MULTIPLE IMPACTS OF OCEAN ACIDIFICATION ON CALCIFYING MARINE INVERTEBRATES

by

Sarah Bethune Nienhuis

Hon. B.Sc., Trent University, 2006

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

The Faculty of Graduate Studies

(Zoology)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

September 2009

ABSTRACT

Increasing anthropogenic atmospheric CO_2 is altering the chemistry of surface seawater worldwide, resulting in ocean acidification. Experiments have begun to demonstrate the detrimental consequences that a CO_2-mediated decline in ocean pH can have on the growth and survival of calcifying organisms. However, significant knowledge gaps exist both in our understanding of the mechanisms driving the observed reductions in shell growth rates of organisms exposed to increases in CO_2, and in our ability to predict how these individual-level effects could scale-up to the population or community-level.

Using laboratory exposure experiments in which levels of dissolved CO_2 were carefully manipulated, we tested the effects of climatically relevant increases in CO_2 levels on a) both shell deposition rate and shell dissolution rate in the intertidal snail, *Nucella lamellosa*, and b) the growth and feeding behaviour of juvenile red sea urchins, *Strongylocentrotus franciscanus*. Based on the results of the former study, we found that shell weight gain per day in live snails decreased linearly with increasing CO_2 level while shell weight loss per day in empty shells more than doubled over this same range. These results suggest that for some species, elevated CO_2 levels may have a much greater effect on shell dissolution than shell deposition. In the latter study, although we found no effect of a doubling of current CO_2 concentration on the individual feeding rates or absorption efficiency of juvenile urchins, there was a significant reduction in relative growth rates at the higher CO_2 concentration after 4 months of exposure.

Applying the urchin growth data to a simple demographic matrix model, and incorporating empirical relationships between urchin test diameter, biomass and kelp consumption rates to the model outputs, we estimated that if current CO_2 levels were to double by the end of the century, it would take significantly longer for urchins to reach reproductive and harvestable sizes, and their per capita kelp grazing rates would be significantly reduced. These simple model applications illustrate how CO_2-mediated reductions in individual growth rates could indirectly impact important population-level attributes such as time to first reproduction, and could have community- or ecosystem-level effects by moderating the importance of top-down biological control.

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ACKNOWLEDGEMENTS

First and foremost I thank Dr. C. D. G. Harley for his supervision and support, and for introducing me to the wonders of the Pacific Northwest and its colourful inhabitants. His enthusiasm for all things intertidal was contagious, and sparked the sense of curiosity and awe that remains with me to this day. I am grateful also to Dr. M. Berrill who inspired me to slip the surly bonds of analytical chemistry and pursue instead the more rewarding field of marine ecology. I would like to thank the members of my supervisory committee, Dr. J. B. Shurin and Dr. C. J. Brauner, for their input and advice at various stages in the evolution of this thesis. Dr. A. R. Palmer was a wonderful mentor, and I am grateful to have had the opportunity to collaborate with him.

The Harley and Shurin lab groups, as well as various member of Bamfield Marine Sciences Centre Graduate Student Society, helped guide and shape the ideas underlying this thesis though constructive criticism and open dialogue. L. White in particular offered invaluable advice and support.

I extend much gratitude towards B. Cameron, S. Pakula, C. Haylock, J. Radoslovich, R. Zohner and the rest of the staff at B.M.S.C. for their tireless assistance with various technical aspects of the work that I conducted there, and for making my experience at the station enjoyable and forever memorable. I thank W.L. Liao, J. T. M. Nienhuis, M. Davis, K. Lee and E. Tang for their assistance with field collections and in the lab.

Finally, sincerest thanks are owed to my family and friends whose encouragement and support motivated me to continue my work through some very difficult times. Thanks to my parents for staying strong, R. Torrie for her friendship, and my sister for making me smile. Last but not least, I am forever grateful to D. DeMarsh whose love, loyalty and patience saw me through the past three years.

CO-AUTHORSHIP STATEMENT

The body of experimental work detailed herein was primarily designed, performed analyzed and written by myself, Sarah Nienhuis, though the manuscripts presented in Chapters 2 and 3 were co-authored.

Chapter 2 details a collaborative study conducted by myself and A.R. Palmer, who developed the non-destructive weighing protocol which allowed us to quantify *in situ* rates of shell deposition in live animals, as well as the dissolution rates of empty shells, upon which the success of this study was critically dependant. He was responsible for field collections, all statistical analysis and reporting of shell weight change data, and in the preparation of those aspects of the manuscript that pertained to his weighing technique. I was responsible for the design, set-up and implementation of the CO_2 exposure experiment, including the collection and analysis of all seawater physicochemical data, as well as assisting with live-weight measurements of snails. I also outlined the framework of the paper and prepared the initial draft of the manuscript. C.D.G. Harley also contributed to the conceptual design and to critical revision of the manuscript.

The study outlined in Chapter 3 was co-authored by C.D.G. Harley, who gave input into the experimental design, suggested the use of a demographic matrix model, assisted with much of the statistical analysis and edited several versions of the manuscript. I was solely responsible in conducting the laboratory experiment and collecting all data, running the models, and preparing the manuscript.

CHAPTER 1: GENERAL INTRODUCTION

CLIMATE CHANGE: THE HISTORICAL CONTEXT

Over the past 65 million years, our planet's climate has experienced continuous change, alternating repeatedly between glaciation events and brief interglacial periods. Such major shifts in Earth's climate system, which have tended to occur with periodic frequency through time, have largely been driven by rhythmic oscillations in Earth's orbital geometry and plate tectonics (Zachos *et al*. 2001), which in turn have influenced changes in continental geography, ocean circulation and concentrations of atmospheric greenhouse gases (Crowley and Burke 1998). Greenhouse gases, including methane and carbon dioxide, are components of the atmosphere that, owing to the nature and geometry of their chemical bonds, absorb radiant energy from the sun and release it in the form of heat. Consequently, there is a very strong positive correlation between the concentration of greenhouse gases in the atmosphere and global temperatures.

Since 1750, and especially since 1990, there has been a consistent, increasingly and unusually rapid increase in global average temperatures (IPCC 2007), unrelated to any known changes in orbital parameters or plate tectonics. The current scientific consensus is that most of the observed warming over the last 50 years is likely due to the increase in greenhouse gas concentrations (IPCC 2001; McCarthy *et al*. 2001) resulting from the industrial and land-use activities of humankind since the beginning of the Industrial Revolution (Oreskes 2004). As a result of the direct effects of increased anthropogenic atmospheric CO_2 concentrations, the current period of global climatic change is occurring at a much greater rate and magnitude than ever before, making it very different from the changes in climate that have occurred in the past (Williams 2000).

While atmospheric CO_2 levels were maintained between 200-280 ppm for nearly 400,000 years prior the Industrial Revolution (Feely *et al*. 2004), there has been an abrupt increase from 280 ppm to 380 ppm in the last 200 years alone (Houghton *et al*. 2001). According to the study of sedimentary archives and ice core data, this increase far exceeds the natural range of the last 650,000 years (IPCC 2007). To put the magnitude of these recent changes into geological perspective, an oscillation of 100 ppm represents the difference between an ice age (typical [CO_2]~180 ppm) and an interglacial period (typical [CO_2]~280

ppm) (Tyrrell 2007). What is most worrisome, however, is that based on current CO_2 emission rates and those projected by the IPCC under conservative scenarios for the near future, it is estimated that by the end of the century, atmospheric CO_2 levels could well reach an unprecedented 800 ppm.

The recent, rapid increase in atmospheric greenhouse gases is already contributing to a number of observable and potentially catastrophic aspects of climate change. Not only are air temperatures on the rise, but sea surface temperatures are increasing, the polar ice caps are melting and the ocean is expanding and its levels rising. Storms, both on and off shore are increasing in frequency and severity. In addition, it is now recognized that rising greenhouse gas concentrations are dramatically altering ocean biogeochemistry, with potentially significant consequences for marine organisms. Often referred to as "the other CO_2 problem" (Doney *et al*. 2009) the widespread phenomenon of ocean acidification, despite having been relatively underrepresented in the scientific literature until very recently, may well prove to be one of the biggest threats to marine life.

OCEAN ACIDIFICATION: CHEMICAL CONCEPTS, TRENDS AND PREDICTIONS

In order to explain how changes in atmospheric CO_2 levels translate to so-called ocean acidification, a brief review of some basic chemistry is required. Atmospheric carbon dioxide equilibrates rapidly with surface water, such that in accordance with Henry's Law, the concentration of dissolved CO_2 at the sea surface is directly proportional to the partial pressure of CO_2 (pCO_2) in air. Given the huge extent of their total surface area, and the intimate contact between sea and air at that surface, the world's oceans therefore represent a major sink for anthropogenic CO_2 emissions. Based on data derived from extensive global oceanographic surveys that mapped the flux and circulation of carbon in the world's oceans, it has been calculated that more than 120 Gt C have diffused across the air-sea interface since 1800 (Sabine *et al*. 2004; Sabine and Feely 2007), implying that the ocean has already taken up approximately one half of atmospheric carbon emissions since the beginning of the Industrial Revolution (Doney 2006).

When carbon dioxide dissolves in water, carbonic acid is produced according to the following equilibrium equation:

$$CO_2 + H_2O \Leftrightarrow H_2CO_3$$

Because it is a weak acid, carbonic acid readily dissociates to form the more stable bicarbonate ion, releasing H^+ and lowering pH in the process. It follows then, that the pH of the surface ocean is intimately linked to levels of CO_2 in the atmosphere. Although carbonate minerals in ocean sediments provide a pool of bicarbonate ions that serve to buffer pH changes over the long term, when atmospheric CO_2 concentrations fluctuate over time periods less than 10,000 years, ocean pH becomes much more sensitive to additional CO_2 loading (Caldeira and Wickett 2003), due in part to the pronounced lag in time required for the upward circulation of deep-ocean waters.

Concomitant with the recent rapid increase in anthropogenic atmospheric CO_2 over the past two centuries, the surface pH of modern oceans has dropped ~0.1 units relative to pre-industrial levels (Orr *et al*. 2005; Feely *et al*. 2008). With atmospheric CO_2 concentrations predicted to reach 800 ppm by the end of this century (Feely *et al.* 2004), a cumulative decrease in surface water pH of up to 0.5 units by the year 2100 is probable (Miles *et al*. 2007). This being the case, "oceanic absorption of CO_2 from fossil fuels may result in larger pH changes over the next several centuries than any inferred from the geological record of the past 300 million years" (Caldeira and Wickett 2003).

One of the more critical consequences of this dramatic CO_2-mediated drop in ocean pH is the resultant decrease in the concentration of dissolved carbonate ions, which are readily consumed by excess protons in the formation bicarbonate ions:

$$CO_3^{2-} + H^+ \rightarrow HCO_3^-$$

Owing to the increased dissolution of anthropogenic CO_2, $[CO_3]$ in modern surface waters has already been reduced by more than 10% (Orr *et al*. 2005), and is expected to decline by more than 50% relative to pre-industrial levels by the end of the century if the IS92a scenario of atmospheric CO_2 reaching 788 ppm is realised (Orr *et al*. 2005; Doney 2006).

