and Software, **27**, 233–249.

Fraterrigo JM, Rusak JA (2008) Disturbance-driven changes in the variability of ecological patterns and processes. *Ecology Letters*, **11**, 756–770.

Frieder CA, Nam SH, Martz TR, Levin LA (2012) High temporal and spatial variability of dissolved oxygen and pH in a nearshore California kelp forest. *Biogeosciences*, **9**, 3917–3930.

Frieder CA, Applebaum SL, Pan TF, Hedgecock D, Manahan DT (2017) Metabolic cost of calcification in bivalve larvae under experimental ocean acidificatio. *ICES Journal of Marine Science*, **74**, 941–954.

Gambi MC, Musco L, Giangrande A, Badalamenti F, Micheli F, Kroeker KJ (2016) Distribution and functional traits of polychaetes in a CO_2 vent system: Winners and losers among closely related species. *Marine Ecology Progress Series*, **550**, 121–134.

Garilli V, Rodolfo-Metalpa R, Scuderi D et al. (2015) Physiological advantages of dwarfing in surviving extinctions in high-CO_2 oceans. *Nature Climate Change*, **5**, 1–6.

Garrard SL, Gambi MC, Scipione MB et al. (2014) Indirect effects may buffer negative responses of seagrass invertebrate communities to ocean acidification. *Journal of Experimental Marine Biology and Ecology*, **461**, 31–38.

Gartner HN, Murray CC, Frey MA, Nelson JC, Larson KJ, Ruiz GM, Therriault TW (2016) Non-indigenous invertebrate species in the marine fouling communities of British Columbia , Canada. **5**, 205–212.

Gattuso J-P, Gao K, Lee K, Rost B, Schulz KG (2011) Approches and tools to manipulate the carbonate chemistry. In: *Guide to best practices for ocean acidification research and data reporting* (ed Riebesell U, Fabry VJ, Hansson L GJ-P), pp. 41–52. Publications Office of the European Union., Luxembourg.

Gaylord B, Hill T, Sanford E et al. (2011) Functional impacts of ocean acidification in an ecologically critical foundation species. *Journal of Experimental Biology*, **214**, 2586–2594.

Gaylord B, Kroeker K, Sunday J et al. (2014) Ocean acidification through the lens of ecological theory. *Ecology*.

Gaylord B, Kroeker KJK, Sunday JMJ et al. (2015) Ocean acidification through the lens of ecological theory. *Ecology*, **96**, 3–15.

Gazeau F, Gattuso J-P, Dawber C et al. (2010) Effect of ocean acidification on the early life stages of the blue mussel *Mytilus edulis*. *Biogeosciences*, **7**, 2051–2060.

Gelman A, Stern H (2006) The difference between "significant" and "not significant" is not itself statistically significant. *The American Statistician*, **60**, 328–331.

Ghedini G, Connell SD (2016) Organismal homeostasis buffers the effects of abiotic change on community dynamics. *Ecology*, **97**, 2671–2679.

Gleser LJ, Olkin I (2009) Stochastically dependent effect sizes. *The handbook of research synthesis and meta-analysis (2nd ed.).*, 357–376.

Gobler C, DePasquale E, Griffith A, Baumann H (2014) Hypoxia and acidification have additive and synergistic negative effects on the growth, survival, and metamorphosis of early life stage bivalves. *PLoS ONE*, **9**, e83648.

Godbold JA, Solan M (2013) Long-term effects of warming and ocean acidification are modified by seasonal variation in species responses and environmental conditions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **368**, 1–11.

Goldman JAL, Bender ML, Morel FMM (2017) The effects of pH and pCO2 on photosynthesis and respiration in the diatom *Thalassiosira weissflogii*. *Photosynthesis Research*, **132**, 83–93.

Goodwin C, Rodolfo-Metalpa R, Picton B, Hall-Spencer JM (2014) Effects of ocean acidification on sponge communities. *Marine Ecology*, **35**, 41–49.

Gounand I, Kefi S, Mouquet N, Gravel D (2016) Trait selection during food web assembly: the roles of interactions and temperature. *Theoretical Ecology*, **9**, 417–429.

Grey E (2011) Relative effects of environment and direct species interactions on the population growth rate of an exotic ascidian. *Oecologia*, **166**, 935–947.

Grosholz E (2002) Ecological and evolutionary consequences of coastal invasions. *Trends in ecology & evolution*, **17**, 22–27.

Guadayol Ò, Peters F, Marrasé C et al. (2009) Episodic meteorological and nutrient-load events as drivers of coastal planktonic ecosystem dynamics: A time-series analysis. *Marine Ecology Progress Series*, **381**, 139–155.

Gurevitch J, Morrison JA, Hedges L V (2000) The interaction between competition and predation: a meta-analysis of field experiments. *The American Naturalist*, **155**, 435–453.

Habeck CW, Schultz AK (2015) Community-level impacts of white-tailed deer on understory plants in North American forests: a meta-analysis. *AoB plants*, **7**, plv119.

Hadfield MG (2011) Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlement sites. *Annual Review of Marine Science*, **3**, 453–470.

Hale R, Calosi P, McNeill L, Mieszkowska N, Widdicombe S (2011) Predicted levels of future ocean acidification and temperature rise could alter community structure and biodiversity in marine benthic communities. *Oikos*, **120**, 661–674.

Hall-Spencer JM, Allen R (2015) The impact of CO_2 emissions on " nuisance " marine species. *Research and Reports in Biodiversity Studies*, **4**, 33–46.

Hall-Spencer JM, Rodolfo-Metalpa R, Martin S et al. (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature*, **454**, 96–99.

Hallett LM, Jones SK, MacDonald AAM et al. (2016) codyn: An r package of community

dynamics metrics. *Methods in Ecology and Evolution*, **7**, 1146–1151.

Halpern BS, Walbridge S, Selkoe K a et al. (2008) A global map of human impact on marine ecosystems. *Science*, **319**, 948–52.

Halpern BS, Frazier M, Potapenko J et al. (2015) Spatial and temporal changes in cumulative human impacts on the world's ocean. *Nature Communications*, **6**, 7615.

Harley CD, Hughes AR, Kristin M et al. (2006) The impacts of climate change in coastal marine systems. *Ecology Letters*, **9**, 228–241.

Harley CD, Anderson KM, Demes KW, Jorve JP, Kordas RL, Coyle TA, Graham MH (2012) Effects of climate change on global seaweed communities. *Journal of Phycology*, **48**, 1064–1078.

Harvey P, Gwynn-Jones D, Moore P (2013) Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. *Ecology and Evolution*, **3**, 1016–1030.

Harvey BP, McKeown NJ, Rastrick SPS et al. (2016) Individual and population-level responses to ocean acidification. *Scientific Reports*, **6**, 20194.

Hedges L V., Gurevitch J, Curtis PS (1999) The meta-analysis of response ratios in experimental ecology. *Ecology*, **80**, 1150–1156.

Henson SA, Beaulieu C, Ilyina T et al. (2017) Rapid emergence of climate change in environmental drivers of marine ecosystems. *Nature Communications*, **8**, 14682.

Hettinger A, Sanford E, Hill TM, Hosfelt JD, Russell a. D, Gaylord B (2013) The influence of food supply on the response of Olympia oyster larvae to ocean acidification. *Biogeosciences*, **10**, 6629–6638.

Higgins JPT, Green S (eds.) (2008) *Cochrane handbook for systematic reviews of interventions.* Wiley, Chichester, England.

Hillebrand H, Bennett D, Cadotte M (2008) Consequences of dominance: a review of evenness effects on local and regional ecosystem processes. *Ecology*, **89**, 1510–1520.

Hoegh-Guldberg O, Bruno JJ (2010) The impact of climate change on the world's marine ecosystems. *Science*, **1523**, 1523–1528.

Hofmann GE, Smith JE, Johnson KS et al. (2011) High-frequency dynamics of ocean pH: a multi-ecosystem comparison. *PLoS ONE*, **6**, e28983.

Hofmann LC, Straub S, Bischof K (2012) Competition between calcifying and noncalcifying temperate marine macroalgae under elevated CO_2 levels. *Marine Ecology Progress Series*, **464**, 89–105.

Hönisch B, Ridgwell A, Schmidt DN et al. (2012) The geological record of ocean acidification. *Science*, **335**, 1058–63.

Hurst TP, Laurel BJ, Hanneman E, Scott A, Ottmar ML (2017) Elevated CO_2 does not exacerbate nutritional stress in larvae of a Pacific flatfish. *in Press*, 1–21.

Iles AC, Gouhier TC, Menge BA, Stewart JS, Haupt AJ, Lynch MC (2012) Climate-driven trends and ecological implications of event-scale upwelling in the California Current System. *Global Change Biology*, **18**, 783–796.

IPCC (2013) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.

Jackson ST, Blois JL (2015) Community ecology in a changing environment: Perspectives from the Quaternary. *Proceedings of the National Academy of Sciences*, **112**, 4915–4921.

Jax K (2005) Function and "' functioning '" in ecology : what does it mean ? *Oikos*, **111**, 641–648.

Jin P, Wang T, Liu N et al. (2015) Ocean acidification increases the accumulation of toxic phenolic compounds across trophic levels. *Nature Communications*, **6**, 8714.

Johnson V, Russell B, Fabricius KE, Brownlee C, Hall-Spencer JM (2012) Temperate and tropical brown macroalgae thrive, despite decalcification, along natural CO_2 gradients. *Global Change Biology*, **18**, 2792–2803.

Johnson R, Brownlee C, Rickaby R, Graziano M, Milazzo M, Hall-Spencer JM (2013) Responses of marine benthic microalgae to elevated CO_2. *Marine Biology*, **160**, 1813–1824.

Johnson M, Moriarty V, Carpenter R (2014) Acclimatization of the crustose coralline alga Porolithon onkodes to variable pCO_2. *PLoS ONE*, **9**, 1–11.

Johnson V, Brownlee C, Milazzo M, Hall-Spencer J (2015) Marine microphytobenthic assemblage shift along a natural shallow-water CO_2 gradient subjected to multiple environmental stressors. *Journal of Marine Science and Engineering*, **3**, 1425–1447.

Jokiel P, Rodgers K, Kuffner I, Andersson AJ, Cox E, Mackenzie F (2008) Ocean acidification and calcifying reef organisms: a mesocosm investigation. *Coral Reefs*, **27**, 473–483.

Joullié M, Leonard MS, Portonovo P, Liang B, Ding X, Clair JJ La, Joullie MM (2003) Chemical defense in ascidians of the *Didemnidae* family. *Bioconjugate Chemistry*, **14**, 30–37.

Jurgens LJ, Gaylord B (2016) Edge effects reverse facilitation by a widespread foundation species. *Scientific Reports*, **6**, 37573.

Katzenberger M, Hammond J, Duarte H, Tejedo M, Calabuig C, Relyea RA (2014) Swimming with predators and pesticides: How environmental stressors affect the thermal physiology of tadpoles. *PLoS ONE*, **9**, 1–11.

King A, Jenkins B, Wallace J, Liu Y, Wikfors G, Milke L, Meseck S (2015) Effects of CO_2 on growth rate, C:N:P, and fatty acid composition of seven marine phytoplankton species. *Marine Ecology Progress Series*, **537**, 59–69.

Konstantopoulos S (2011) Fixed effects and variance components estimation in three-level meta-analysis. *Research Synthesis Methods*, **2**, 61–76.

Kooijman S a. LM (2010) *Dynamic Energy Budget theory for metabolic organisation : Summary of concepts of the third edition*, Vol. 365. 68 pp.

Kordas RL, Harley CD, O'Connor MI, Connor MIO (2011) Community ecology in a warming world: The influence of temperature on interspecific interactions in marine systems. *Journal of Experimental Marine Biology and Ecology*, **400**, 218–226.

Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecology Letters*, **13**, 1419–1434.

Kroeker KJ, Micheli F, Gambi M-C, Martz TR (2011) Divergent ecosystem responses within a benthic marine community to ocean acidification. *Proceedings of the National Academy of Sciences*, **108**, 14515–14520.

Kroeker KJ, Kordas RL, Crim R et al. (2013a) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology*, **19**, 1884–1896.

Kroeker KJ, Gambi M-C, Micheli F (2013b) Community dynamics and ecosystem simplification in a high-CO_2 ocean. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 12721–6.