Carbonate ions play a very important role in ocean biomineralization processes, as their concentration in seawater dictates the solubility of calcium carbonate ($CaCO_3$) the predominant structural mineral used by marine invertebrates (Wilt *et al*. 2003). Calcium carbonate is formed via the following simplified reaction equation:

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3$$

The saturation state (Ω) of this abundant biomineral is proportional to the solubility product constant (Ksp) for calcium carbonate at a given temperature and pressure, and on the ion product of the concentrations of both calcium and carbonate ions.

$$\Omega_{CaCO3} = \frac{[Ca^{2+}]\,[CO_3^{2-}]}{Ksp}$$

In saline ocean waters, calcium ion concentrations remain relatively constant, and are generally present far in excess of carbonate ion concentrations, meaning that the saturation state of calcium carbonate is predominantly dependant upon the availability of CO_3^{2-} ions. Therefore, by reducing the relative abundance of CO_3^{2-} ions (and thereby the value of Ω) it follows that an increase in dissolved CO_2 indirectly increases the solubility of calcium carbonate.

It is worth noting that the calcium carbonate secreted by marine organisms exists in several distinct mineral phases or polymorphs; calcite, high-magnesian calcite and aragonite. These different forms vary not only in the structure of their crystal lattice, and in the proportion of magnesium ions therein, but also in their relative propensities for dissolution. Each form has a different Ksp value, and therefore a different saturation state in seawater. The saturation states for aragonite and high-magnesian calcite, for example, always have lower values than the saturation state for calcite, and as a result the former are nearly 50% more soluble in seawater than the latter (Mucci 1983, cited in Feely *et al*. 2004). In consequence, the particular form of $CaCO_3$ that a marine calcifier deposits will influence its relative susceptibility to the negative impacts of ocean acidification.

Also important to note is the fact that the saturation state of seawater with respect to the various forms of calcium carbonate is not uniform throughout the ocean, owing largely to the

wide range of temperature and pressure that exist at different latitudes and depths, and the importance of these two physical factors in dictating the value of Ksp. In brief, the solubility of a given mineral in water increases with increasing pressure and decreasing temperature. At the ocean's surface, the saturation state of the carbonate minerals is lower in the colder, higher latitude regions than, for example, in the warmer tropical regions. At great depths where the pressure is greater and the water colder, many areas are in fact currently undersaturated with respect to $CaCO_3$ while the shallow, warmer surface waters of the world remain supersaturated. Though Ω_{CaCO3} is predicted to decline in all parts of the ocean as pCO_2 increases, it is evident that the degree to which it decreases will be strongly influenced by both geographic location and depth.

The saturation horizon is the depth at which seawater transitions from supersaturated to undersaturated waters, and below which calcium carbonate will dissolve. Recent increases in dissolved CO_2 have already caused the saturation horizons for aragonite and calcite to shoal, or shift closer to the surface, by 50 to 200 meters from the Atlantic through the Indian to Pacific Oceans since the 1800's, significantly reducing the volume of the "shell-friendly" portion of the world's oceans (Feely *et al.* 2004; Doney 2006). Using models of the ocean carbon cycle to assess calcium carbonate saturation under the IS92a scenario, Orr *et al*. (2005) predict that over the course of this century, the aragonite saturation horizon in the Southern Ocean could shoal from its present average depth of 730m all the way to the surface, while it may shoal from 120m in the subarctic Pacific to the surface, and in the North Atlantic from 2,600m to 115m. Because its saturation state is higher than that of aragonite, the calcite saturation horizon will experience less dramatic shoaling, though the surface waters in some areas may still become undersaturated by 2100 (Orr *et al*. 2005).

Despite the fact that most of the surface ocean is expected to remain supersaturated with respect to $CaCO_3$ (or at least with respect to calcite) even under the IPCC's worst case scenarios for future greenhouse gas emissions, the saturation state of $CaCO_3$ in surface waters has already begun to decline significantly. In fact, average global surface Ω_{CaCO3} has already decreased from 5.3 to 4.4 over the past century and will continue to decline as pCO_2 increases (Kleypas *et al*. 1999, 2006; Orr *et al*. 2005). As for the more soluble aragonitic form of the biomineral, model projections imply that Southern Ocean surface waters may in fact become undersaturated by the year 2050 under the IS92a business-as-usual scenario for future carbon

emissions, while the subarctic Pacific Ocean may follow suit by the year 2100 (Orr *et al*. 2005).

IMPLICATIONS FOR BIOGENIC CALCIFICATION

From a purely physicochemical standpoint, all polymorphs of $CaCO_3$ should precipitate spontaneously when the ocean waters are supersaturated with respect to that particular phase of the mineral (ie. $\Omega > 1$). Conversely, because a solution with a low Ω is not energetically favourable for $CaCO_3$ precipitation (Simkiss 1976) carbonate minerals should start to dissolve when the oceans become undersaturated (ie. $\Omega < 1$). However, it has been recently demonstrated that the degree of supersaturation has a significant effect on the rate at which calcification can occur (Kleypas *et al*. 1999; Feely *et al*. 2004). In almost every case in which it has been investigated, calcification rates in a wide variety of marine calcifying taxa were found to decrease with a decreasing $CaCO_3$ saturation state even when $\Omega_{CaCO3} > 1$ (Leclercq *et al*. 2000; Langdon and Atkinson 2005; Feely *et al*. 2004; Orr *et al*. 2005). By indirectly decreasing the saturation state of calcium carbonate, an increase in the dissolution of anthropogenic CO_2 will have significant impacts on global calcification rates.

The benefits that calcified structures confer to marine organisms are many and varied, and include among other things, structural and mechanical support, reduced vulnerability to predation and erosion, and increased buoyancy. While it is one of the most fundamental processes regulating the ocean carbon cycle, and is widespread across the vast majority of marine taxa, the precise cellular and molecular mechanisms controlling biocalcification remain poorly understood (Allemand *et al*. 2004; Wilt *et al*. 2003). What is clear is that calcification is both a highly complex and tightly regulated process, requiring biological initiation even in supersaturated seawater (Raven *et al*. 2005). Because it is integral to the biology of the vast majority of marine biota, any decrease in calcification as a result of increased CO_2 is likely to have significant consequences for these species.

The take home story here is that the predictable changes in ocean chemistry wrought by the increased dissolution of carbon dioxide at the sea surface could hinder/impair the ability of calcifying marine organisms to precipitate $CaCO_3$. Though the degree to which such organisms will suffer from reduced calcification rates will depend upon the particular polymorph of $CaCO_3$ that they use in shell construction, and will be influenced by the depth, latitude and

temperature of the water that they inhabit, there is a growing body of experimental evidence to suggest that ocean acidification will have important implications for the growth and survival of calcifying species (Kleypas *et al*. 2006).

EXPERIMENTAL FINDINGS IN THE FIELD

The number of ocean acidification papers published in the scientific literature has increased steadily from the handful of studies that existed prior to about 2005, to the hundreds that are currently being published in a wide range of high-impact journals. This recent explosion of publications in the field reflects a growing awareness within the scientific community that the current and predictable future changes to ocean chemistry resulting from increased anthropogenic CO_2 could in fact represent a more worrisome threat to marine species and ecosystems than the much more publicized impacts of global warming. The results of exposure experiments with experimentally manipulated [CO_2] and pH have certainly begun to demonstrate that for much of the oceans' fauna, such concerns may well be justified. The following is a brief review of the experimental ocean acidification studies that have been published to date, with a focus on the growth and calcification response of various taxa to increased pCO_2.

Experimental studies that have manipulated levels of dissolved CO_2 and pH have begun to demonstrate the pronounced physiological effects that changes in these chemical factors can have on marine organisms. For water breathing organisms, where CO_2 enters via diffusion, elevated seawater CO_2 raises the level of CO_2 in body compartments, which in turn leads to an almost immediate drop in extracellular pH and an increase in bicarbonate levels as the organisms' ion-regulatory processes attempt to re-establish acid-base equilibria in the body fluids (Portner *et al*. 2004). Such changes to extracellular acid-base parameters can slow down or hinder several critical physiological processes. At the subcellular level, for example, acidification of body fluids (hypercapnia) upon exposure to increased levels of CO_2 has been shown to hinder such important processes as protein synthesis and ion exchange in a number of marine animals (Langenbuch and Portner 2002, 2005). In addition, there is evidence to suggest that tissue acidosis can decrease cellular energy use and lower respiration rates (Raven *et al*. 2005).

It has recently been shown that chronic reduction of seawater pH to below 7.5 was severely detrimental to the acid-base balance of an intertidal sea urchin species, while a pH of 6.63 caused significant mortality (Miles *et al*. 2007). In a similar study with a marine mussel, long-term hypercapnia caused by a reduction in seawater pH to a value of 7.3 caused a permanent reduction in haemolymph pH (despite partial compensation via internal dissolution of shell $CaCO_3$) which led to decreased rates of oxygen consumption indicating a lower metabolic rate (Michaelidis *et al*. 2005). Reduced metabolic activity is a common survival strategy among invertebrates exposed to periods of elevated CO_2. However, chronic metabolic depression, resulting in reduced rates of protein synthesis, respiration, and feeding for example, would necessarily restrict organism growth and survival. Fortunately, most of the experimental studies reporting significant physiological or metabolic effects have involved CO_2 concentrations far exceeding those predicted from projected future CO_2 emission scenarios.

Although pronounced metabolic depression and death tends to be elicited only at extremely high CO_2 concentrations, reductions in calcification or growth rates among marine invertebrates occur with much smaller increases in CO_2. For example, an experiment with marine gastropods and sea urchins demonstrated a significant reduction in growth after 6-months of exposure to CO_2 levels elevated by only 200 ppm (which caused a pH drop of 0.03 units) (Shirayama and Thornton 2005). This is largely owing to the fact that the solubility of calcium carbonate is sensitive to even subtle changes in seawater carbonate chemistry, and that soft tissue growth in invertebrates encased in hard skeletons is often limited by shell extension rates (Palmer 1992). As highlighted in recent summary reports, experimental and biogeochemical studies have clearly demonstrated a significant, positive correlation between the saturation state of seawater with respect to calcium carbonate and calcification rates in marine calcifiers (Gattuso *et al*. 1999; Kleypas *et al*. 1999, 2006).

Many recent laboratory and mesocosm experiments focussed on those species of greatest global ecological consequence have shown that climatically relevant increases in CO_2 (and corresponding decreases in $[CO_3^{2-}]$ and Ω_{CaCO3}) significantly reduce calcification and linear extension rates for various species of scleractinian and reef-building corals (Marubini and Atkinson 1999; Leclercq and Gattuso 2000; Reynaud *et al*. 2003; Schneider and Erez 2006; Hoegh-Guldberg *et al*. 2007; Jokiel *et al*. 2008), as well as for the planktonic calcifiers including coccolithiphores (Riebsell *et al*. 2000; Zondervan *et al*. 2001,2002; Engel *et al*.

2005) foraminiferans (Bijma *et al*. 1999, 2002; Erez 2003) and pteropods (Orr *et al*. 2005). Although these groups have received the most attention to date, significant reductions in growth and calcification upon exposure to moderately elevated CO_2 have also been documented for mussels (Berge *et al*. 2006; Gazeau *et al*., 2007) oysters (Gazeau *et al*. 2007) marine snails (Shirayama and Thornton 2005; Nienhuis and Palmer (unpublished data)) and sea urchins (Shirayama and Thornton 2005).

Recently, several authors have reported increased growth or calcification rates with moderate increases in CO_2 (Gutowska *et al*. 2008; Wood *et al*. 2008; Gooding *et al*. 2009) in a species of cephalopod, brittle star and sea star, respectively. All of these studies were carried out over the course of 10 weeks or less, which suggests that certain organisms may be able to increase metabolic and calcification rates in response to elevated CO_2 over the short-term. However, it is not clear whether enhanced calcification would be sustainable over the long-term (Doney *et al*. 2009).

Most of the experimental studies cited above have examined various impacts of elevated CO_2 on adult individuals only. Until very recently, surprisingly little was known about the long term impact of predicted ocean chemistry changes on the early developmental stages of marine invertebrates, despite their known sensitivity to environmental disturbances in general (Dupont and Thorndyke 2009). Results of exposure studies (primarily with sea urchin embryos and larvae) are now beginning to demonstrate that increases in CO_2 predicted of the next several centuries can cause reduced fertilization, cleavage and hatching rates, as well as decreased egg production, larval size, survival and developmental speed (Kurihara and Shirayama 2004; Kurihara *et al*. 2004; Havenhand *et al*. 2008; Kurihara 2008). Because the health and survival of early life-history stages are essential for recruitment success and population maintenance of a given species, the fact that these stages may be most vulnerable to coming ocean chemistry changes is especially worrisome.

STATEMENT OF PURPOSE, HYPOTHESES AND PREDICTIONS

To date, most of the studies that have sought to assess the impacts of ocean acidification on marine organisms have involved short-term laboratory experiments on single species, often with CO_2 levels far exceeding those predicted for the next several centuries

(IPCC 2001, 2007). The response variables that tend to be measured in the majority of these experiments are overall changes in calcification or growth and survival. Based on the body of experimental evidence that currently exists, we know that ocean acidification has the potential to reduce calcification and growth rates in a variety of calcifying organisms. What we don't know is how this reduction in skeletal growth is manifested, whether it is through decreased rates of shell deposition, increased rates of shell dissolution, or a combination of both. Furthermore, we have yet to appreciate or understand how changes to individual growth rates could scale up to population or community levels. This is particularly relevant considering that among many invertebrates reproductive output and feeding rates are size dependant. Having identified these important knowledge gaps, the studies detailed in Chapters 2 and 3 were designed and conducted.

Although a CO_2-induced lowering of ocean pH and calcium carbonate saturation states may influence the growth rates of mineralized skeletons in a wide range of marine organisms, the relative impact of elevated CO_2 on shell deposition and dissolution rates are not known for many large-bodied organisms. The aim of the study described in Chapter 2 was therefore to test the effects of increased CO_2 levels on both shell deposition rate and shell dissolution rate in a marine mollusc, the intertidal snail, *Nucella lamellosa*. In order to do this, we ran a short-term exposure experiment using three levels of CO_2 during which we quantified *in situ* rates of shell deposition in live animals, as well as the dissolution rates of empty shells, under three different levels of CO_2 over the short term. We predicted that shell deposition rates among live animals would decrease while shell dissolution rates among empty shells would increase, with increasing CO_2 concentrations. We further predicted that the relative reduction in shell deposition upon exposure to higher CO_2 levels would be less than the dissolution rates of empty shells, owing to the compensatory mechanisms exhibited by numerous calcifying species which enable them to maintain $CaCO_3$ supersaturation in extracellular tissues despite undersaturated external seawater conditions.

On the other end of the scale, the aim of the study described in Chapter 3 was to determine the impact of a moderate increase in dissolved CO_2 on the growth and feeding behaviour of juvenile red sea urchins, *Strongylocentrotus franciscanus*, and to apply this information to a simple demographic matrix model to predict the potential population and community-level consequences of ocean acidification. Specifically, we asked a) what is the

effect of long-term (4-months) exposure to a doubling of current atmospheric CO_2 levels on the growth, feeding rates and assimilation efficiency of juvenile red sea urchins? and b) How would a CO_2-mediated reduction in juvenile growth rates impact the biomass of potentially harvestable urchins and their per capita kelp grazing rates? We predicted that upon exposure to 780ppm CO_2, urchin growth rates would decline to a similar extent as reported for other species of urchin in comparable studies (Shirayama and Thornton 2005). Furthermore, because elevated CO_2 has been reported to reduce feeding rates and conversion efficiency in other shellfish species (Bamber 1990; Siikavuopio *et al*. 2007), we expected that the urchins exposed to higher CO_2 levels would consume less kelp, and experience reduced absorption efficiency relative to those under ambient conditions. We further predicted that reduced individual growth rates would translate to a reduced biomass of reproductive and harvestable urchins, and reduced per-capita grazing pressure on kelp resources, given the size-dependence of these parameters.

The following chapters detail these two independent studies, which together comprise the basis of the thesis.

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CHAPTER 2: ELEVATED CO_2 AFFECTS SHELL DISSOLUTION RATE BUT NOT CALCIFICATION RATE IN A MARINE SNAIL[1]

INTRODUCTION

Many conspicuous and ecologically significant marine animals produce massive skeletons of calcium carbonate, including corals, molluscs, tube-dwelling polychaetes, crustaceans, bryozoans, and echinoderms (Milliman 1974). These skeletons provide structural support for individuals and colonies and are often essential components of morphological defense in the ever-escalating evolutionary 'arms race' between prey and their durophagous predators (Vermeij 1987). Because calcium carbonate structures also provide important habitat for a wide diversity of organisms, calcifying species are often ecologically important and play a central role in determining local species richness and community structure (e.g., Kimbro and Grosholz 2006, Yap 2009).

Although calcium carbonate may not be energetically expensive to produce (Palmer 1992) the rate of shell deposition may limit the maximum rate of body growth in some groups (Palmer 1981), which might affect both growth rate and survival. A decrease in the calcium carbonate saturation state (Ω_{CaCO3}), along with decreased ocean pH levels due to increased dissolved CO_2, could therefore have a profound impact on rates of growth, or on extent of skeletal development (via slower deposition rates or increased dissolution rates), in many marine animal groups.

The degree to which future increases in ocean CO_2 levels will reduce calcification rates in marine animals remains unclear. For example, at tropical and temperate latitudes, even at current rates of CO_2 increase, the predicted saturation state remains > 2 for pure calcite, and >1 for aragonite and high-Mg calcite, beyond the end of the current century either with or without increased temperatures predicted by current global warming trends (Andersson *et al*. 2008). Only at high latitudes does the predicted saturation state drop below 1 by the end of the century (Orr *et al*. 2005), and this is a) only true for aragonite and high-Mg calcite, not pure calcite and b) sensitive to ocean temperature predictions. So, for at least the next century, the saturation state of $CaCO_3$ should remain favourable for biogenic calcification at most latitudes given the

[1] A version of this chapter will be submitted for publication. Nienhuis, S.B., Palmer, A.R. and Harley, C.D.G. Elevated CO_2 affects shell dissolution rate but not calcification rate in a marine snail

current rate of CO_2 increase. However, the degree of calcite supersaturation has a significant effect on the rate at which calcification can occur (Kleypas *et al*. 1999; Feely *et al*. 2004). In almost every case studied, calcification rates in a wide variety of marine taxa decreased with decreasing $CaCO_3$ saturation state even when $\Omega_{CaCO3} > 1$ (Leclercq *et al*. 2000; Langdon and Atkinson 2005; Feely *et al*. 2004; Orr *et al*. 2005).

In prior ocean acidification studies, where marine calcifiers were exposed to experimentally elevated levels of dissolved CO_2, many taxa exhibited lower growth and calcification rates in response to climatically relevant increases in $[CO_2]$ (reviewed in Raven *et al*. 2005; Fabry *et al*. 2008; Doney *et al*. 2009). This widely observed phenomenon is largely explained by predictable shifts in the carbonate equilibrium with changes in $[CO_2]$. As more CO_2 dissolves in seawater to form H_2CO_3, the rapid dissociation of this unstable acid causes an increase in proton concentrations, resulting in lower pH and a decrease in $[CO_3^{2-}]$:

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^-$$

and

$$H^+ + CO_3^{2-} \Leftrightarrow HCO_3^-$$

Marine organisms typically utilize the more readily available sink of bicarbonate ions (HCO_3^-) to form calcium carbonate ($CaCO_3$):

$$Ca^{2+} + HCO_3^- \Leftrightarrow CaCO_3 + H^+$$

However, calcification appears to be "intimately linked" to the calcium carbonate saturation state (Ω), which depends on the relative concentrations of Ca^{2+} and CO_3^{2-} in seawater (Allemand *et al*. 2004):

$$\Omega_{CaCO3} = \frac{[Ca^{2+}]\,[CO_3^{2-}]}{Ksp}$$

(where Ksp= stoichiometric solubility product of calcium carbonate at a given temperature and pressure).

Because the [Ca^{2+}] in seawater is roughly 100-fold that of [CO_3^{2-}] and has changed very little over time, Ω_{CaCO3} is largely determined by [CO_3^{2-}] (Reynaud *et al.* 2003). The more significant of future climate projections, therefore, is the approx. 60% decrease in dissolved surface-water CO_3^{2-} expected if atmospheric CO_2 reaches 800 ppm by the end of the century (Feely *et al.* 2004). Thus, as [CO_3^{2-}] decreases, marine calcifying organisms may have increasing difficulty secreting calcium carbonate, suggesting that calcification rates will decline along with [CO_3^{2-}] (Orr *et al*. 2005), particularly because pH changes seem to be more pronounced in surface seawater (Dore *et al.* 2009).

Though critical to the growth and survival of most skeletonized marine invertebrates, the process of biomineralization is still not fully understood (Allemand *et al*. 2004; Wilt *et al*. 2003). From a purely physicochemical standpoint, $CaCO_3$ should precipitate spontaneously when $\Omega > 1$, and start to dissolve when $\Omega < 1$. However, biogenic calcium carbonate deposition is not this straightforward. It is an active, highly controlled and far more complex process. Not only must organisms obtain Ca^{2+} and HCO_3^- from ambient seawater, which requires active transport across cell membranes, but they must also eliminate excess protons released during mineralization (Allemand *et al*. 2004). To precipitate $CaCO_3$ organisms must also actively transport H^+ against a concentration gradient so that the internal environment at the calcification site remains supersaturated with respect to $CaCO_3$.