Kroeker KJ, Micheli F, Gambi M-C (2013c) Ocean acidification causes ecosystem shifts via altered competitive interactions. *Nature Climate Change*, **3**, 156–159.

Kroeker K, Gambi M-C, Micheli F (2013d) Community dynamics and ecosystem simplification in a high-CO2 ocean. *Proceedings of the National Academy of Sciences*, **110**, 12721–12726.

Kroeker KJ, Sanford E, Jellison BM, Gaylord B (2014) Predicting the effects of ocean acidification on predator-prey interactions: a conceptual framework based on coastal molluscs. *The Biological bulletin*, **226**, 211–222.

Kroeker KJ, Kordas RL, Harley CDG (2017) Embracing interactions in ocean acidification research: Confronting multiple stressor scenarios and context dependence. *Biology Letters*, **13**, 1–4.

Krumhansl KA, Lee JM, Scheibling RE (2011) Grazing damage and encrustation by an invasive bryozoan reduce the ability of kelps to withstand breakage by waves. *Journal of Experimental Marine Biology and Ecology*, **407**, 12–18.

Kuffner I, Andersson AJ, Jokiel P, Rodgers K, Mackenzie F (2007) Decreased abundance of crustose coralline algae due to ocean acidification. *Nature Geoscience*, **1**, 114–117.

Kurihara H, Asai T, Kato S, Ishimatsu A (2008) Effects of elevated *p*CO_2 on early development in the mussel *Mytilus galloprovincialis*. *Aquatic Biology*, **4**, 225–233.

LaJeunesse MJ (2011) On the meta-analysis of response ratios for studies with correlated and multi-group designs. *Ecology*, **92**, 2049–2055.

Lancaster L, Morrison G, Fitt R (2016) Life history trade-offs, the intensity of competition, and coexistence in novel and evolving communities under climate change. *Philosophical Transactions of the Royal Society B*.

Lane AC, Mukherjee J, Chan VBS, Thiyagarajan V (2013) Decreased pH does not alter metamorphosis but compromises juvenile calcification of the tube worm *Hydroides elegans*. *Marine Biology*, **160**, 1983–1993.

Langer JAF, Sharma R, Schmidt SI et al. (2017) Community barcoding reveals little effect of ocean acidification on the composition of coastal plankton communities: Evidence from a long-term mesocosm study in the Gullmar Fjord, Skagerrak. *PLoS ONE*, **12**, 1–20.

Leclercq N, Gattuso J-P, Jaubert J (2002) Primary production, respiration, and calcification of a coral reef mesocosm under increased CO2 partial pressure. *Limnology and Oceanography*.

Leduc AOA, Munday PL, Brown GE, Ferrari MCO (2013) Effects of acidification on olfactory-mediated behaviour in freshwater and marine ecosystems: a synthesis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **368**, 1–14.

Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia*, **129**, 271–280.

Lennon JT, Smith VH, Dzialowski AR (2003) Invasibility of plankton food webs along a trophic state gradient. *Oikos*, **103**, 191–203.

Lesser MP, Shumway SE, Cucci T, Smith J (1992) Impact of fouling organisms on mussel rope culture: interspecific competition for food among suspension-feeding invertebrates. *Journal of Experimental Marine Biology and Ecology*, **165**, 91–102.

Li C, Chan V, He C, Meng Y (2014) Weakening mechanisms of the serpulid tube in a high CO_2 world. *Environmental Science & Technology*, **48**, 14158–14167.

Li F, Wu Y, Hutchins DA, Fu F, Gao K (2016) Physiological responses of coastal and oceanic diatoms to diurnal fluctuations in seawater carbonate chemistry under two CO_2 concentrations. *Biogeosciences*, **13**, 6247–6259.

Li W, Yang Y, Li Z, Xu J, Gao K (2017) Effects of seawater acidification on the growth rates of the diatom Thalassiosira (Conticribra) weissflogii under different nutrient, light, and UV radiation regimes. *Journal of Applied Phycology*, **29**, 133–142.

Lidbury I, Johnson V, Hall-Spencer JM, Munn C, Cunliffe M (2012) Community-level response of coastal microbial biofilms to ocean acidification in a natural carbon dioxide vent ecosystem. *Marine Pollution Bulletin*, **64**, 1063–1066.

Liess A, Lange K, Schulz F, Piggott JJ, Matthaei CD, Townsend CR (2009) Light, nutrients and grazing interact to determine diatom species richness via changes to productivity, nutrient state and grazer activity. *Journal of Ecology*, **97**, 326–336.

Linares C, Vidal M, Canals M et al. (2015) Persistent natural acidification drives major distribution shifts in marine benthic ecosystems. *Proceedings of the Royal Society B*, **282**, e20150587.

Lischka S, Bach LT, Schulz KG, Riebesell U (2017) Ciliate and mesozooplankton community response to increasing CO2 levels in the Baltic Sea: Insights from a large-scale mesocosm experiment. *Biogeosciences*, **14**, 447–466.

Liu N, Tong S, Yi X et al. (2017) Carbon assimilation and losses during an ocean acidification mesocosm experiment, with special reference to algal blooms. *Marine Environmental Research*, **129**, 229–235.

Lodge D (1993) Biological invasions: Lessons for ecology. *Trends in ecology & evolution*, **8**, 133–137.

Lombardi C, Rodolfo-Metalpa R, Cocito S, Gambi M-C, Taylor P (2011) Structural and geochemical alterations in the Mg calcite bryozoan Myriapora truncata under elevated seawater pCO_2 simulating ocean acidification. *Marine Ecology*, **32**, 211–221.

Lortie CJ, Callaway RM (2006) Re-analysis of meta-analysis: Support for the stress-gradient hypothesis. *Journal of Ecology*, **94**, 7–16.

Lucey NM, Lombardi C, Florio M et al. (2016) An in situ assessment of local adaptation in a calcifying polychaete from a shallow CO_2 vent system. *Evolutionary Applications*, 1–140.

Luhring TM, Delong JP (2016) Predation changes the shape of thermal performance curves for population growth rate. *Current Zoology*, **62**, 501–505.

Macarthur R, Levins R (1967) The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist*, **101**, 377–385.

MacArthur R (1970) Species packing and competitive equilibrium for many species. *Theoretical Population Biology*, **1**, 1–11.

Maier C, Popp P, Sollfrank N, Weinbauer MG, Wild C, Gattuso J-P (2016) Effects of elevated pCO_2 and feeding on net calcification and energy budget of the Mediterranean cold-water coral *Madrepora oculata*. *The Journal of Experimental Biology*, **2**, jeb.127159.

Mainka SA, Howard GW (2010) Climate change and invasive species: double jeopardy. *Integrative Zoology*, **5**, 102–111.

Martin S, Rodolfo-Metalpa R, Ransome E, Rowley S, Buia M, Gattuso J-P, Hall-Spencer JM (2008) Effects of naturally acidified seawater on seagrass calcareous epibionts. *Biology Letters*, **4**, 689–692.

Mata TM, Haddad NM, Holyoak M (2013) How invader traits interact with resident

communities and resource availability to determine invasion success. *Oikos*, **122**, 149–160.

Maury O, Poggiale JC (2013) From individuals to populations to communities: A dynamic energy budget model of marine ecosystem size-spectrum including life history diversity. *Journal of Theoretical Biology*, **324**, 52–71.

McCormick MI, Watson S-A, Munday PL (2013a) Ocean acidification reverses competition for space as habitats degrade. *Sci. Rep.*, **3**, 3280.

McCormick MI, Watson S-AS-A, Munday PL (2013b) Ocean acidification reverses competition for space as habitats degrade. *Sci. Rep.*, **3**, 3280.

McCoy SJ, Allesina S, Pfister CA (2016) Ocean acidification affects competition for space: projections of community structure using cellular automata. *Proceedings of the Royal Society B: Biological Sciences*, **283**, 20152561.

McLean KM, Todgham AE, J M et al. (2015) Effect of food availability on the growth and thermal physiology of juvenile Dungeness crabs (*Metacarcinus magister*). *Conservation Physiology*, **3**, cov013.

Meadows AS, Ingels J, Widdicombe S, Hale R, Rundle SD (2015) Effects of elevated CO_2 and temperature on an intertidal meiobenthic community. *Journal of Experimental Marine Biology and Ecology*, **469**, 44–56.

Melzner F, Gutowska MA, Langenbuch M (2009a) Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosciences (*.

Melzner F, Gutowska MA, Langenbuch M et al. (2009b) Physiological basis for high CO 2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosciences (*, **6**, 2313–2331.

Melzner F, Stange P, Trübenbach K et al. (2011) Food supply and seawater pCO_2 impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. *PLoS ONE*, **6**.

Menge B (2000) Recruitment vs. postrecruitment processes as determinants of barnacle population abundance. *Ecological Monographs*, **70**, 265–288.

Menge BA, Sutherland JP (1987) Community regulation: Variation in disturbance, competition, and predation in relation to environmental stress and recruitment. *American Naturalist*, **130**, 730–757.

Micheli AF, Cottingham KL, Bascompte J et al. (1999) The Dual Nature of Community Variability. *Nordic Society Oikos*, **85**, 161–169.

Milazzo M, Rodolfo-Metalpa R, Chan VBS et al. (2014) Ocean acidification impairs vermetid reef recruitment. *Scientific Reports*, **4**, 1–7.

Milazzo M, Cattano C, Alonzo SH et al. (2016) Ocean acidification affects fish spawning but not paternity at CO_2 seeps. *Proceedings of the Royal Society B*, **283**, 20161021.

Millero FJ (2010) Carbonate constants for estuarine waters. *Marine and Freshwater Research*, **61**, 139–142.

Monaco CJ, Wethey DS, Helmuth B (2014) A Dynamic Energy Budget (DEB) model for the keystone predator Pisaster ochraceus. *PLoS ONE*, **9**.

Montagnes DJS, Berges JA, Harrison PJ, Taylor FJR (1994) Estimating carbon, nitrogen, protein, and chlorophyll a from volume in marine phytoplankton. *Limnology and Oceanography*, **39**, 1044–1060.

Mook D (1981) Removal of Suspended Particles by Fouling Communities . *Marine Ecology Progress Series*, **5**, 279–281.

Morin P (2011) *Community Ecology*. John Wiley & Sons, Hoboken, NJ.

Morris WF, Hufbauer RA, Agrawal AA et al. (2007) Direct and indirect effects of enemies and mutualists on plant performance: a meta-analysis. *Ecology*, **88**, 1021–1029.

Motegi C, Tanaka T, Piontek J, Brussaard CPD, Gattuso J-P, Weinbauer MG (2013) Effect of CO_2 enrichment on bacterial metabolism in an Arctic fjord. *Biogeosciences*, **10**, 3285–3296.

Muller EB, Nisbet RM (2014) Dynamic energy budget modeling reveals the potential of future growth and calcification for the coccolithophore Emiliania huxleyi in an acidified ocean. *Global Change Biology*, **20**, 2031–2038.

Mullin MM, Sloan PR, Eppley RW (1966) Relationship between carbon content, cell volume, and area in phytoplankton. *Limnology and Oceanography*, **11**, 307–311.

Nagelkerken I, Connell SD (2015) Global alteration of ocean ecosystem functioning due to increasing human CO_2 emissions. *Proceedings of the National Academy of Sciences*, **2015**, 201510856.

Nash MC, Opdyke BN, Troitzsch U et al. (2012) Dolomite-rich coralline algae in reefs resist dissolution in acidified conditions. *Nature Climate Change*.

Navarro JM, Torres R, Acuña K et al. (2013) Impact of medium-term exposure to elevated pCO_2 levels on the physiological energetics of the mussel *Mytilus chilensis*. *Chemosphere*, **90**, 1242–1248.

Needles L, Wendt D (2013) Big changes to a small bay: introduced species and long-term compositional shifts to the fouling community of Morro Bay (CA). *Biological Invasions*, **15**, 1231–1251.

Nisbet RM, McCauley E, Johnson LR (2010) Dynamic energy budget theory and population ecology: lessons from Daphnia. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**, 3541–3552.

Nogueira P, Gambi MC, Vizzini S, Califano G, Tavares AM, Santos R, Martínez-Crego B (2017) Altered epiphyte community and sea urchin diet in Posidonia oceanica meadows in the vicinity of volcanic CO_2 vents. *Marine Environmental Research*, **127**, 102–111.