Two competing processes affect skeletal mass of calcified marine invertebrates: shell deposition at the site of mineralization, which is actively mediated by the living organism, and shell dissolution at the outer surface. Barring erosion due to scouring and attack by shell-boring organisms, dissolution is a physicochemical process driven largely by a) the solubility of the biomineral, b) chemical characteristics of the external seawater environment, and c) potential effects of metabolic by-products released by adhering biofilm. By comparing shell deposition rates of live animals and shell dissolution rates of empty shells we could gauge the relative contribution of these two processes to overall shell mass change under increased CO_2 levels.

We set out to determine the effect of climatically relevant increases in [CO_2] on rates of both $CaCO_3$ deposition and dissolution in a common rocky intertidal whelk, *Nucella lamellosa*. By employing a technique that reliably estimates shell mass of living snails non-destructively (Palmer 1982), we were able to quantify short-term *in situ* rates of shell deposition in live animals, as well as dissolution rates of empty shells, under three CO_2 levels.

We predicted that shell deposition rates of live snails would decrease at rates comparable to studies using other mollusc species (e.g., Shirayama and Thornton 2005; Berge *et al*. 2006; Gazeau *et al*. 2007) and that shell dissolution rates of empty shells would increase, with increasing CO_2 concentrations. However, we expected that the declines in shell deposition rate under higher CO_2 levels would be less than the increases in dissolution rates of empty shells, given the ability of most species to regulate the concentration of physiologically important ions under unfavourable external conditions.

MATERIALS AND METHODS

Collection, Feeding, and Initial Measurements

Thick-shelled *Nucella lamellosa* were collected intertidally from the shore of Grappler Inlet (48°50'00"N, 125°06'49"), a quiet-water inlet near the Bamfield Marine Sciences Center (BMSC), on the west coast of Vancouver Island, British Columbia, Canada on July 10, 2008. Only large-sized, actively growing snails showing more than ¼ whorl of recent shell growth were collected. Snails were transported to BMSC, placed in large plastic tubs (40 x 25 x 15 cm) with lids where they were held in running seawater (pH= 8.00 ± 0.02, temperature= 9 ± 1°C, salinity= 35.0 ± 0.5 psu) so that they could not crawl out of the water. For 40 days prior to the experiment (except days 19 - 27 when starved during a pilot study) snails were held continuously immersed and fed their preferred prey, the barnacle *Balanus glandula*, on small stones. Fresh barnacles were provided every 10 - 12 days and greatly exceeded the numbers eaten.

Five days after collection, 100 snails were tagged, measured and weighed. Snails were removed from seawater, scrubbed to remove encrusting organisms, rinsed briefly in tap water and allowed to dry for 3 hr, during which time they were a) individually tagged using numbered Brady Wire Markers coated with cyanoacrylate glue, b) measured for shell length (apex to tip of siphonal canal) to the nearest 0.01mm with digital calipers (Fowler Ultracal), and c) weighed in air (see *Weighing Protocol*). Snails were returned to holding tubs and underwater weights (see *Weighing Protocol*) recorded the next day, to avoid potentially confounding effects of air in the mantle cavity.

Weighing Protocol

Body wet weights and shell dry weights were estimated non-destructively on live snails following Palmer (1982). In brief, two weights were recorded for each snail: a) total weight in air after extravisceral water was removed, and b) underwater weight (weight while suspended in seawater on a wire hanging from underneath the balance). Weight in air includes both body wet weight and shell dry weight, so body wet weight was estimated by subtracting shell dry weight from total weight in air. The empirically derived correction for the buoyant effect of seawater yields a highly accurate estimate of shell dry weight: Shell dry weight= 1.572 * underwater weight (r^2= 0.9998; Palmer 1982). All weights were recorded to ±1 mg with a digital balance (Mettler, BB240).

Shell Deposition and Dissolution Rates

Baseline rate of shell deposition was estimated prior to the CO_2 treatments by measuring underwater weight (see *Weighing Protocol*) at the start and end of a 48 hr period of starvation, immediately before starting the CO_2 treatments. Rate of shell deposition under the three CO_2 treatments was estimated by measuring underwater weight (see *Weighing Protocol*) of live *Nucella lamellosa* at the start and end of a 6-day period of starvation. Shell dissolution rates were obtained by weighing thoroughly dried, empty *N. lamellosa* shells in air before and after the experiment. Empty shells were obtained by removing the flesh from live snails similar in size to those used in the experiment that had been collected with the experimental snails. Empty shells were thoroughly scrubbed and then cleaned in heated tap water for 1 hr in a 1 litre ultrasonic cleaner to remove residual organic material, and dried for 24 hr prior to weighing.

Experimental Design

From 100 tagged snails, only 72 individuals showing the greatest rates of shell weight gain during the preceding 13 days while feeding were selected. This ensured that all individuals used had experienced positive and measurable rates of shell growth. To estimate baseline rates of shell deposition, these snails were removed from food, weighed underwater (see *Weighing Protocol*), returned to their cleaned and otherwise empty holding tubs in running seawater (see *Collection, Feeding, and Initial Measurements*), and weighed underwater again after 48 hr.

Snails were assigned to treatments so that shell lengths, body weights, shell weights, and baseline shell deposition rates were similar in each CO_2 treatment (Table 2.1). The 24 snails in each treatment were divided equally among six plastic containers (four snails per container). One pre-weighed, empty *Nucella* shell was also placed in each container. Empty shells were assigned to containers so that average shell dry weights were approximately the same in the three CO_2 treatments (Table 2.2). Underwater weights (see *Weighing Protocol*) of live snails were measured at the beginning and end of six days exposure to the three CO_2 treatments.

Each 25 x 15 x 15 cm polypropylene container used in the exposure experiment had a tightly fitting lid through which two small holes were drilled, one for PVC tubing fitted with an airstone through which the appropriate gas mixture was bubbled, and one to allow the gas mixture to escape. To keep snails fully submerged throughout the experiment (thereby eliminating emersion time as a variable that would confound shell deposition rates; Palmer unpublished), plastic mesh was fitted just below the lid of each container to prevent access to small air pockets at the lid corners. Containers were randomly arranged in three rows of six in an indoor sea table bearing 5 cm of continuously flowing seawater to stabilize water temperature.

Manipulation of pH and CO_2

The three CO_2 treatments included a) a control, in which ambient air was bubbled into the tanks (1X ambient [CO_2]= 385 ppm (NOAA 2008), b) moderately increased CO_2, where 400 ppm of CO_2 was added to the ambient air being bubbled into the tanks (2X ambient [CO_2]= 785 ppm), and c) high CO_2, where 1200 ppm of CO_2 was added to the ambient air (4X ambient [CO_2]= 1585 ppm). These treatments were chosen to approximate future CO_2 concentrations predicted for the year 2100 under the IS92a scenario for carbon emissions (IPCC 2001, Feely *et al*. 2004) and within the range projected to occur between 2100-2200 (Wigley *et al*. 1996, Caldeira and Wickett 2003), respectively. To achieve the desired CO_2 concentrations in the 2X and 4X treatments, paired mass flow controllers (Sierra Instruments, Model 810C Mass-Trak, Mass Flow Controllers, 0-100 sccm, and 0-5 slpm) were programmed to combine carbon dioxide from a pre-mixed compressed gas cylinder (2.0% CO_2: 20.4% O_2: balance N_2, PRAXAIR) and ambient air from the in-house compressor in the appropriate

proportions. A fifth mass flow controller (0-5 slpm) regulated air flow between the compressor and the 1X CO_2 tanks.

Bubbling rate was maintained at ~150mL/min. Among treatments, daily fluctuations in pH were small, and correlated with slight variations in bubbling rates. One half of the volume of each container was replaced with fresh seawater daily; equilibrium pH values re-established within approximately 1 hour. pH was measured using an Accumet AP63 pH meter, calibrated daily with pH_{NBS} 4.0, 7.0 and 10.0 buffers (Fisher Scientific). pH, temperature, salinity and bubbling rates were recorded at least once every 24 hours.

Statistical Analysis

Rates of dissolution and deposition were computed as mg dry shell weight change per snail per day. Differences in shell deposition rates of live snails among CO_2 treatments were tested with a one-factor ANCOVA (dependent variable: mg/d shell weight change per snail over the six day experiment; covariate: baseline mg/d shell weight change per snail during the two days prior to the experiment; grouping factor: CO_2 treatment; Type III Sum of Squares) (SuperANOVA 1.11, Abacus Concepts, Inc., Berkeley, CA, USA). All remaining statistical tests for differences in snail characteristics among treatments were conducted with a one-factor ANOVA (SuperANOVA 1.11). Tests for differences in seawater properties among treatments were conducted with a one-factor ANOVA (JMP 4.0.4, SAS Inst.).

RESULTS

Water Chemistry

As predicted, the pH decreased significantly (ANOVA: $F_{2,15} = 297.97$, $p < 0.0001$) from ambient levels where [CO_2] was increased experimentally (Fig. 2.1a). When averaged across all measurements, the mean (±SE) pH of the control containers bubbled with ambient air was 7.98 ± 0.012, while those bubbled with 2X and 4X current [CO_2] had average (±SE) pH values of 7.80 ± 0.015 and 7.54 ± 0.009 respectively.

Although all containers varied slightly over time (Fig. 2.1b-d), differences between treatments in temperature (ANOVA: $F_{2,15} = 0.001$, $p > 0.99$), salinity (ANOVA: $F_{2,15} = 0.122$, $p = 0.89$) and bubbling rates (ANOVA: $F_{2,15} = 2.800$, $p = 0.09$) were not significant.

Shell Deposition and Dissolution

Baseline shell deposition rate of individual *Nucella lamellosa* prior to the CO_2 treatments explained 73% to 83% of the variation in shell weight gain among snails within the CO_2 treatments (Fig. 2.2). The relationship was linear and deviations about the regression appeared to be uniform across the range of baseline deposition rates (Fig. 2.2) so we statistically removed among-individual variation in baseline shell deposition rate via ANCOVA. Slopes did not differ among the three treatments ($P > 0.99$; Fig. 2.2, Table 2.3a) therefore tests for deviations of mean shell weight change from a single common slope were justified.

Least-square mean shell weight change — shell weight change during the experiment standardized to a common baseline deposition rate by ANCOVA —differed significantly among CO_2 treatments ($P = 0.009$, Table 2.3a): weight change decreased roughly linearly with increasing CO_2 level (Fig. 2.3). Shell weight change of empty shells also varied significantly among CO_2 treatments ($P < 0.001$, Table 2.3b): in all cases it was negative, which indicated dissolution, and dissolution rate increased roughly linearly with increasing CO_2 level (Fig. 2.3). When the entire average amount of shell lost to dissolution per gram of shell was added to the shell weight change of live *Nucella lamellosa* over the experiment (open squares, Fig. 2.3), differences in estimated rates of shell deposition among CO_2 levels were no longer significant ($P = 0.79$). When only half of the shell lost to dissolution was added (solid squares, Fig. 2.3), to better estimate dissolution from only the outside surface of the shell, estimated rates of shell deposition did decrease with increasing CO_2 but these differences were also not significant ($P = 0.18$).