Norberg JJ, Swaney DPDP, Dushoff JJ, Lin JJ, Casagrandi RR, Levin SASA (2001) Phenotypic diversity and ecosystem functioning in changing environments: a theoretical framework. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, **98**, 11376–11381.

O'Brien JM, Krumhansl KA, Scheibling RE (2013) Invasive bryozoan alters interaction between a native grazer and its algal food. *Journal of the Marine Biological Association of the United Kingdom*, **93**, 1393–1400.

O'Donnell MJ, George MN, Carrington E (2013) Mussel byssus attachment weakened by ocean acidification. *Nature Climate Change*, **3**, 1–4.

Occhipinti-Ambrogi A (2007) Global change and marine communities: Alien species and climate change. *Marine Pollution Bulletin*, **55**, 342–352.

Odum EP, Finn JT, Franz EH (1979) Perturbation theory and the subsidy-stress gradient. *BioScience*, **29**, 349–352.

Oksanen AJ, Blanchet FG, Kindt R et al. (2015a) vegan: Community Ecology package.

Oksanen J, Blanchet FG, Friendly M et al. (2015b) vegan: Community Ecology Package.

Olyarnik S V, Bracken MES, Byrnes JE, Hughes AR, Hultgren KM, Stachowicz JJ (2009) Ecological Factors Affecting Community Invasibility. In: *Biological Invasions in Marine Ecosystems* (eds Rilov G, Crooks JA), pp. 215–238. Springer-Verlag Berlin Heidelberg.

Orejas C, Rossi S, Peralba À, García E, Gili JM, Lippert H (2013) Feeding ecology and trophic impact of the hydroid *Obelia dichotoma* in the Kongsfjorden (Spitsbergen, Arctic). *Polar Biology*, **36**, 61–72.

Osman RW, Whitlach RB (1995) The influence of resident adults on recruitment: a comparison to settlement. *Journal of Experimental Marine Biology and Ecology*, **190**, 169–198.

Osman R, Whitlatch R, Zajac R (1989) Effects of resident species on recruitment into a community larval settlement versus post-settlement mortality in the oyster *Crassostrea virginica*. *Marine Ecology Progress Series*, **54**, 61–73.

Pajusalu L, Martin G, Põlumäe A (2013) Results of laboratory and field experiments of the direct effect of increasing CO_2 on net primary production of macroalgal species in brackish-water ecosystems. *Proceedings of the*.

Pan T-CF, Applebaum SL, Manahan DT (2015) Experimental ocean acidification alters the allocation of metabolic energy. *Proceedings of the National Academy of Sciences*, **112**, 4696–4701.

Pansch C, Schaub I, Havenhand JN, Wahl M, Sciences B (2014) Habitat traits and food availability determine the response of marine invertebrates to ocean acidification. *Global Change Biology*, **20**, 765–777.

Parker LM, Ross PM, O'Connor WA, Borysko L, Raftos DA, Portner HO (2012) Adult exposure

influences offspring response to ocean acidification in oysters. *Global Change Biology*, **18**, 82–92.

Peck LS, Clark MS, Power D, Reis J, Batista FM, Harper EM (2015) Acidification effects on biofouling communities: winners and losers. *Global Change Biology*, **21**, 1907–1913.

Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L (2008) Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. *Journal of Clinical Epidemiology*, **61**, 991–996.

Petersen JK (2007) Ascidian suspension feeding. *Journal of Experimental Marine Biology and Ecology*, **342**, 127–137.

Pickett M, Andersson AJ (2015) Dissolution rates of biogenic carbonates in natural seawater at different pCO_2 conditions: a laboratory study. *Aquatic Geochemistry*, **21**, 459–485.

Pierrot D, Lewis E, Wallace DWR (2006) MS Excel program developed for CO_2 system calculations.

Piggott JJ, Townsend CR, Matthaei CD (2015) Reconceptualizing synergism and antagonism among multiple stressors. *Ecology and Evolution*, **5**, 1538–1547.

Platt WJ, Connell JH (2003) Natural disturbances and directional replacement of species. *Ecological Monographs*, **73**, 507–522.

Poloczanska ES, Burrows MT, Brown CJ et al. (2016) Responses of marine organisms to climate change across oceans. *Frontiers in Marine Science*, **3**, 1–21.

Polovina JJ, Howell EA, Abecassis M (2008) Ocean's least productive waters are expanding. *Geophysical Research Letters*, **35**, 2–6.

Poore A, Graba-Landry A, Favret M, Brennand H, Byrne M, Dworjanyn S (2013) Direct and indirect effects of ocean acidification and warming on a marine plant–herbivore interaction. *Oecologia*, **173**, 1113–1124.

Porzio L, Buia MC, Hall-spencer JM, Cristina M (2011) Effects of ocean acidification on macroalgal communities. *Journal of Experimental Marine Biology and Ecology*, **400**, 278–287.

Porzio L, Garrard S, Buia M (2013) The effect of ocean acidification on early algal colonization stages at natural CO_2 vents. *Marine Biology*, **160**, 2247–2259.

Przeslawski R, Byrne M, Mellin C (2015) A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Global change biology*, **21**, 2122–2140.

Rabalais NN, Turner RE, Díaz RJ, Justić D (2009) Global change and eutrophication of coastal waters. *Ices Journal of Marine Science*, **66**, 1528–1537.

R Development Core Team (2009) R: a language and environment for statistical computing.

Rahel FJ, Olden JD (2008) Assessing the effects of climate change on aquatic invasive species.

Conservation Biology, **22**, 521–533.

Ramajo L, Marbà N, Prado L et al. (2015) Biomineralization changes with food supply confer juvenile scallops (*Argopecten purpuratus*) resistance to ocean acidification. *Global Change Biology*, **22**, 2025–2037.

Ramajo L, Pérez-León E, Hendriks IE et al. (2016) Food supply confers calcifiers resistance to ocean acidification. *Scientific Reports*, **6**, 19374.

Range P, Piló D, Ben-hamadou R et al. (2012) Seawater acidification by CO_2 in a coastal lagoon environment: Effects on life history traits of juvenile mussels Mytilus galloprovincialis. *Journal of Experimental Marine Biology and Ecology*, **424**–**425**, 89–98.

Raulf FF, Fabricius K, Uthicke S, de Beer D, Abed RMM, Ramette A (2015) Changes in microbial communities in coastal sediments along natural CO_2 gradients at a volcanic vent in Papua New Guinea. *Environmental Microbiology*, **17**, 3678–3691.

Reum J, Alin SR, Feely RA, Newton J, Warner M, McElhany P (2014) Seasonal carbonate chemistry covariation with temperature, oxygen, and salinity in a fjord estuary: implications for the design of ocean acidification experiments. *PLoS ONE*, **9**, e89619.

Ricciardi A, Cohen J (2007) The invasiveness of an introduced species does not predict its impact. *Biological Invasions*, **9**, 309–315.

Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. *Geology*, **37**, 1131–1134.

Riley LA, Dybdahl MF (2015) The roles of resource availability and competition in mediating growth rates of invasive and native freshwater snails. *Freshwater Biology*, **60**, 1308–1315.

Rodríguez-Romero A, Jarrold MD, Massamba-N'Siala G, Spicer JI, Calosi P (2015) Multi-generational responses of a marine polychaete to a rapid change in seawater pCO_2. *Evolutionary Applications*, 1–14.

Rohatgi A (2015) WebPlotDigitizer.

Roleda MY, Cornwall CE, Feng Y, McGraw CM, Smith AM, Hurd CL (2015) Effect of ocean acidification and pH fluctuations on the growth and development of coralline algal recruits, and an associated benthic algal assemblage. *Plos One*, **10**, e0140394.

Romanuk TN, Kolasa J (2005) Resource limitation, biodiversity, and competitive effects interact to determine the invasibility of rock pool microcosms. *Biological Invasions*, **7**, 711–722.

Ross P, Parker L, O'Connor W, Bailey EA (2011) The Impact of ocean acidification on reproduction, early development and settlement of marine organisms. *Water*, **3**, 1005–1030.

Ross PM, Parker L, Byrne M (2016) Transgenerational responses of molluscs and echinoderms to changing ocean conditions. *ICES Journal of Marine Science*, **73**, 537–549.

Rossoll D, Bermúdez R, Hauss H, Schulz KG, Riebesell U, Sommer U, Winder M (2012) Ocean acidification-induced food quality deterioration constrains trophic transfer. *PLoS ONE*, **7**,

e34737.

Roughgarden J, Gaines SD, Possingham H (1988) Recruitment dynamics in complex life cycles. *Science*, **241**, 1460–1466.

Russell B, Connell SD (2012) Origins and consequences of global and local stressors: incorporating climatic and non-climatic phenomena that buffer or accelerate ecological change. *Marine Biology*, **159**, 2633–2639.

Russell BD, Thompson JI, Falkenberg LJ, Connell SD (2009) Synergistic effects of climate change and local stressors: CO_2 and nutrient-driven change in subtidal rocky habitats. *Global Change Biology*, **15**, 2153–2162.

Russell BD, Connell SD, Findlay HS, Tait K, Widdicombe S, Mieszkowska N (2013) Ocean acidification and rising temperatures may increase biofilm primary productivity but decrease grazer consumption. *Proceedings of the National Academy of Sciences of the United States of America*, **368**.

Saderne V, Wahl M (2013) Differential responses of calcifying and non-calcifying epibionts of a brown macroalga to present-day and future upwelling pCO_2. *PLoS ONE*, **8**, e70455.

Sams MA, Keough MJ (2012) Contrasting effects of variable species recruitment on marine sessile communities. *Ecology*, **93**, 1153–1163.

Sanford E, Gaylord B, Hettinger A, Lenz EA, Meyer K, Hill TM (2014) Ocean acidification increases the vulnerability of native oysters to predation by invasive snails. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 1–8.

Sarmento VC, Souza TP, Esteves a. M, Santos PJP (2015) Effects of seawater acidification on a coral reef meiofauna community. *Coral Reefs*, **34**, 955–966.

Schaalje GB, McBride JB, Fellingham GW (2002) Adequacy of approximations to distributions of test statistics in complex mixed linear models. *Journal of Agricultural, Biological, and Environmental Statistics*, **7**, 512–524.

Schneider CA, Rasb WS, Eliceiri KW, Rasband WS (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, **9**, 671–675.

Schulz K, Bellerby RGJ, Brussaard C et al. (2013) Temporal biomass dynamics of an Arctic plankton bloom in response to increasing levels of atmospheric carbon dioxide. *Biogeosciences*, **10**, 161–180.

Shaw E, Munday P, McNeil B (2013a) The role of CO_2 variability and exposure time for biological impacts of ocean acidification. *Geophysical Research Letters*, **40**, 4685–4688.

Shaw EC, Munday PL, McNeil BI (2013b) The role of CO_2 variability and exposure time for biological impacts of ocean acidification. *Geophysical Research Letters*, **40**, 4685–4688.

Short J, Kendrick GA, Falter J, McCulloch MT (2014) Interactions between filamentous turf algae and coralline algae are modified under ocean acidification. *Journal of Experimental*

Marine Biology and Ecology, **456**, 70–77.

Sih A, Englund G, Wooster D (1998) Emergent impacts of multiple predators on prey. *Trends in Ecology and Evolution*, **13**, 350–355.

Skaug H, Fournier D, Nielsen A, Magnusson A, Bolker B (2013) glmmADMB: generalized linear mixed models using AD model builder. R package version 0.7.7.4.

Small DP, Milazzo M, Bertolini C, Graham H, Hauton C, Hall-spencer JM, Rastrick SPS (2015) Temporal fluctuations in seawater pCO_2 may be as important as mean differences when determining physiological sensitivity in natural systems. *Ices Journal of Marine Science*, **73**, 1–9.

Smith AM, Garden CJ (2013) Being a bimineralic bryozoan in an acidifying ocean. In: *Bryozoan Studies*, Vol. 143 (eds Ernst A, Schafer P, Scholz J), pp. 137–153. Berlin, Heidelberg.

Smith AM, Key MM, Gordon DP (2006) Skeletal mineralogy of bryozoans: Taxonomic and temporal patterns. *Earth-Science Reviews*, **78**, 287–306.

Smith AM, Riedi MA, Winter DJ (2013) Temperate reefs in a changing ocean: Skeletal carbonate mineralogy of serpulids. *Marine Biology*, **160**, 2281–2294.

Smith JN, De'ath G, Richter C, Cornils A, Hall-Spencer JM, Fabricius KE (2016) Ocean acidification reduces demersal zooplankton that reside in tropical coral reefs. *Nature Climate Change*, **1**.

Sokolova IM (2013) Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. *Integrative and comparative biology*, **53**, 597–608.

Sokolova IM, Frederich M, Bagwe R, Lannig G, Sukhotin AA (2012) Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Marine Environmental Research*, **79**, 1–15.

Sorte CJ, Williams SL, Carlton JT (2010) Marine range shifts and species introductions: comparative spread rates and community impacts. *Global Ecology and Biogeography*, **19**, 303–316.

Sousa WP (1979a) Experimental investigations of disturbance and ecological succession in a rocky intertidal algal community. *Ecological Monographs*, **49**, 227–254.

Sousa WP (1979b) Disturbance in marine intertidal boulder fields: the nonequilibrium maintenance of species diversity. *Ecology*, **60**, 1225–1239.

Sperling EA, Frieder CA, Levin LA (2016) Biodiversity response to natural gradients of multiple stressors on continental margins. *Proceedings of the Royal Society B: Biological Sciences*, **283**, 20160637-.

Stachowicz JJ, Byrnes JE (2006) Species diversity, invasion success, and ecosystem functioning: disentangling the influence of resource competition, facilitation, and extrinsic factors. *Marine Ecology Progress Series*, **311**, 251–262.

Stachowicz JJ, Whitlatch RB, Osman RW (1999) Species diversity and invasion resistance in a marine ecosystem. *Science*, **286**, 1577–1579.

Stachowicz JJ, Terwin JR, Whitlatch RB, Osman RW (2002a) Linking climate change and biological invasions: Ocean warming facilitates nonindigenous species invasions. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 15497–15500.

Stachowicz J, Fried H, Oman RW, Whitlatch R (2002b) Biodiversity, invasion resistance, and marine ecosystem function: reconciling pattern and process. *Ecology*, **83**, 2575–2590.

Standing J (1976) Fouling community structure: effects of the hydroid, *Obelia dichotoma*, on larval recruitment. In: *Coelenterate Ecology and Behavior* (ed Mackie GO), pp. 155–164. Springer Science & Business Media, New York.

Stewart RI a., Dossena M, Bohan D a. et al. (2013) Mesocosm experiments as a tool for ecological climate-change research. In: *Advances in Ecological Research*, 1st edn, Vol. 48 (eds Woodward G, O'Gorman EJ), pp. 71–181. Elsevier Ltd., London, UK.

Stumpp M, Hu MY, Melzner F, Gutowska MA, Dorey N (2012a) Acidified seawater impacts sea urchin larvae pH regulatory systems relevant for calcification.

Stumpp M, Trübenbach K, Brennecke D, Hu MY, Melzner F (2012b) Resource allocation and extracellular acid–base status in the sea urchin *Strongylocentrotus droebachiensis* in response to CO_2 induced seawater acidification. *Aquatic Toxicology*, **110**–**111**, 194–207.

Sui Y, Kong H, Huang X et al. (2016) Combined effects of short-term exposure to elevated CO_2 and decreased O_2 on the physiology and energy budget of the thick shell mussel *Mytilus coruscus*. *Chemosphere*, **155**, 207–216.

Sunday JM, Crim RN, Harley CD, Hart MW (2011) Quantifying rates of evolutionary adaptation in response to ocean acidification. *PLoS ONE*, **6**, e22881.

Sunday JM, Calosi P, Dupont S, Munday PL, Stillman JH, Reusch TBH (2014) Evolution in an acidifying ocean. *Trends in ecology & evolution*, **29**, 117–125.

Sunday JM, Fabricius KE, Kroeker KJ et al. (2017) Ocean acidification can mediate biodiversity shifts by changing biogenic habitat. *Nature Climate Change*, **7**, 81–85.

Sutherland JP (1974) Multiple stable points in natural communities. *American Naturalist*, **108**, 859–873.

Swezey DS, Bean JR, Ninokawa AT, Hill TM, Gaylord B, Sanford E (2017a) Interactive effects of temperature, food and skeletal mineralogy mediate biological responses to ocean acidification in a widely distributed bryozoan. *Proceedings of the Royal Society B*, **284**, 20162349.

Swezey DS, Bean JR, Hill TM, Gaylord B, Ninokawa AT, Sanford E (2017b) Plastic responses of bryozoans to ocean acidification. *The Journal of Experimental Biology*, jeb.163436.

Tanaka T, Alliouane S, Bellerby RGJ et al. (2013) Effect of increased pCO_2 on the planktonic metabolic balance during a mesocosm experiment in an Arctic fjord. *Biogeosciences*, **10**, 315–325.

Tans P (2009) An accounting of the observed increase in oceanic and atmospheric CO_2 and the outlook for the future. *Oceanography*, **22**, 26–35.

Taylor JR, Lovera C, Whaling PJ, Buck KR, Pane EF, Barry JP (2014) Physiological effects of environmental acidification in the deep-sea urchin *Strongylocentrotus fragilis*. *Biogeosciences*, **11**, 1413–1423.

Taylor PD, Lombardi C, Cocito S (2015) Biomineralization in bryozoans: present, past and future. *Biological Reviews*, **90**, 1118–1150.

Teo SLM, Ryland JS (1995) Potential antifouling mechanisms using toxic chemicals in some British ascidians. *Journal of Experimental Marine Biology and Ecology*, **188**, 49–62.

Thomsen JJ, Casties I, Pansch C, Körtzinger A, Melzner F (2013) Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis* : laboratory and field experiments. *Global Change Biology*, **19**, 1017–1027.

Thomson RE (1981) *Straight of Georgia and Fraser River Hydrology Report*. 165-185 pp.

Tilman D (1990) Constraints and tradeoffs: toward a predictive theory of competition and succession. *Oikos*, **58**, 3–15.

Tilman D (1999) The ecological consequences of changes in biodiversity: a search for general principles. *Ecology*, **80**, 1455–1474.

Tilman D (2004) Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. *Proceedings of the National Academy of Sciences*, **101**, 10854–10861.

Towle EK, Enochs IC, Langdon C (2015) Threatened Caribbean coral is able to mitigate the adverse effects of ocean acidification on calcification by increasing feeding rate. *PLoS ONE*, **10**, 1–17.

Turner LM, Ricevuto E, Massa-Gallucci A, Gambi M-C, Calosi P (2015) Energy metabolism and cellular homeostasis trade-offs provide the basis for a new type of sensitivity to ocean acidification in a marine polychaete at a high-CO_2 vent: adenylate and phosphagen energy pools versus carbonic anhydrase. *Journal of Experimental Biology*, **218**, 2148–2151.

Vallejo G, Fernandez MP, Tuero E, Livacic-Rojas PE (2010) Analyzing repeated measures using resampling methods. *Anales de Psicologia*, **26**, 400–409.

Vaz-Pinto F, Olabarria C, Gestoso I, Cacabelos E, Incera M, Arenas F (2013) Functional diversity and climate change: effects on the invasibility of macroalgal assemblages. *Biological Invasions*, **15**, 1833–1846.

Verity PG, Robertson CY, Tronzo CR, Andrews MG, Nelson JR, Sieracki ME (1992)

Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. *Limnology and Oceanography*, **37**, 1434–1446.

Viechtbauer W (2010) Conducting meta-analyses in R with the metafor package. *Statiscal Software*, **36**, 1–48.

Vitousek PM (1990) Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. *Oikos*, **57**, 7–13.

Vizzini S, Di Leonardo R, Costa V, Tramati CDD, Luzzu F, Mazzola A (2013) Trace element bias in the use of CO_2 vents as analogues for low pH environments: Implications for contamination levels in acidified oceans. *Estuarine, Coastal and Shelf Science*, **134**, 19–30.

Vizzini S, Martinez-Crego B, Andolina C, Massa-Gallucci A, Connell SD, Gambi M (2017) Ocean acidification as a driver of community simplification via the collapse of higher-order and rise of lower-order consumers. *Scientific Reports*, **7**, 4018.

Volkman JK, Jeffrey SW, Nichols PD, Rogers GI, Garland CD (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *Journal of Experimental Marine Biology and Ecology*, **128**, 219–240.

Van de Waal DB, Verspagen JM, Finke JF et al. (2011) Reversal in competitive dominance of a toxic versus non-toxic cyanobacterium in response to rising CO_2. *The ISME journal*, **5**, 1438–1450.

Wahl M, Schneider Covachã S, Saderne V, Hiebenthal C, Müller JD, Pansch C, Sawall Y (2017) Macroalgae may mitigate ocean acidification effects on mussel calcification by increasing pH and its fluctuations. *Limnology and Oceanography*.

Walther G-R, Roques A, Hulme P et al. (2009) Alien species in a warmer world: risks and opportunities. *Trends in ecology & evolution*, **24**, 686–693.

Wang Y, Li L, Hu M, Lu W (2015) Physiological energetics of the thick shell mussel *Mytilus coruscus* exposed to seawater acidification and thermal stress. *Science of the Total Environment*, **514**, 261–272.

Wang X, Wang M, Xu J, Jia Z, Liu Z, Wang L, Song L (2017) Soluble adenylyl cyclase mediates mitochondrial pathway of apoptosis and ATP metabolism in oyster *Crassostrea gigas* exposed to elevated CO_2. *Fish and Shellfish Immunology*, **66**, 140–147.

Warwick RM, Clarke KR (1993) Increased variability as a symptom of stress in marine communities. *Journal of Experimental Marine Biology and Ecology*, **172**, 215–226.

Webster N, Uthicke S, Botté E, Flores F, Negri A (2013) Ocean acidification reduces induction of coral settlement by crustose coralline algae. *Global Change Biology*, **19**, 303–315.

Wernberg T, Smale D, Thomsen MS (2012) A decade of climate change experiments on marine organisms: procedures, patterns and problems. *Global Change Biology*, **18**, 1491–1498.

Widdicombe S, Spicer JI (2008) Predicting the impact of ocean acidification on benthic

biodiversity: What can animal physiology tell us? *Journal of Experimental Marine Biology and Ecology*, **366**, 187–197.

Widdicombe S, Dashfield SL, McNeill CL (2009) Effects of CO_2 induced seawater acidification on infaunal diversity and sediment nutrient fluxes. *Marine Ecology Progress Series*, **379**, 59–75.

Williams GJ, Price NN, Ushijima B et al. (2014) Ocean warming and acidification have complex interactive effects on the dynamics of a marine fungal disease. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20133069–20133069.

Willis TJ, Berglof KTL, Mcgill RAR et al. (2017) Kleptopredation : a mechanism to facilitate planktivory in a benthic mollusc. *Biology Letters*, **13**, 20170447.

Winder M, Schindler DE (2004) Climate change uncouples trophic interactions in an aquatic ecosystem. *Ecology*, **85**, 2100–2106.

Wittmann AC, Pörtner H-O (2013) Sensitivities of extant animal taxa to ocean acidification. *Nature Climate Change*, **3**, 995–1001.

Wood H, Spicer JI, Widdicombe S (2008) Ocean acidification may increase calcification rates, but at a cost. *Proceedings. Biological sciences / The Royal Society*, **275**, 1767–1773.

Wootton JT (2001) Local interactions predict large-scale pattern in empirically derived cellular automata. *Nature*, **413**, 841–844.

Wootton J, Pfister C (2012) Carbon system measurements and potential climatic drivers at a site of rapidly declining ocean pH. *PLoS ONE*, **7**, e53396.

Wright DH (1983) Species-energy theory: an extension of species-area theory. *Nordic Society Oikos*, **41**, 496–506.

Xu X, Yang F, Zhao L, Yan X (2016) Seawater acidification affects the physiological energetics and spawning capacity of the Manila clam *Ruditapes philippinarum* during gonadal maturation. *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology*, **196**, 20–29.

Zhan A, Briski E, Bock DG, Ghabooli S, Macisaac HJ (2015) Ascidians as models for studying invasion success. *Marine Biology*, **162**, 2449–2470.

Zhang H, Cheung SG, Shin PKS (2014) The larvae of congeneric gastropods showed differential responses to the combined effects of ocean acidification, temperature and salinity. *Marine Pollution Bulletin*, **79**, 39–46.