DISCUSSION

Our results suggest that elevated ocean CO_2 levels may have a much more profound effect on shell dissolution rates than on shell deposition rates, at least for temperate marine molluscs at lower temperatures (~10°C or less). Significantly, our results reveal how estimates of both deposition and dissolution rate are essential in studies of manipulated ocean CO_2 or pH levels impacts on skeletal growth. Had we not measured the rate of shell dissolution, we might have been tempted to interpret the decline in shell weight gain per snail per day with increasing CO_2 levels (Fig. 2.3) as reduced ability to produce new shell. In fact, after accounting for the

average rates of shell dissolution at increased CO_2 levels, net rates of shell deposition did not appear to differ among treatments. So, elevated CO_2 levels within a climatically relevant range clearly affected shell dissolution rates but had little or no effect on shell deposition rates in *Nucella*. We cannot say with certainty that elevated CO_2 levels had no effect on deposition rate because dissolution rate per gram of shell for empty shells may differ from that in occupied shells (see *Methodological issues* below). But regardless of whether all or only half of the shell lost to dissolution was added back to the shell weight change of live snails, differences in estimated rates of shell deposition did not differ significantly among CO_2 levels (Fig. 2.3).

Many invertebrates can regulate extracellular pH and maintain high rates of calcification during periods of hypercapnia. For example, even at 10X and 15X current CO_2 levels — levels much higher than in our experiment — the cuttlefish *Sepia officinalis* grew and deposited new skeleton at the same rate as controls (Gutowska *et al*. 2008). Wood *et al*. (2008) similarly reported increased calcification rates in a brittle star exposed to reduced seawater pH, even at pH 6.8. In addition, upon exposure to acidic external conditions many shellfish can dissolve their inner-shell surfaces, thereby increasing the extracellular concentration of bicarbonate ions, to buffer tissue pH (Michaelidis *et al* 2005). Therefore many species can actively generate an internal micro-environment that is supersaturated with respect to calcium carbonate despite being exposed to an under-saturated external environment. This would explain the seemingly impossible ability of marine molluscs a) to deposit calcium carbonate shells despite living in naturally acidic habitats (such as near volcanic vents and in some estuarine areas) (Hall-Spencer *et al*. 2008; Marshall *et al*. 2008; Tunnicliffe *et al*. 2009), and b) to have survived periods of elevated CO_2 in the past (e.g., Fraiser and Bottjer 2007), in seawater highly under-saturated with respect to calcium carbonate.

Clearly, calcifying invertebrates can regulate extracellular concentrations of physiologically important ions, and exercise tight control over cellular processes including calcification. Furthermore, the site of calcification is typically isolated from the external seawater (Tambutte *et al*. 2007), and would not directly experience the ambient saturation state (McConnaughey *et al*. 2000). So the ability of calcifying species to mediate precipitation of new shell material under inhospitable external seawater conditions, at least over the short-term, is perhaps unsurprising. However, shell dissolution at the external surface is not regulated by

the organism, and may be much more sensitive to changes in the carbonate saturation state of the seawater with which it is in direct contact.

Like many temperate marine molluscs (Lowenstam 1954) the shell of *Nucella lamellosa* is mainly calcite (Vermeij 1995) and therefore it should be less soluble in seawater than aragonitic or high-Mg calcite shells (Andersson *et al*. 2008). Nonetheless, despite exposing snails to experimental conditions where the predicted saturation state for calcite (Ω_{CaCO3}) is greater than 1 (11°C, CO_2 < 1600 ppm; Andersson *et al.* 2008) we observed significant increases in shell dissolution rate more or less linearly with increasing CO_2 levels (Fig. 2.3). This trend is consistent with the increase in dissolution rates of 'dead' or detached brittle star arms and dead coralline algal thalli exposed to increasingly acidic seawater over a similar range of pH values (Wood *et al*. 2008; Martin and Gattuso 2009). Shell dissolution therefore appears to be highly sensitive to decreases in saturation state even when the seawater to which the shell is exposed is supersaturated with respect to calcite. Alternatively, dissolution rates may be influenced by factors other than the purely physicochemical properties of seawater, such as the proportion of organic matrix in the shell, the relative size of the calcite crystals (Harper 2000), or dissolution effects of adhering biofilm.

Recent findings that CO_2-mediated declines in ocean pH may not affect calcification rates seem to contradict many prior experimental studies where skeletal growth and calcification rates decreased with increased CO_2 for a wide range of invertebrates (e.g. Shirayama and Thornton 2005; Gazeau *et al*. 2007; Jokiel *et al*. 2008). But this apparent contradiction may be illusory. Our results reveal that shell dissolution may have a greater impact on changes in shell mass under elevated CO_2 levels than declines in biologically mediated rates of shell deposition. Thus, measurements of overall changes in shell mass, although relevant endpoints to report, do not necessarily reflect changes in calcification or shell deposition rates *per se*. Clearly, both shell dissolution and deposition rates must be monitored to understand how changes in seawater carbonate chemistry will affect the ability of marine calcifying organisms to grow and maintain essential skeletal structures.

Finally, our results highlight some of the broader ecological implications of ocean acidification. Dissolution of non-growing shell regions can result in structural weakness in these skeletal elements (e.g., for barnacle tests; McDonald *et al*. 2009), and may increase susceptibility to certain predators and bio-eroders. Furthermore, shells and skeletons of reef-

forming corals and bivalves provide important habitat even after the animals have died, and gastropod shells may house hermit crabs and other organisms for many years after the death of the original occupant. Increased dissolution rates, as measured here, will reduce the lifespan of these biogenic structures, and may have significant ecological consequences.

Methodological Issues

The success of this study depended critically on two convenient qualities of *Nucella*. First, well-fed snails continue to deposit shell at easily measurable rates (>2mg/d) for up to three weeks even after removal of food (e.g., Appleton and Palmer 1988; Palmer 1990). Therefore we could measure shell deposition rates under various conditions with no confounding effects of variation in feeding rates. Second, underwater weight permits an accurate estimate of shell dry weight of live *Nucella lamellosa* because a) the shell makes up a significant fraction of the total weight in air (e.g., 83.1% in snails used in this study; see Table 2.1 below) and b) shell material is much denser than seawater whereas snail tissue is nearly neutrally buoyant, so underwater weight of a live snail is due almost entirely to the shell (Palmer 1982).

Estimates of baseline shell deposition rate for each snail were essential prior to the CO_2 treatments to control for among-individual variation in rates of shell deposition. For reasons that remain unclear, shell deposition rates vary considerably among similar-sized *Nucella* even when held under the same conditions (e.g., baseline shell deposition rate ranged from 0.8 to 20.5 mg/d, CV= 88.2%; Fig. 2.1,Table 2.1). The baseline rates of shell deposition (Fig. 2.2) are arbitrary, as average rate of shell deposition depends on the amount of food snails have eaten recently, water temperature, and other factors (Palmer 1990).

Shell dissolution rate of unoccupied shells may overestimate dissolution rate in occupied shells, when computed per gram of shell, because both the outer and inner surface of unoccupied shells are exposed to seawater whereas only the outer surface of an occupied shell is. We cannot know for sure, though, whether the inner and outer surfaces dissolve at the same rate. *Nucella* shells have two distinct layers of different microstructure, a thick outer homogeneous layer and a thin inner crossed-lamellar layer (Avery and Etter 2006). Although both layers are calcite (Vermeij 1995), microstructure and organic matrix content may have a greater effect on shell dissolution rate than mineralogy (Harper 2000). For aragonitic bivalve shells, dissolution rates of homogeneous and crossed-lamellar microstructures do not appear to

differ under either sterile or non-sterile conditions (Harper 2000), but we do not know if this is also true for the two microstructural types of *Nucella*. We therefore estimated the shell deposition rate, in the absence of dissolution, by adding back to the weight change of live snails either 0.5 or 1.0 of the shell lost to dissolution (Fig. 2.3).

ACKNOWLEDGEMENTS

We thank the staff at Bamfield Marine Sciences Centre for their assistance in various aspects of the research conducted at the facility, in particular Randy Zohner for his help with transportation, and Bruce Cameron for technical support. This study was supported by grants from the Bamfield Marine Sciences Centre, Natural Sciences and Engineering Research Council of Canada Discovery Grants (A7245 to ARP and 22R43158 to CDGH), and a Canada Foundation for Innovation grant (to CDGH). SN and ARP conceived and designed the study, conducted the experiments and analyses, and drafted the article. CDGH contributed to the conceptual design and to critical revision of the manuscript.

Table 2.1: Initial sizes and baseline shell deposition rates of *Nucella lamellosa* used in the CO_2 treatments.

		Shell Length (mm)		Body Wet Wt. (g)		Shell Dry Wt. (g)		Baseline Shell Deposition Rate* (mg/d)	
Treatment	N	mean	SE	mean	SE	mean	SE	mean	SE
Control	24	34.9	0.48	1.17	0.044	5.51	0.212	4.36	0.963
2X CO_2	24	34.9	0.42	1.13	0.030	5.83	0.228	5.44	0.931
4X CO_2	24	34.6	0.43	1.13	0.037	5.54	0.196	5.50	0.814

Differences among treatments were not significant for any measurement (P= 0.87, P= 0.70, P= 0.50, P= 0.60, respectively).

* Baseline shell deposition rate of starved snails over two days immediately prior to the CO_2 treatments.

Table 2.2: Initial weights of empty *Nucella lamellosa* shells.

		Shell Dry Wt. (g)	
Treatment	N	mean	SE
Control	6	5.23	0.401
2X CO_2	6	5.83	0.375
4X CO_2	6	5.53	0.525

Differences among treatments were not significant (P= 0.64).

Table 2.3: Results from statistical tests for differences among CO_2 treatments.

a) ANCOVA of shell weight change of live snails (mg/d) among three CO_2 levels with baseline shell deposition rate as a covariate (data in Fig. 2.2).

Source	df	MS	F	P
CO_2 level	2	5.97	5.03	0.0094
Covariate	1	268.20	226.06	<0.0001
Residual	63	1.18		
Covariate x treatment	2	0.0009	0.0008	>0.99

b) ANOVA of weight change of empty shells (mg/d) among three CO_2 levels (means in Fig. 2.3).

Source	df	MS	F	P
Between groups	2	1.093	12.53	0.0006
Within groups	15	0.087		

c) ANOVA of residual shell weight change of live shells (mg/d) among three CO_2 levels after estimated amount of shell lost to dissolution (Fig. 2.3, empty shells) was added back.