Appendix A: Supplementary Information for Chapter 2

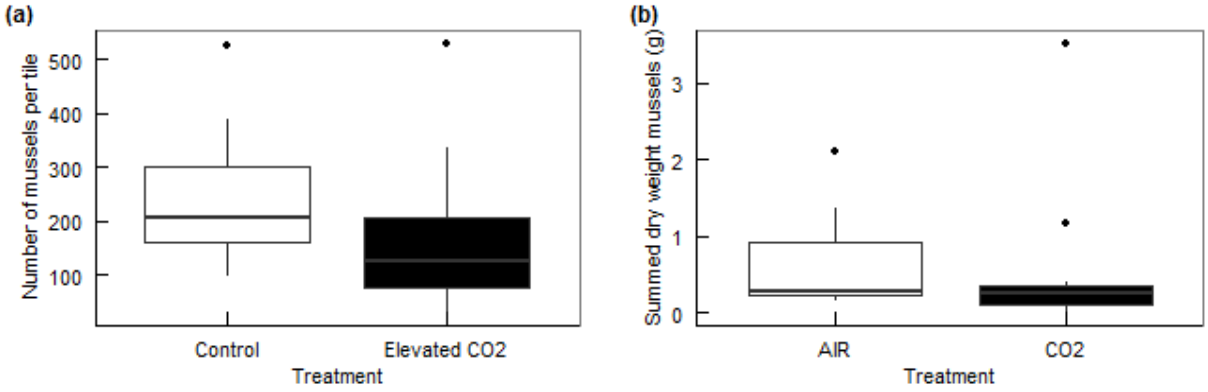

Fig. S2.1. (a) The number of mussels per tile (GLMM, P=0.018) and (b) summed dry weight (g) of mussels (QR, tau=0.5, P>0.05) for ambient and elevated CO_2 treatments at week 10.

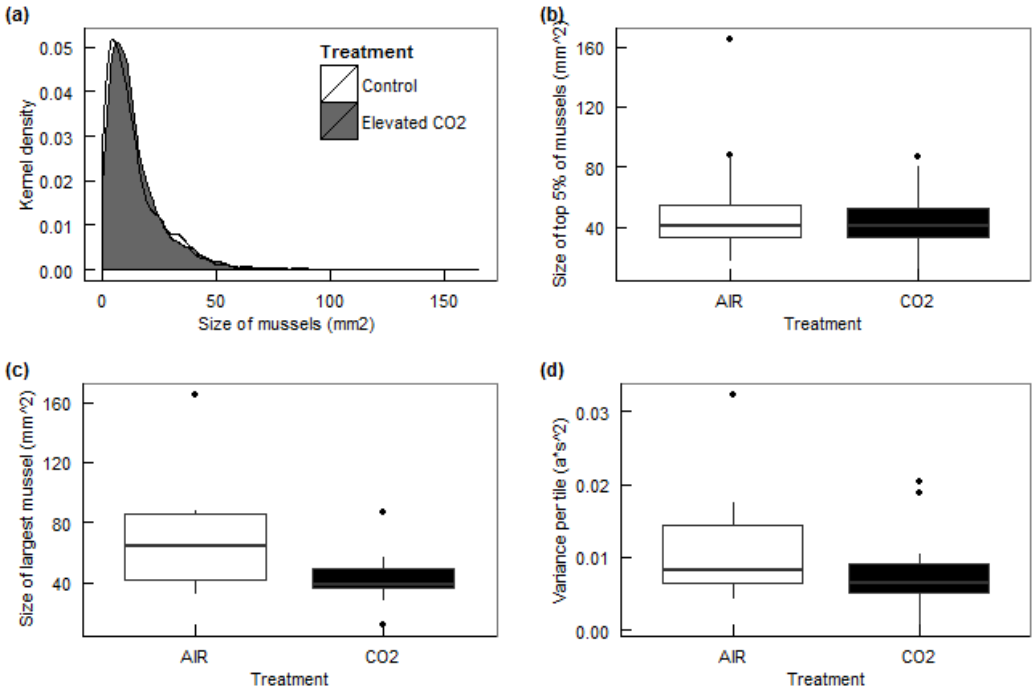

Fig. S2.2. Effects of experimental acidification on *Mytilus trossulus* size distribution at the end of the 10-week experiment: (a) mussel size (cm^2) (GLMM, P>0.05), (b) largest 5% of mussels (GLMM, P>0.05), (c) size of the single largest mussel (GLM, P=0.03), and (d) variance of mussel size (GLM, P>0.05).

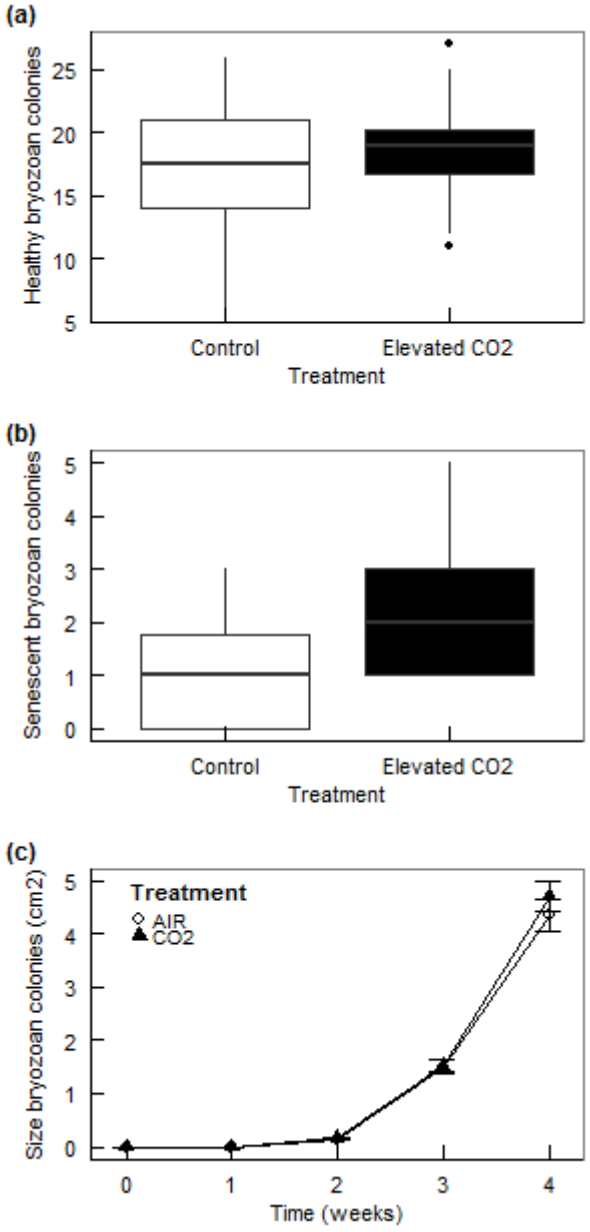

Fig. S2.3. Effects of experimental acidification on *Membranipora membranacea*: (a) healthy colonies (GLMM, $P>0.05$) and (b) senescent colonies (GLMM, $P=0.0009$) after 10 weeks in ambient (n=11) and elevated (n=12) CO_2 conditions. (c) Size of *Membranipora* colonies (cm^2) in acidified and control conditions over the first four weeks of the experiment (RMANOVA, $P>0.05$).

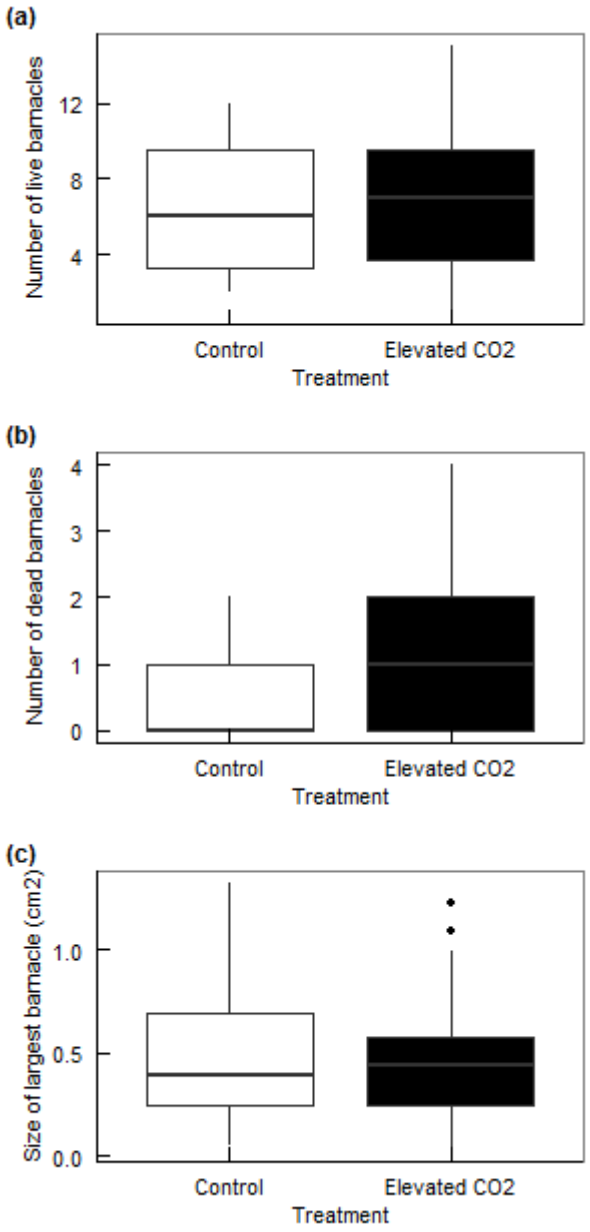

Fig. S2.4. Effects of experimental acidification on *Balanus crenatus* after 10 weeks: (a) live barnacles (GLMM, $P>0.05$), and (b) dead barnacles (GLMM, $P=0.051$) at week 10. (c) Basal area of the largest barnacle (GLMM, $P>0.05$) at week 10 in acidified and control conditions (n=23).

Appendix B: Supplementary Information for Chapter 3

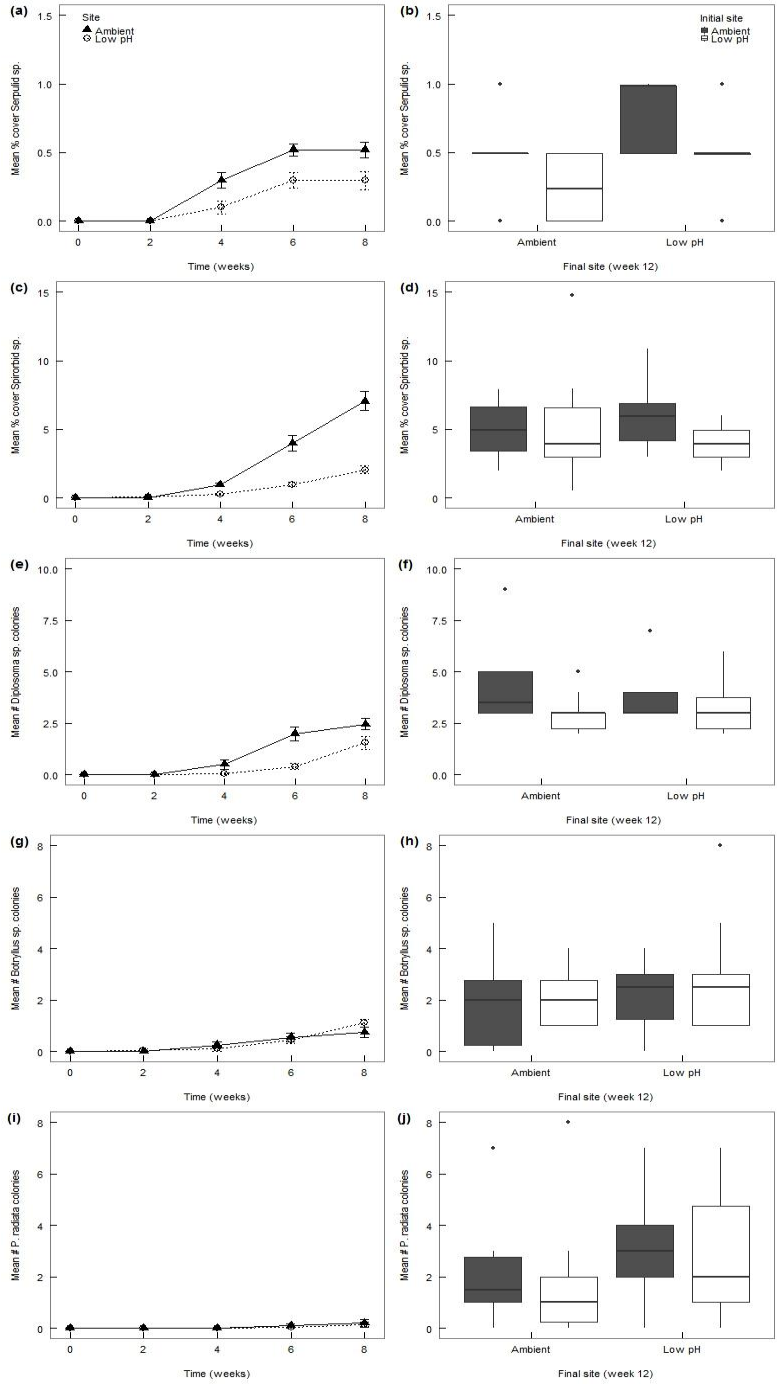

Fig. S3.1. Abundance of selected primary and secondary colonizers in ambient and low pH sites over time, left-hand panels are up to week 8 (n=20) and right-hand panels are at week 12 (n=10) of both transplanted and non-transplanted tiles. For the right-hand panels, shading indicates initial site and position on x-axis indicates final site. Species are: (a, b) Serpulid, (c, d) spirorbid, (e, f) *Diplosoma* sp., (g, h) *Botryllus* sp., and (i,j) *Patinella radiata*. Error bars indicate standard error.