Source	df	MS	F	P
Between groups	2	0.273	0.23	0.79
Within groups	64	1.170		

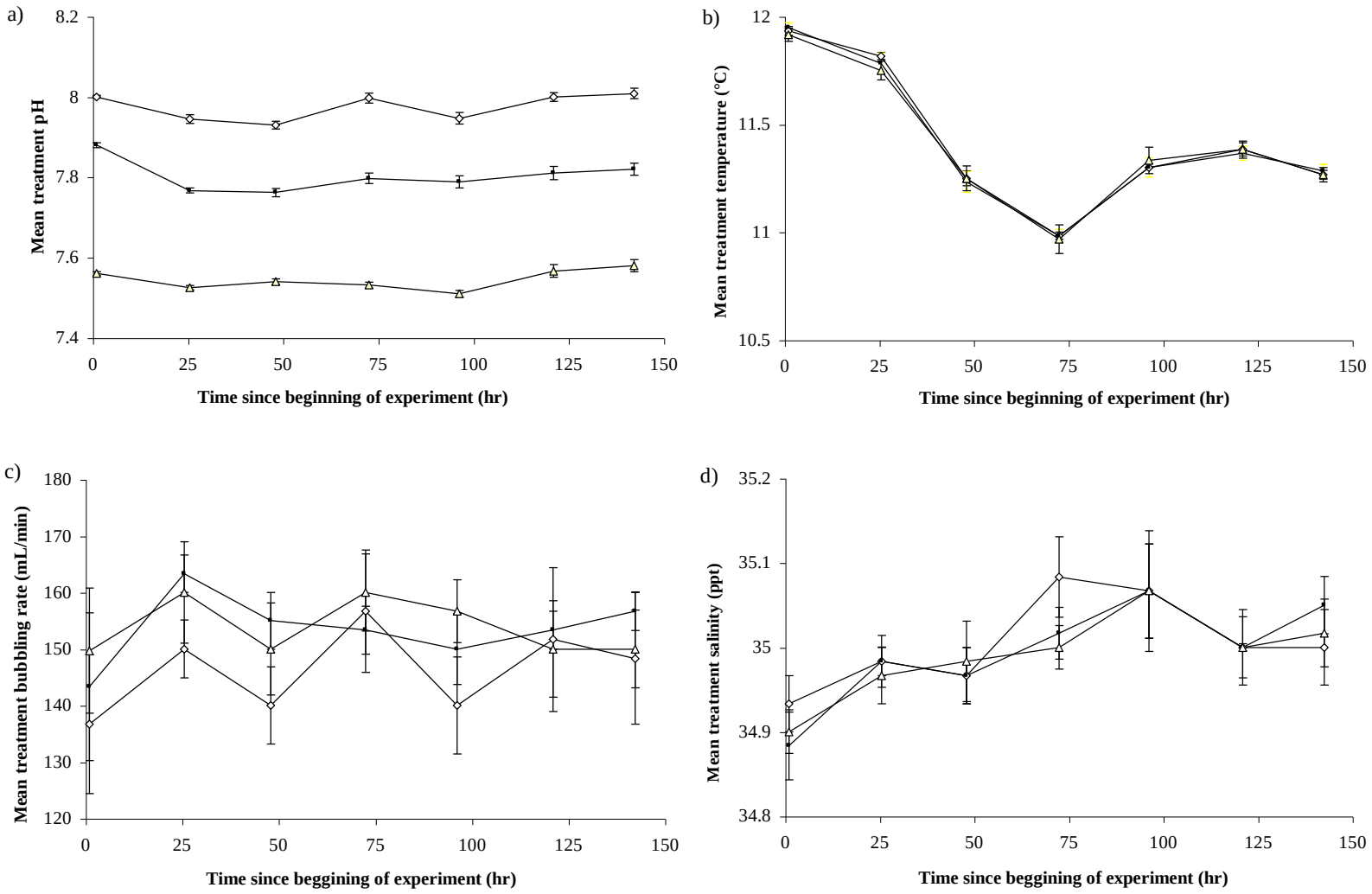

Figure 2.1: Variation in seawater properties (including a) pH_{NBS} b) temperature (°C) c) bubbling rate (mL/min) and d) salinity (psu)) in the 1X CO_2 (open diamonds), 2X CO_2 (solid squares) and 4X CO_2 (open triangles) tanks across the six-day duration of the CO_2 exposure experiment. All values are the mean (±SE) of all tanks in each treatment (N=6).

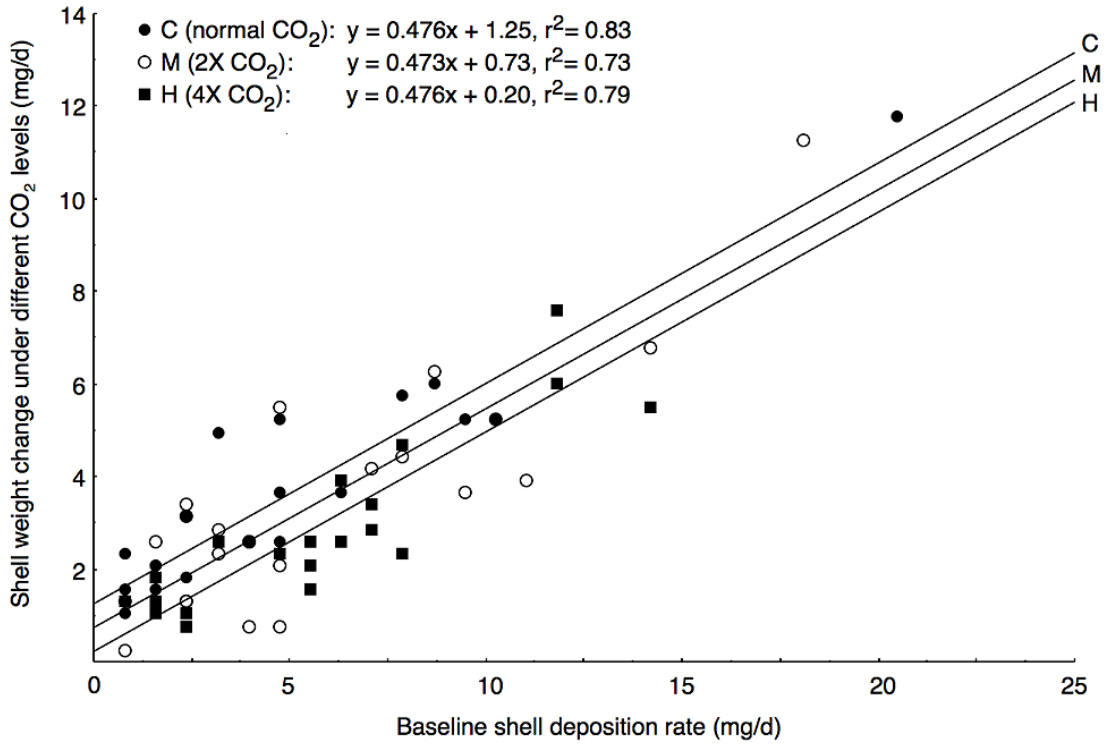

Figure 2.2: Shell weight change of live *Nucella lamellosa* held under three CO_2 levels for six days as a function of baseline shell deposition rate (shell deposited in the absence of food for two days in ambient seawater prior to the experiment). Each point represents an individual snail. Least-squares linear regression equations describe the relationship for each treatment (all three were highly significant, $P < 0.001$). Five snails that exhibited a baseline shell deposition of 0 mg/d or less were excluded from the analysis.

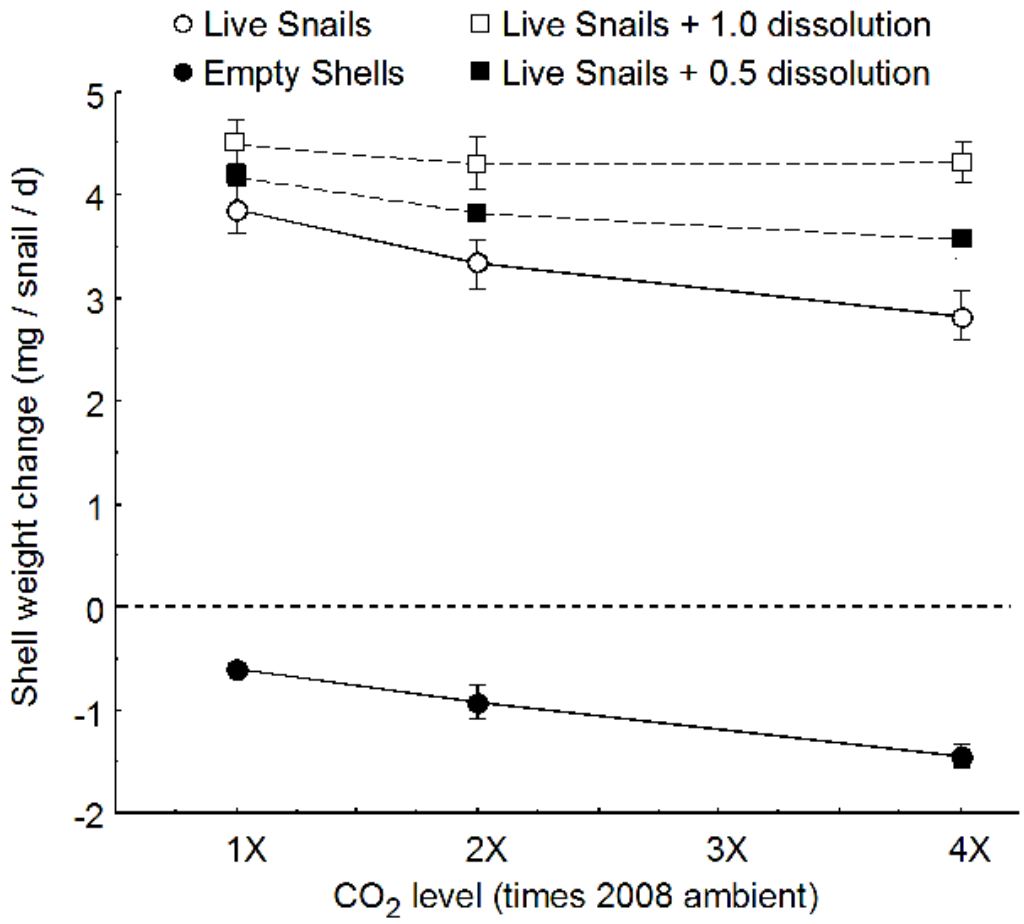

Figure 2.3: Circles and solid lines: average weight change of live and empty *Nucella lamellosa* shells held under three CO_2 levels for six days (mean ± SE; N= 22, 23 and 22 respectively for live shells; N= 6 for dead shells). Squares and dashed lines: estimated shell deposition rate of live *N. lamellosa* after correcting for dissolution (i.e., after adding either 0.5 or 1.0 times the shell weight change per gram of empty shells). Error bars for the solid squares were omitted for clarity.