Appendix C: Supplementary Information for Chapter 4

Regression with delta CO_2 analysis (Fig. S4.1)

I tested for differences in response to CO_2 (slopes) across experimentally imposed changes in CO_2 between food supply levels using Q_M tests (here the test statistic is referred to as $Q_{Mslopes}$). Models included deltaCO_2 as a co-variate and were tested as in the multilevel analysis (Fig. 4.2).

Repeatability analysis (Fig. S4.2)

Here I used exactly the same papers as the original analysis and included intermediate food supply levels. When possible, I used data extracted by Ramajo *et al.*(Ramajo *et al.*, 2016), courtesy of Laura Ramajo, and then extracted the additional data points for "low food, low pH" ourselves (but see Table S4.1). I followed statistical methods as presented in Ramajo *et al.*(Ramajo *et al.*, 2016). I present the data as bias-corrected bootstrapped confidence intervals and plotted these on top of bias-corrected means (BCa method). I used tests of heterogeneity described by the moderators (Q_M tests) on a random-effects model (study as random) to test differences between food supply levels for effect sizes. I inferred the Q_M method from their results although it is not described their methods. I first recreated the Ramajo *et al.* analysis, using a common denominator of "Control CO_2, High food" treatment for all LnRR calculations (i.e. at every food level this was the common denominator) (Fig. 4.3 left hand panels). I then used the same data but the proper LnRR calculation (Fig. 4.3 right hand panels). This repeatability analysis contains a few known errors (see Table S4.1), so should be interpreted only as a comparison of the two LnRR techniques, not as a reliable result.

Repeatability analysis using factorial meta-analysis (Fig. S4.3)

Here, I calculated the factorial LnRR using the same methods as above for Fig. 4.3, using data from the repeatability analysis, Fig. S4.2. I conducted both the repeated analysis with a LnRR calculation error and a repeated analysis with this error corrected. Although factorial meta-analysis uses a different calculation than the analyses in the main paper, I assumed the same common denominator of "Control CO_2, High food" treatment employed by Ramajo *et al.* (2016) as the control in the factorial LnRR calculations. This analysis is for illustration purposes only as Ramajo *et al.* (2016) did not employ this analysis in their paper. The results of this analysis demonstrate how the interaction effect is exaggerated when LnRR is calculated using a common control.

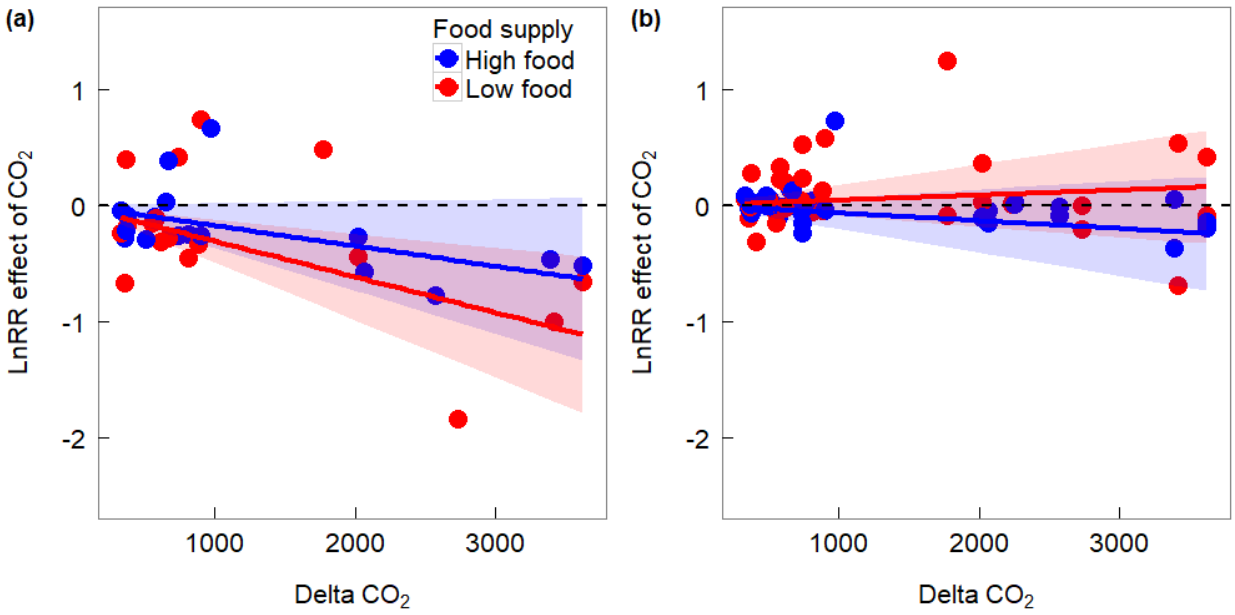

Fig. S4.1. Relationship between magnitude of CO_2 effect imposed in the study on LnRR of (a) calcification (N=11) and (b) growth (N=16) measures under high (blue lines) and low food supply (red lines) conditions. The regression line and confidence intervals are predicted estimates and confidence intervals based on an REML model. Across food supply levels, calcification response ratios declined with increasing experimental change in CO_2 (Q_M = 10.79, *P*=0.0045, slope high food = -0.003, slope low food = - 0.002). However, the rate of change of calcification LnRR with increasing delta CO_2 (i.e. slope) did not differ between food treatments ($Q_{Mslopes}$= 1.51, *P*= 0.22). Growth LnRR did not change with increasing experimental delta CO_2 (Q_M= 2.37, *P*=0.30, slope high food = -0.0001, slope low food = 0.00) and there was no difference in slopes between food treatments ($Q_{Mslopes}$= 2.35, *P*= 0.12).

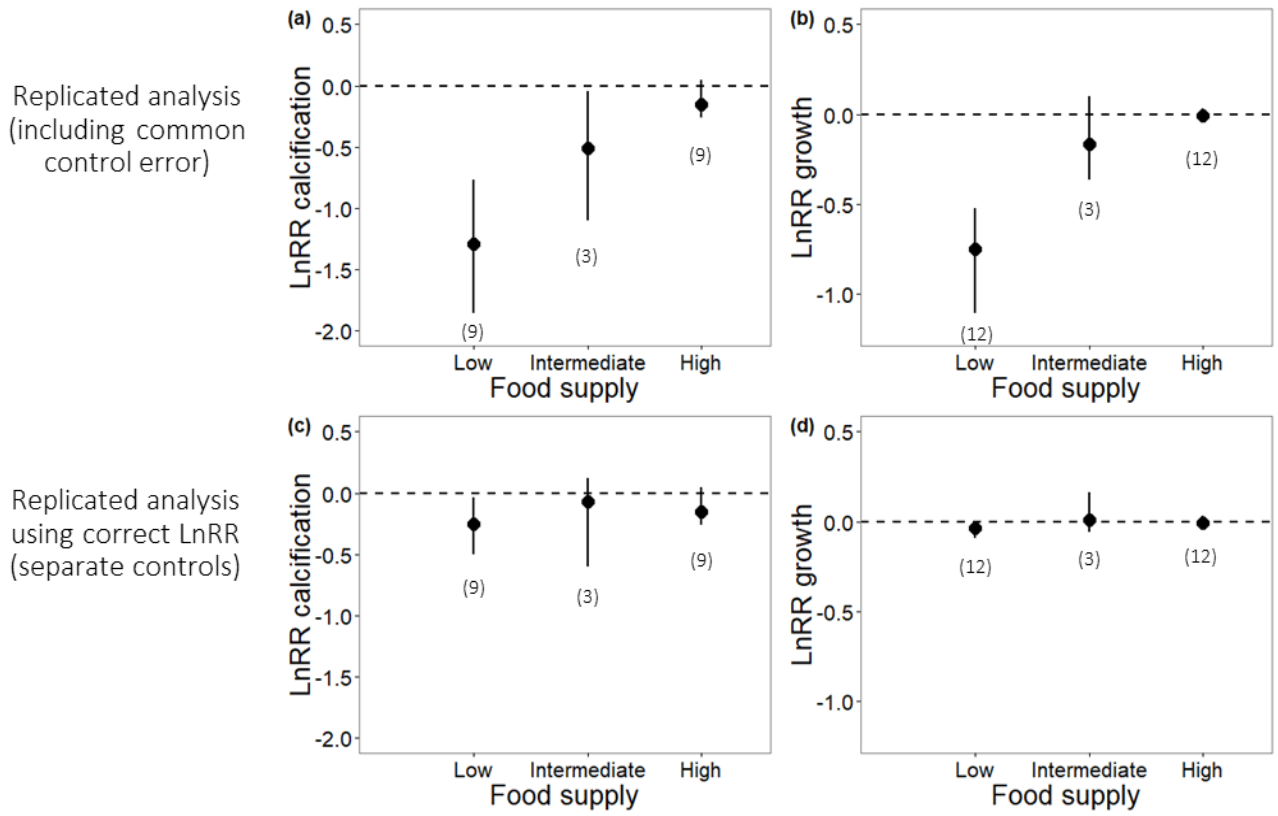

Fig. S4.2. Repeatability analysis. Mean effect size (LnRR) of (a,b) calcification and (c,d) growth responses to CO_2 under low, intermediate, and high food supply levels using exact data from Ramajo *et al.* (2016)(see table S1). (a) and (b) show the analysis using the incorrect LnRR calculation employed by Ramajo *et al.* and (c) and (d) show how a corrected LnRR dramatically alters the results. Error bars shown are the bias-corrected bootstrapped 95% confidence intervals around bias-corrected estimates. In this analysis, if the 95% confidence intervals do not overlap between food supply levels a significant difference in CO_2 response is inferred between those food supply levels. Therefore, the incorrect analysis (a) and (b) suggests that the response to CO_2 is significantly different between low and high food supply for calcification ($Q_{M(coef)}$=5.56, P= 0.018) and growth ($Q_{M(coef)}$=6.45, P= 0.011); whereas the correct analysis (c) and (d) shows that the response to CO_2 is not significantly different between food supply levels for calcification ($Q_{M(coef)}$=0.60, P= 0.44) and growth ($Q_{M(coef)}$=0.28, P= 0.61). Numbers in brackets indicate the number of studies contributing to the LnRR.

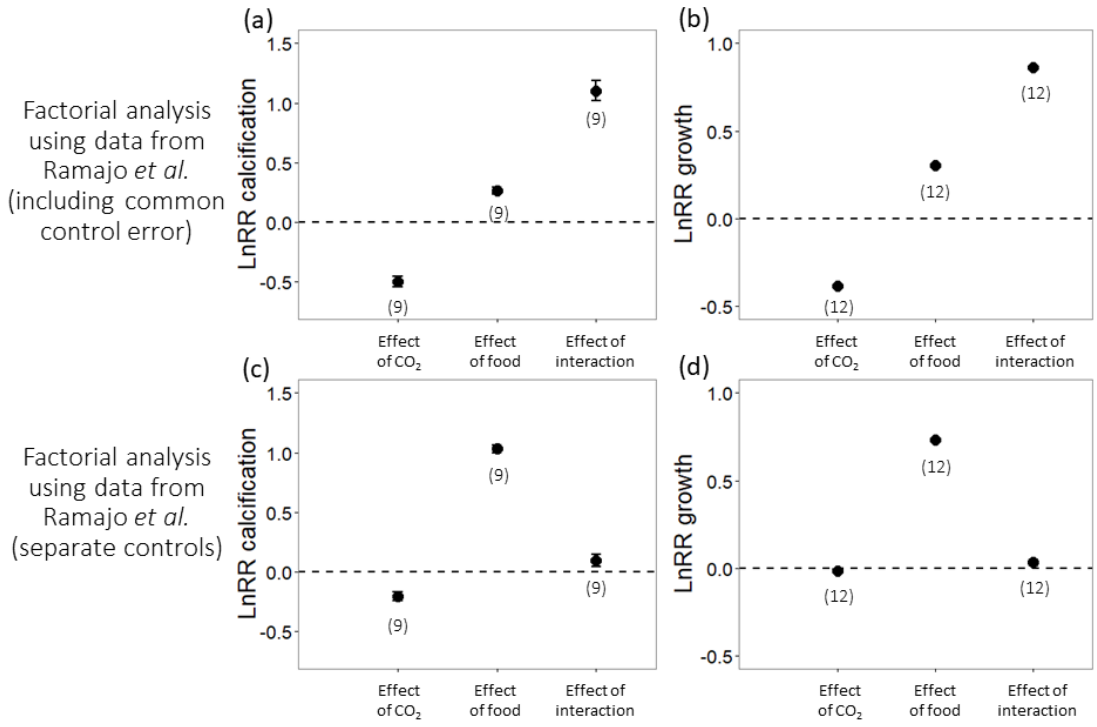

Fig. S4.3. Factorial repeatability analysis. Overall and interactive effect sizes for CO_2 and food supply for (a,c) calcification and (b,d) growth responses using exact data from Ramajo *et al.*(Ramajo *et al.*, 2016)(see table S1). (a) and (b) show the analysis using the incorrect LnRR calculation employed by Ramajo *et al.* and (c) and (d) show how a corrected LnRR dramatically alters the results. (a) For calcification, the incorrect analysis shows an overall significant negative effect of CO_2 (LnRR=-0.50, lower=-0.54, upper=-0.45), a significant positive effect of food supply (LnRR=0.26, lower=0.24, upper=0.29), and a very large positive interaction effect of CO_2 and food supply (LnRR=1.10, lower=1.02, upper=1.19). (c) The corrected analysis shows a small significant negative effect of CO_2 (LnRR=-0.20, lower=-0.24, upper=-0.17), a large significant positive effect of food supply (LnRR=1.03, lower=1.00, upper=1.07), and a much smaller positive effect of the interaction between CO_2 and food supply (LnRR=0.10, lower=0.043, upper=0.15). (b) For growth, the incorrect analysis shows an overall significant negative effect of CO_2 (LnRR=-0.39, lower=-0.39, upper=-0.38), a significant positive effect of food supply (LnRR=0.30, lower=0.29, upper=0.31), and a large positive interaction effect of CO_2 and food supply (LnRR=0.86, lower=0.85, upper=0.87). (d) The corrected growth analysis shows a small significant negative effect of CO_2 (LnRR=-0.014, lower=-0.024, upper=-0.0044), a large significant positive effect of food supply (LnRR=0.73, lower=0.72, upper=0.74), and a small positive effect of the interaction between CO_2 and food supply (LnRR=0.03, lower=0.015, upper=0.048). I note that this interaction is in the opposite direction as calculated with more data in Fig. 4.2, however when calculated without extraction errors (Table 1) this effect becomes non-interactive. In both calcification and growth responses, the incorrect common control analysis inflated the interaction effect. Error bars are 95% confidence intervals calculated from standard error for small sample sizes. If the error bars do not overlap zero then a significant response is inferred. Numbers in brackets indicate the number of studies contributing to the LnRR.

Table S4.1. Differences between datasets used in Ramajo *et al*.(2016), the repeatability analysis, factorial analyses, and main analysis in Chapter 4.

	Ramajo et al. original analysis, Repeated analysis, Factorial analysis using Ramajo et al. data	*Main analysis*	*Factorial main analysis*
Calcification			
Hettinger et al. 2013	total dry weight	shell growth	shell growth
Edmunds 2011	Calcification mg/cm^2/day	Calcification mg/mg/day	Calcification mg/mg/day
Comeau et al. 2013	Reversed feeding level for high CO_2 for calcification measure (incorrectly used 0.22 mg $CaCO_3$ day^{-1} mg^{-1} instead of 0.11)	Used correct high and low CO_2 for calcification measure	Used correct high and low CO_2 for calcification measure
Edmunds 2011 & Thomsen et al. 2013	Used SE incorrectly as SD, artificially reducing variability	Used correct value for SD	Used correct value for SD
Maier et al. 2016	---	net calcification	net calcification
Swezey et al. 2017	---	Proportion zooids Mg/Ca > 12	Proportion zooids Mg/Ca > 12
Growth			
Melzner et al. 2011	length	length & somatic growth	somatic growth
Hettinger et al. 2013	shell growth	shell growth & total dry weight	total dry weight
Pansch et al. 2014	growth (mm)	growth (mm) & dry weight	dry weight
Thomsen et al. 2013	shell length	shell length & organic mass	organic mass
Drenkard et al. 2013	septa diameter	septa diameter & tissue lipid weight	tissue lipid weight
Edmunds 2011	Low temp as control	High temp as control (as specified in Edmunds 2011)	High temp as control (as specified in Edmunds 2011)
Crook et al., Drenkard et al. & Edmunds 2011	Used SE incorrectly as SD, artificially reducing variability	Used correct value for SD	Used correct value for SD
Oddvarsdotter 2014 (unpublished)	carbon (ug C/L)	Not included, unpublished data	Not included, unpublished data
Taylor et al. 2014	---	% change width included for LnRR effect of CO_2	Not included, ln (negative number) = NA
Büscher et al. 2017	---	growth (% per day)	growth (% per day)

Cole et al. 2016	---	shell length	shell length
Hurst et al. 2017	---	Larval growth (mm/day) and mass growth (mg/day)	Larval growth (mm/day) and mass growth (mg/day)
Swezey et al. 2017	---	growth efficiency	growth efficiency
All papers	Included intermediate food levels No correction for multiple comparisons to control CO_2	Excluded intermediate food levels Corrected for multiple comparisons to control CO_2	

Appendix D: Supplementary Information for Chapter 5

Table S5.1. Seawater carbonate chemistry in ambient (n=30) and elevated (n=29) CO_2 treatments. Mean midday values averaged over the duration of the experiment. Asterisks indicate calculated values in the CO2-SYS program (Pierrot *et al.*, 2006).

Seawater parameter	Control	Low pH
Temperature (°C)	15.6 ± 0.1	15.6 ± 0.1
Salinity	24.7 ± 0.1	24.7 ± 0.1
pH_{NBS}	8.00 ± 0.01	7.64 ± 0.02
Alkalinity (μmol kg^{-1})	1596.9 ± 9.0	1622.6 ± 11.46
pCO_2 (μatm)*	544.6 ± 22.3	1604.9 ± 97.1
DIC (μmol kg^{-1})*	1509.5 ± 8.9	1625.2 ± 11.1
HCO_3^- (μmol kg^{-1})*	1416.7 ±8.7	1520 ± 10.4
Ω Calcite*	1.8 ± 0.06	1.03 ± 0.06
Ω Aragonite*	1.13 ± 0.04	0.65 ± 0.03

Table S5.2. Mean and SE of abundance (percent cover, # individuals or # colonies) of all species at week 12 in each treatment.

Species abundances	Ambient food Ambient CO_2		Ambient food Ambient CO_2		Low-quality diet Ambient CO_2		Low-quality diet Elevated CO_2		High-quality diet Ambient CO_2		High-quality diet Elevated CO_2	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
% *Obelia dichotoma*	23.70	8.09	21.90	7.05	21.60	6.69	19.20	5.28	36.90	8.37	12.44	5.05
% *Folliculina* sp.	17.10	3.99	7.80	1.55	15.10	3.48	19.50	5.62	14.50	3.75	11.22	5.91
% *Botryllus schlosseri*	11.50	3.51	28.45	7.24	9.50	1.71	14.00	3.86	6.45	3.91	20.78	5.34
% *Membranipora membranacea*	8.45	5.85	4.20	1.69	8.60	5.40	5.40	2.50	12.45	6.95	3.67	3.09
% Caprellid	5.20	2.32	7.30	2.52	8.70	2.06	2.30	0.91	7.50	2.51	9.56	2.40
# *Balanus crenatus*	3.00	1.93	1.80	0.81	3.20	0.73	2.30	0.87	2.30	0.56	0.89	0.35
# *Disporella sp.*	2.50	0.43	1.90	0.46	1.70	0.45	1.80	0.55	2.00	0.52	2.56	0.41
# Serpulidae	0.60	0.27	0.30	0.21	0.30	0.15	0.10	0.10	1.10	0.43	0.00	0.00
% *Hermissenda* eggs	0.60	0.43	0.00	0.00	0.75	0.29	1.00	0.45	0.60	0.34	0.33	0.24
# *Corella inflata*	0.50	0.22	0.10	0.10	0.30	0.15	0.30	0.15	0.00	0.00	0.00	0.00
% *Hermissenda crassicornis*	0.45	0.30	0.15	0.11	0.00	0.00	0.05	0.05	0.05	0.05	0.11	0.11
# *Schizoporella japonica*	0.30	0.21	0.10	0.10	0.30	0.21	0.20	0.13	0.20	0.13	0.22	0.15
% *Didemnum* sp.	0.10	0.10	1.00	0.89	1.00	0.61	0.70	0.50	0.20	0.20	0.00	0.00
% orange sponge	0.10	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% *Corambe* nudibranch	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% *Mytilus* sp.	0.00	0.00	0.00	0.00	0.30	0.15	0.00	0.00	0.00	0.00	0.00	0.00
% brown sponge	0.00	0.00	0.00	0.00	0.30	0.30	0.00	0.00	0.00	0.00	0.00	0.00
% Cribrilina sp.	0.00	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% flatworm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% anemone	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.10	0.00	0.00	0.00	0.00
% isopod	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% annelid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% seastar	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.11
% *Bowerbankia* sp.	0.00	0.00	1.20	1.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table S5.3. Statistical results from generalized linear models using X^2 statistic and linear models using F statistic from percent cover or counts of a given species or community measure. These models are designed as ANCOVAs to test the overall and interactive effects of acidification (measured as minimum 10th percentile [H^+]) and food supply. Corrected P refers to P values that have been adjusted for false discovery rate. Bold represents significant results at P<0.05.

Species or community measure	**Effect of minimum 10th percentile [H^+]**			**Effect of food treatment**			**Effect of food treatment * minimum 10th percentile [H^+]**		
	Chisq	df	Corrected P	Chisq	df	P	Chisq	df	P
% *Obelia*	20.54	1	**1.46e-05**	100.88	2	**< 2.2e-16**	96.92	2	**< 2.2e-16**
% *Botryllus*	27.52	1	**7.75e-07**	36.52	2	**1.176e-08**	18.47	2	**9.778e-05**
% *Folliculina*	11.43	1	**0.0012**	20.72	2	**3.172e-05**	66.45	2	**3.713e-15**
% Caprellid	18.82	1	**2.88e-05**	129.85	2	**3.31e-07**	51.72	2	**5.887e-12**
% *Membranipora*	68.62	1	**2.2e-15**	11.54	2	**0.0031**	5.3584	2	**0.019**
# *Balanus*	5.41	1	**0.025**	2.136	2	0.34	7.065	2	**0.029**
# *Disporella*	0.34	1	0.56	0.70	2	0.71	0.15	2	0.93
# Serpulidae	26.12	1	**1.07e-06**	9.41	2	**0.0091**	7.51	2	**0.023**
% *Didemnum*	1.14	1	0.31	11.66	2	**0.0029**	12.91	2	**0.0016**
***Hermissenda* % (adults and egg sacks)**	7.77	1	**0.0076**	1.486	2	0.476	3.75	2	0.15
Richness	6.39	1	**0.011**	0.54	2	0.76	0.57	2	0.75
Evenness	0.79 (F)	1	0.38	5.89 (F)	2	**0.0049**	5.28 (F)	2	**0.0081**
% cover occupied space	25.56	1	**4.298e-07**	93.64	2	**<2e-16**	78.36	2	**<2e-16**
Dry Biomass (per tile)	0.89 (F)	1	0.35	1.59 (F)	2	0.21	0.86 (F)	2	0.43

Table S5.4. Statistical results from pairwise generalized linear models using X^2 statistic and linear models using F statistic from percent cover or counts of a given species or community measure. These models are designed as ANCOVAs to test the overall and interactive effects of acidification (here measured as minimum 10th percentile [H^+]) and each food supply independently. Corrected P refers to P values that have been adjusted for false discovery rate. Bold represents significant results at P<0.05.