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CHAPTER 3: THE IMPACT OF ELEVATED CO_2 ON THE GROWTH AND FEEDING RATES OF RED SEA URCHINS: IMPLICATIONS FOR RED URCHIN POPULATIONS AND KELP FOREST COMMUNITIES[2]

INTRODUCTION

Anthropogenic climate change is having a wide array of increasingly well-publicized impacts on the oceans (Harley *et al*. 2006; Hoegh-Guldberg *et al*. 2007). While sea surface temperatures continue to rise and ocean storms occur with greater frequency and severity, physical changes are not the only new conditions being imposed upon marine life. The recent increase in atmospheric greenhouse gas concentrations is also altering the chemistry of the oceans dramatically, resulting in ocean acidification (Feely *et al*. 2004; Doney *et al*. 2009). The world's oceans represent a major sink for anthropogenic CO_2, and have already sequestered nearly one half of atmospheric carbon emissions since the beginning of the industrial revolution (Doney 2006). Concomitant with this increased oceanic absorption of anthropogenic atmospheric CO_2 -- which results in the production of carbonic acid -- the surface pH of modern oceans has dropped ~0.1 units relative to pre-industrial levels (Orr *et al*. 2005). Global atmospheric CO_2 concentrations are currently 380 ppm, up from a pre-industrial value of 280 ppm. With the predicted increase of atmospheric CO_2 to nearly 800 ppm by the end of this century (Feely *et al*. 2004), a cumulative decrease in surface water pH of 0.3 to 0.5 units by the year 2100 is probable (Orr *et al*. 2005; Miles *et al*. 2007).

Unlike changes in temperature and physical disturbance regimes, which will be strongly influenced by location, a decline in sea-surface pH is expected to occur ocean-wide, though colder, higher latitude regions like the Southern Ocean and the North Pacific will suffer undersaturation with respect to $CaCO_3$ first (Orr *et al*. 2005; Fabry *et al*. 2008; Andersson *et al*. 2008). While certain organisms may be able to shift their geographic ranges in response to temperature change, shallow-water marine species will find little refuge from these increasingly acidic conditions. The recent decline in ocean pH (by about 0.0017 ± 0.0004 pH units/year) has already been documented by direct measurements (Santana-Casiano *et al*. 2007;

[2] A version of this chapter has been submitted for publication. Nienhuis, S.B. and Harley, C.D.G. (2009) The impact of elevated CO_2 on the growth and feeding rates of red sea urchins: implications for red urchin populations and kelp forest communities

Wootton *et al*. 2008). Despite the fact that ocean acidification is ongoing and further pH change is unavoidable, very little is actually known about how this stress will affect marine populations and communities.

One of the more critical consequences of the current CO_2-mediated drop in ocean pH is the resultant decrease in the concentration of dissolved carbonate ions [CO_3^{2-}]. Carbonate ions play a central role in ocean biomineralization processes, as they are required for the production of calcium carbonate ($CaCO_3$), the predominant structural mineral used by marine invertebrates (Wilt *et al*. 2003). It is primarily the calcium carbonate saturation state that controls calcification rates in these organisms (Fabry *et al*. 2008). Because the saturation state of calcium carbonate is directly proportional to the concentration of carbonate ions in seawater, the ability of calcifying marine organisms to precipitate $CaCO_3$ is dependant upon the availability of CO_3^{2-} in the ocean. However, owing to the nature of the carbonate equilibrium, as the CO_2 concentration in the ocean increases, the amount of CO_3^{2-} available for biogenic calcification decreases. Thus, an increase in dissolved CO_2 indirectly increases the solubility of calcium carbonate. According to recent climate models, if the concentration of atmospheric CO_2 reaches 800 ppm by the end of the century, the corresponding decrease in dissolved surface-water CO_3^{2-} concentrations would be approximately 60% (Feely *et al*. 2004). For marine calcifiers, this dramatic decline in the availability of carbonate ions is predicted to reduce calcification rates, resulting in weaker shells, slower extension rates and greater susceptibility to breakage or erosion (Kleypas *et al*. 1999). By hindering calcification as such, ocean acidification may have detrimental impacts on the growth and survival of these species.

Experimental studies that have manipulated levels of dissolved CO_2 and pH are beginning to demonstrate the pronounced physiological effects that changes in these chemical parameters can have on marine organisms. Long-term moderate hypercapnia induced by lowering seawater pH by 0.5 pH units or more has been shown to decrease metabolic rate (Langenbuch and Portner 2004; Michaelidis *et al*. 2005) and compromise acid-base balance (Miles *et al*. 2007) in various invertebrate species, with significant impacts on their growth and survival. While studies such as these often involve increasing seawater CO_2 to levels much greater than expected of the next century, significant effects on growth and calcification rates have been documented for much more subtle changes in seawater chemistry. Experiments with marine gastropods and sea urchins demonstrated a significant reduction in both growth and

survivorship after 6-months of exposure to CO_2 levels elevated by only 200 ppm (which caused a pH drop of 0.03 units) (Shirayama and Thornton 2005). In the latter experiment, the authors also discovered that both species experienced a significant reduction in mass after only 3 months of exposure to water with 550 ppm CO_2, an effect that they attributed to reduced rates of shell formation in these calcium-depositing organisms.

Certainly, the ability of moderately elevated seawater CO_2 concentrations to reduce calcification and skeletal growth rates is well documented for a variety of marine invertebrate taxa, from corals (Leclercq *et al*. 2000; Kleypas *et al*. 2006; Jokiel *et al*. 2008) to planktonic calcifiers (Riebesell *et al*. 2000; Orr *et al*. 2005) to molluscs (Berge *et al*. 2006; Gazeau *et al*. 2007; Nienhuis and Palmer (in preparation)) and echinoderms (Shirayama and Thornton 2005). Though most of these experiments were performed on adult individuals, a number of recent studies with invertebrate embryos and larvae indicate that these early life stages may be particularly vulnerable to increases in seawater CO_2 levels (Kurihara *et al*. 2004; Kurihara and Shirayama 2004; Havenhand *et al*. 2008; Kurihara 2008; Portner and Farrell 2008; O'Donnell *et al*. 2009).

The majority of studies that have sought to assess the impacts of ocean acidification on marine organisms have involved short-term laboratory experiments on individuals of a single species (Doney 2006; Fabry *et al*. 2008; Hall-Spencer 2008). There remains an important knowledge gap regarding the potential consequences of long-term chronic exposure to climatically relevant CO_2 concentrations, especially at the population or community level (Kleypas *et al*. 2006; Raven *et al*. 2005; Fabry *et al*. 2008; Widdicombe and Spicer 2008). While it is critical to quantify the effect that ocean acidification will have on the survival and growth rates of individuals, it is also important to understand how such changes might affect the population dynamics of those species. Changes in population size, coupled with changes in behaviour (e.g., Sanford 1999), will in turn determine climatic impacts on community structure. Studies which focus on strongly interacting species, such as keystone consumers (e.g., Gooding *et al*. 2009) will be key to providing a more complete understanding of the acidification-mediated community- and ecosystem-level changes that are already underway.

Here, we investigate the potential impacts of ocean acidification on the population dynamics and community impacts of the red sea urchin, *Strongylocentrotus franciscanus*. *S. franciscanus* is an ideal test organism for several reasons. First, sea urchins deposit high-

magnesian calcite in their test, teeth and spines (Weber 1969; Magdans and Gies 2004). Because high magnesian calcite is more soluble than other forms of calcium carbonate used to build skeletal material (Davis *et al*. 2000), organisms which utilize high-Mg calcite could suffer a disproportionately greater risk of shell, or test, dissolution (Miles *et al*. 2007; Andersson *et al*. 2008). Second, red sea urchins are arguably the most important herbivores in the kelp forest ecosystems on the west coast of North America (Estes *et al*. 1989). This species of urchin feeds on the giant kelp *Macrocystis*, a dominant macrophyte providing much of the canopy structure and biomass in kelp forest communities (North 1971). Through overgrazing, red sea urchins have been shown to cause extensive damage to kelp beds, to the point of complete destruction (Leighton 1971). Therefore, any acidification-induced changes in urchin survival or growth could have ecosystem-scale impacts (Clarke *et al*. 2009). Finally, red sea urchins are an economically valuable species, harvested for their gonads which are considered a delicacy among seafood enthusiasts, particularly in Japan (McBride *et al*. 2004). From its beginnings in the early 1970s, the red sea urchin fishery on the west coast of North America has grown dramatically. In British Columbia alone, the total annual landed value of the red sea urchin fishery reached $14.4 million in 1997/98 (Department of Fisheries and Oceans (DFO) Canada 2001). The red sea urchin fishery in California is one of the state's largest fisheries in terms of both landings and revenue (Quinn *et al*. 1993), while in Washington State annual landings reached 3.6×10^6 kg in 1988 (Pfister and Bradbury 1996). If a CO_2-mediated drop in ocean pH causes a decline in the growth rate of red sea urchins, the number of individuals attaining harvestable size would be expected to decrease as well, with implications for the approach that North American sea urchin harvesters will need to take in managing this valuable fishery.

In this study, we set out to generate quantitative predictions about how sea urchin populations, and by extension kelp forest communities, will respond to changes in ocean chemistry in the decades and centuries to come, and to assess some of the socio-economic consequences of these changes. Specifically, we manipulated CO_2 to determine the impact of a moderate increase in dissolved CO_2 on the growth rate and feeding behaviour of juvenile red sea urchins; a species of both ecological and economic importance along the west coast of North America. We then used our empirical data to model the effects of ocean acidification on several key population- and community-level attributes. We predicted that feeding rates would

be lower in urchins exposed to higher CO_2 levels compared to those in control CO_2 conditions, because increased CO_2 concentrations (hypercapnia) can induce chronic metabolic depression in marine invertebrates (Portner *et al*. 2005; Michaelidis *et al*. 2005; Langenbuch and Portner 2005), and has been linked to suppressed invertebrate feeding activity in several studies (eg. Bamber 1990; Siikavuopio *et al*. 2007). In addition, calcification rates have been shown to decrease with increasing CO_2 (Fabry *et al*. 2008; Doney *et al*. 2009), and for well-fed calcifying organisms encased in a hard mineral shell or test a reduction in shell growth can often limit or reduce the rate of soft tissue growth (Palmer 1992). Thus, we further predicted that juvenile urchins would experience a decline in growth rate comparable to those reported in CO_2 manipulation studies with other calcifying marine invertebrates (e.g., Shirayama and Thornton 2005; Michaelidis *et al*. 2005; Berge *et al*. 2006). Finally, we predicted that reductions in feeding and growth would result in longer times to maturity, reduced urchin biomass, and a reduced impact on kelp resources.

MATERIALS AND METHODS

Sea Urchin Collection and Housing

Approximately thirty juvenile red sea urchins, ranging in size from 10 –50 g wet weight (30-40 mm test diameter) were collected subtidally near Bamfield Marine Sciences Centre on Vancouver Island, British Columbia (48º 51.141'N, 125º 06.839'W) on October 6, 2006. They were transported in a cooler with ice and damp paper towels to the laboratory at the University of British Columbia the following day, where they were housed in a re-circulating artificial sea water system (pH = 7.95, salinity = 32 ppt, temperature = 12ºC). The urchins were fed *ad libitum* with previously collected, cut and frozen drift kelp (*Macrocystis integrifolia*).