Species or community measure		Effect of Food quality			Effect of food quality * min.10.pH		
		Food effect	Z value	Corrected P	Interactive effect	Z value	Corrected P
% *Obelia*	High	+	8.75	**4e-16**	-	-8.94	**4e-16**
	Low		-0.15	0.88		-0.82	0.55
% *Botryllus*	High	-	-5.85	**2.012e-08**	+	3.97	**1.468e-04**
	Low	-	-3.41	**8.67e-04**		-0.24	0.81
% *Folliculina*	High		0.80	0.42		-0.94	0.42
	Low	-	-3.48	**0.001**	+	6.59	**1.72e-10**
% Caprellid	High	+	2.21	**0.036**		1.208	0.34
	Low	+	5.29	**1.058e-06**	-	-5.98	**9.16e-09**
% *Membranipora*	High	+	3.37	**0.003**	-	- 2.73	**0.0126**
	Low		1.55	0.13		- 1.51	0.13
# *Balanus*	High		1.44	0.30		-2.39	0.068
	Low		0.75	0.61		-0.46	0.65
# Serpulidae	High	-	-2.44	**0.046**	-	-2.27	**0.046**
	Low		-0.87	0.52		0.28	0.78
% *Didemnum*	High		0.99	0.33		-0.96	0.33
	Low	+	2.88	**0.0098**	-	-2.81	**0.0098**
Evenness	High	-	-2.54	**0.028**	+	2.87	**0.023**
	Low		0.84	0.53		-0.19	0.85
Occupied space (%)	High	+	-8.74	**4e-16**	-	8.31	**4e-16**
	Low	-	-3.29	**0.0013**	+	3.20	**0.0014**

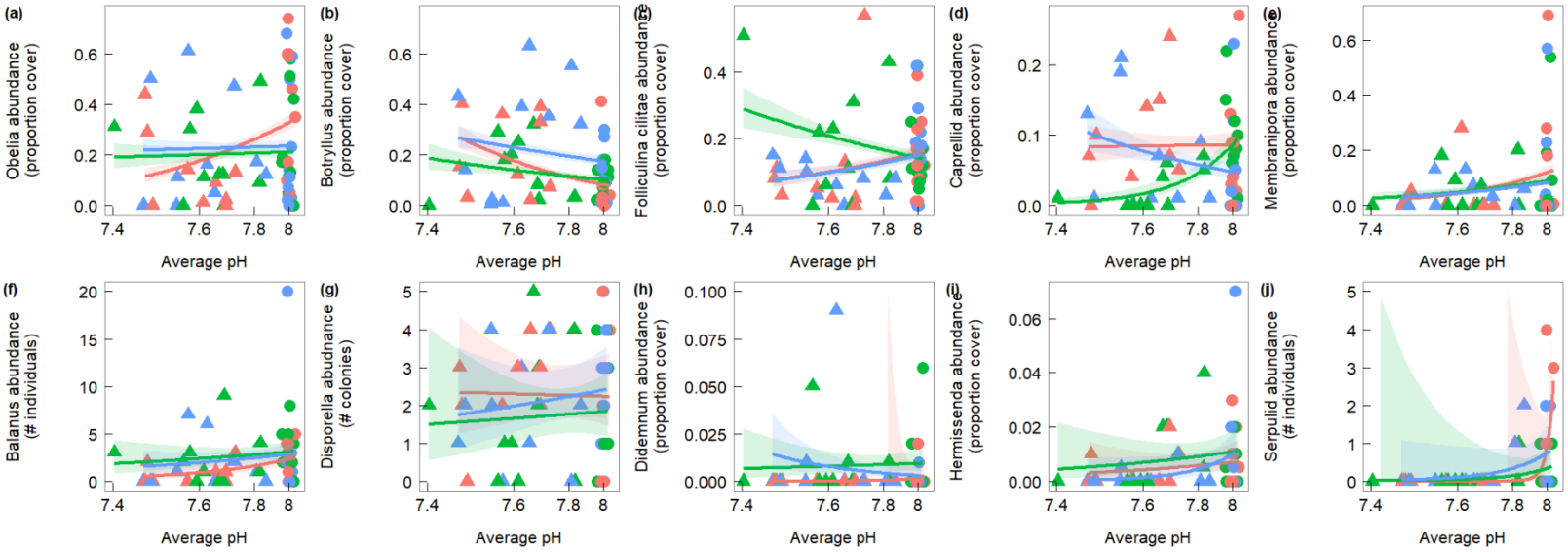

Fig. S5.1: Regressions of species abundance (% cover and # individuals or colonies) across average hydrogen ion concentration values across all weeks (here represented as pH which is -log([H+]) for illustration purposes). Circles represent data from tiles in ambient CO_2 mesocosms and triangles represent those from high CO_2/low pH conditions. Blue symbols and regression lines are from mesocosms with ambient food supply (organisms fed on plankton and other suspended matter coming in from pump), whereas red and green lines and points represent data from added phytoplankton in a high-quality diet (*Thalassiosira* sp.) and low-quality diet (*Tetraselmis* sp.), respectively. Selected species had mean cover greater than 2 %. Error bars represent 95% confidence intervals on a binomial model for % cover and Poisson model for counts (n=10 for all treatments, except 9 for high-quality food, low pH).

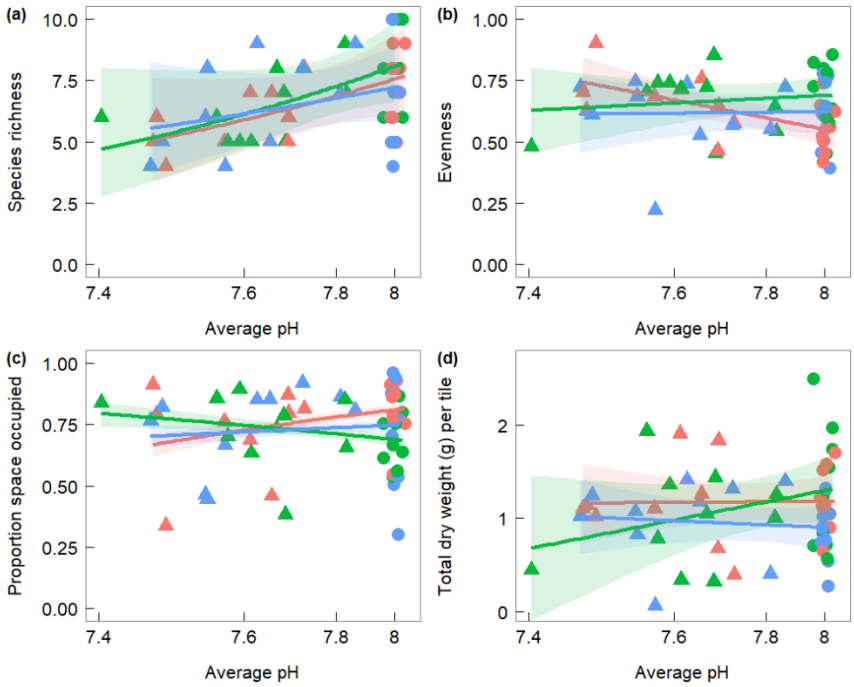

Fig. S5.2: Regression of end of experiment (a) species richness, (b) evenness, (c) proportion space occupied per tile, and (d) biomass per 1% cover of tiles across average hydrogen ion concentration, represented as pH as in Fig. S5.1. Colours and shapes as in Fig. 5.1. Error bars represent 95% confidence intervals on a binomial model for % cover and Poisson model for counts (n=10 for all treatments, expect 9 for high-quality food, low pH).

Table S5.5. Statistical results from generalized linear models using X^2 statistic and linear models using F statistic from percent cover or counts of a given species or community measure. These models are designed as ANCOVAs to test the overall and interactive effects of acidification (here measured as average H^+]) and food supply. Corrected P refers to P values that have been adjusted for false discovery rate. Bold represents significant results at $P<0.05$. Deviations from results in Table S5.3, minimum 10th percentile pH, are indicated with an asterisk.

Species	Effect of average [H^+]			Effect of food treatment			Effect of food treatment * average [H^+]		
	Chisq	df	P	Chisq	df	P	Chisq	df	P
% *Obelia*	32.30	1	**1.320e-08**	52.66	2	**3.680e-12**	41.37	2	**1.038e-09**
% *Botryllus*	72.33	1	**< 2.2e-16**	36.47	2	**1.205e-08**	17.16	2	**0.000188**
% *Folliculina*	7.42	1	**0.0065**	18.21	2	**0.00011**	51.23	2	**7.50e-12**
% Caprellid	13.17	1	**0.0002844**	43.21	2	**4.15e-10**	60.55	2	**7.13e-14**
% *Membranipora*	70.27	1	**<2.2e-16**	8.18	2	**0.017**	3.79	2	0.15*
# *Balanus*	9.87	1	**0.0017**	0.17	2	0.92	2.44	2	0.29*
# *Disporella*	0.32	1	0.57	0.48	2	0.79	0.38	2	0.83
# Serpulidae	20.64	1	**5.55e-06**	4.14	2	0.13*	3.33	2	0.19*
% *Didemnum*	1.06	1	0.30	5.03	2	0.081*	6.26	2	**0.044**
***Hermissenda* % (adults and egg sacks)**	8.22	1	**0.0041**	1.94	2	0.38	2.90	2	0.23
Richness	6.57	1	**0.010**	0.49	2	0.78	0.29	2	0.87
Evenness	0.89 (F)	1	0.35	3.43 (F)	2	**0.040**	2.58 (F)	2	0.085*
% cover occupied space	8.11	1	**0.0044**	58.03	2	**2.51e-13**	41.64	2	**9.10e-10**
Dry Biomass (per tile)	0.66 (F)	1	0.42	1.97 (F)	2	0.15	1.22 (F)	2	0.30

Table S5.6. Statistical results from pairwise generalized linear models using X^2 statistic and linear models using F statistic from percent cover or counts of a given species or community measure. These models are designed as ANCOVAs to test the overall and interactive effects of acidification (here measures as average $[H^+]$) and each food supply independently. Corrected P refers to P values that have been adjusted for false discovery rate. Bold represents significant results at $P<0.05$. Deviations from results in Table S5.4, minimum 10th percentile pH, are indicated with an asterisk.

		Effect of Food quality			**Effect of food quality * min.10.pH**		
Species or community measure	**Food level**	Food effect	Z value	Corrected P	Interactive effect	Z value	Corrected P
% *Obelia*	High	+	5.12	**1.3e-08**	-	-5.64	**3.4e-08**
	Low		-0.79	0.57		-0.061	0.95
% *Botryllus*	High	-	-5.87	**1.76e-08**	+	3.83	**0.00025**
	Low	-	-3.21	**0.0018**		0.16	0.87
% *Folliculina*	High		0.41	0.86		-0.18	0.86
	Low	-	-3.38	**0.0015**	+	5.82	**2.42e-08**
% *Caprella mutica*	High	+	3.54	**0.000528**	-	-2.69	**0.007194***
	Low	+	6.24	**8.86e-10**	-	-6.66	**1.1e-10**
% *Membranipora*	High	+	2.63	**0.034**		- 1.710	0.17*
	Low		0.402	0.92		- 0.081	0.93
% *Didemnum*	High		0.64	0.52		-0.73	0.52
	Low		2.015	0.16*		-1.75	0.16*
Evenness	High		-1.68	0.20*		1.79	0.20*
	Low		0.92	0.48		-0.35	0.72
Occupied space (%)	High	+	3.97	**2.74e-04**	-	-2.97	**0.0030**
	Low	-	-3.81	**0.0002**	+	3.71	**0.00028**