After acclimating to the conditions in the lab for approximately 4 months, the urchins were weighed and subsequently ranked by size. The largest and smallest urchins were excluded from the study, as only 20 individuals were required. The remaining 20 urchins were paired by size, and the pairs were split up into two groups of ten to ensure an equal size distribution of urchins between the two experimental treatments. Initial urchin wet masses ranged from 10.26 to 23.32 g with a mean (±SE) of 16.96 ± 1.34 g for the control treatment and from 11.62 to 23.53 g with a mean of 16.95 ± 1.30 g for the high CO_2 treatment. There was no difference in the means between treatments. Ten glass aquaria (20.82 litre) were placed in the sea table, so

that there were 5 tanks per treatment. The tanks were positioned adjacent to each other in three rows of three, and one row of one, with the control and high CO_2 tanks alternating within the array (Fig. 3.1). Urchins were randomly selected from the appropriate, pre-divided groups and placed two to a tank in each of the ten aquaria.

Manipulation of pH and CO_2

The experimental design consisted of two treatments: a control treatment in which the tanks were bubbled with ambient air (CO_2 concentration 380 ppm), and a high CO_2 treatment in which an additional 400 ppm of CO_2 was added to the air being bubbled into the tanks (total CO_2 concentration 780 ppm). Two gas manifolds, one for each treatment, served to distribute air from air compressors, via PVC tubing into individual aquaria. For the high CO_2 treatment, mass flow controllers upstream of the manifold were programmed to mix carbon dioxide from a cylinder (2% CO_2: 20.6% O_2: balance N_2, PRAXAIR) and ambient air from the compressor to achieve the desired CO_2 concentration in the output (Fig. 3.1).

The rate of bubbling in all of the tanks was regularly monitored and maintained at ~200mL/min. Fluctuations in pH between measurements were small, and were correlated with slight variations in the rate of bubbling in a given aquarium. Regardless of treatment, a faster bubbling rate consistently resulted in higher pH values, though routine adjustments to the rate of bubbling in each tank minimized these variations. All aquaria were fitted with clear plastic lids with only a small hole for the gas line to expedite the dissolution equilibria of the gases. To further ensure adequate equilibration of CO_2 in the aquaria, rather than be supplied with a constant flow of seawater, a partial (one third) volume replacement was performed for each tank every 2-3 days with new seawater. Equilibrium pH values were re-established within approximately 2 hours of these volume replacements. The pH of all of the tanks, as well as of the sea water system itself, was measured to ± 0.01 pH units a minimum of three times a week using a hand-held multi-meter probe (YSI 556-MPS) calibrated prior to each measurement with NBS buffers (pH 4.00, 7.00 and 10.00; Fisher Scientific) pre-equilibrated to tank seawater temperature.

Measurement of Growth

Urchin wet weight was measured for each individual at the onset of the experiment, and thereafter at monthly intervals for the four-month duration of the experiment. Wet weights

were calculated as the average of two measurements made after submersing the urchin, shaking it gently until it stopped dripping, and weighing it to the nearest 0.01 g. Measurements of live urchin test diameter suffered from poor repeatability due to the movement of the urchins' spines and the inherent difficulty of acquiring a precise measurement of this sort on a live individual. Therefore we used wet mass to estimate growth rates, which were calculated as a cumulative growth rate expressed as percent of starting mass, according to the equation from Shirayama and Thornton (2005):

((wet wt at time T)-(initial wet wt))/(initial wet wt) x 100%

A relative rather than an absolute measure of growth is useful in this type of study in order to account for any confounding differences in growth rate associated with the initial size of the urchin.

In order to facilitate comparisons with earlier studies, we also expressed urchin growth rates in terms of test diameter. To do this, we first calculated the allometric relationship between final urchin wet weight (M) and test diameter (D), as measured upon the removal of spines at the end of the experiment. Over a large range of urchin size classes, this relationship is typically described by a power function (Harrold and Reed 1985). However, because this relationship proved to be linear for the relatively small size range of urchins used in our experiment, with no effect of treatment (ANCOVA, treatment effect: $p = 0.8929$), we simply converted growth in grams to growth in mm using our regression line equation, $D = 0.5569*M + 22.679$ ($R^2 = 0.9035$), calculated from values pooled across the two treatments.

Measurement of Feeding Rate and Absorption Efficiency

Sea urchins were fed three times a week on a diet of previously cut and frozen *M. integrifolia* stipes for the entirety of the experiment. The kelp to be added to each tank was thawed, blotted and weighed to the nearest 0.01 g before being fed to the urchins, and all remaining algae were similarly re-weighed 2-3 days later. Typically, three pieces of algae weighing approximately 8 g in total were provided to each tank three times a week. This amount of kelp was chosen to ensure an ample supply of food to the urchins, so that there was always uneaten kelp remaining to be re-weighed. To control for equal accessibility of kelp to the urchins in all tanks, one piece of kelp was placed by hand into the spines of each urchin in

the tank, while the third piece was dropped haphazardly onto the bottom of the tank. Several times throughout the course of the experiment, small pieces of *Macrocystis* were suspended in mesh from the top of each aquarium for several days, and their wet mass before and after exposure to the tanks was recorded. However the change in kelp mass due to decomposition upon immersion was equally negligible for both treatments (data not shown). Feeding rate was calculated on a per weight basis (g/g/day) for each aquarium as the mass of algae consumed (mass added – mass remaining)/total mass of urchins in aquarium/number of days in feeding trial.

When sea urchins are well fed, they produce faecal pellets that can be easily collected and weighed. Over the course of the experiment, each tank was carefully vacuumed every two to three days, and all of the faecal pellets in the wastewater were collected on pre-weighed No. 1 Whatman filters. The filters were dried in a drying oven at 60 ^{o}C for 48 hours, and the dry mass of the egested material from each aquarium was recorded. A wet to dry ratio for the *Macrocystis* stipes comprising the diet of the urchins was obtained at the onset of the experiment using 20 pieces of algae that were similarly dried at 60 ^{o}C for 48 hours. This conversion factor was used to compute the mass of algae ingested per tank over the same time period on a dry weight basis. Absorption efficiency was then calculated based on gravimetric analyses according to the expression used by Vadas (1977):

(dry wt ingested – dry wt egested/dry wt ingested) x 100%

Statistical Analysis

pH data were analyzed using a student's t-test to determine whether there was a significant difference in pH between the control and high CO_2 treatments. To do this we calculated the mean pH value for each tank over time, and then analyzed the mean of these means for each treatment. To test for differences in growth rates, feeding rates, absorption efficiency and morphometric measures between urchins exposed to different CO_2 concentrations, separate, nested ANOVAs with tank nested within treatment were performed. Model output parameters required transformations in order to calculate means and standard errors. Time to size data best approximated a normal distribution following log transformation, while urchin biomass and kelp consumption were square-root transformed in order to better approximate a normal distribution. Even with transformations, however, minor departures from

normality occurred in at least one of the model scenarios for each of the four metrics. Therefore, although transformations were used for calculating means and standard errors (which were subsequently back-transformed), statistical comparisons were made using non-parametric Kruskal-Wallis tests.

Demographic Modelling

To assess how the size distribution of red sea urchin cohorts may change with time as the pH of ocean water decreases, we applied our experimentally determined estimates of the future CO_2-mediated reduction in sea urchin growth rates to a simple demographic model based on the size-structured von Foerster equation. This approach assumes deterministic growth g(l) and mortality D(l) functions, which govern changes in the density of individuals in a size frequency distribution with size (l) and time (t).

The von Bertalanffy equation was used to model red sea urchin growth as it describes the linear reduction in growth increments with increasing size commonly observed in sea urchins and other shellfish species (Ebert and Russell 1993; Smith *et al*. 1998; Morgan *et al*. 2000). This deterministic growth function is defined by the following equation:

$$g(l) = K (L - l)$$

where K is the instantaneous rate of change in growth rate and L is the asymptotic or maximum test diameter in mm. Total mortality was calculated as the sum of natural and fishing mortalities, according to the following:

$$D(l) = Z + F(l)$$

where Z is the instantaneous natural mortality rate and F(l) is the instantaneous fishing mortality rate at size (l).

We used these simple growth and mortality functions to model the change in sea urchin size and survival for a single cohort of 100 newly settled individuals over weekly increments for a 20 year total time period. Thus, the test diameter (d) of a sea urchin at a specific time interval was calculated as:

$$d_{t+1} = d_t + K(L - d_t)$$

while the number of urchins surviving (n) at a given time increment was:

$$n_{t+1} = n_t - D(l)*(n_t)$$

In our simplified model we assumed size-independence for the K and Z parameters, and applied a constant fishing mortality rate F, to all individuals with test diameters greater than 90 mm; the minimum legal size for harvestable urchins in British Columbia (DFO 2001). Values for all model parameters were taken from previous studies that calculated natural growth and mortality rates for red sea urchins in the wild, and are presented in Table 3.1. Using the available estimates of variation for these model parameters (Table 3.1), we were able to calculate the variance around 99 jack-knife iterations for each modelling scenario.

Morgan *et al.*'s (2000) value for K was used to model sea urchin demography under current atmospheric CO_2 concentrations because it was calculated based on a broad size range of red sea urchins in the wild. This K value is therefore more representative of natural growth rates than those that were calculated in our laboratory study from urchins limited to juvenile size ranges. However, in order to estimate the impact of ocean acidification on future growth and survival rates of red sea urchins we applied our experimental estimates of the relative reduction in urchin growth rates upon exposure to ~780 ppm CO_2 to Morgan *et al.*'s value for K, with all other model parameters unchanged. To account for the time-dependant growth effect that was observed between treatments in our exposure experiment, we calculated future urchin growth using two different modelling scenarios. We first used the overall mean difference in relative growth rates between the control and high CO_2-exposed urchins from month 0 to month 4 of the experiment to represent a more conservative future scenario (Scenario A) of reduced urchin growth at elevated CO_2 levels. However, because the treatment effect on urchin growth was not significant for the first two months of the experiment, we also calculated the difference in relative growth rates between months 2 and 4 only, and used this more pronounced growth rate reduction factor to model a second scenario (Scenario B) of reduced urchin growth in a high-CO_2 world. The growth rate reduction factors used for both scenarios are presented in Table 3.1. After running the simple demographic models for

individual cohorts of red sea urchins under current and future CO_2 scenarios, we applied empirically determined relationships between red sea urchin test diameter, biomass and feeding rates to the model outputs. This allowed us to generate simple estimates for the impact of a drop in ocean pH on future harvestable urchin biomass and kelp grazing rates, respectively.

Kawamata (1997) developed a model to predict feeding rate based on test diameter for *Strongylocentrotus nudus*, a species closely related to the red sea urchin. Using data sets from several laboratory feeding experiments wherein urchins of various sizes were fed several species of brown macroalgae, he derived the following equation relating the annual feeding amount (kg kelp) to sea urchin test diameter (d):

$$\text{kg kelp} = 0.0231\ d^{2.169}$$

We used this size-dependant feeding rate function to estimate the mass of kelp consumed at weekly intervals by the individual cohorts of urchins from our "control" and "high CO_2" model scenarios, and summed these amounts over the 20-year time span for which we ran the demographic models. In doing so, we were able to generate and compare estimates of the total amount of kelp consumed in the average lifetime of sea urchin cohorts at the present time, and under future CO_2 scenarios.

The relationship between test diameter (d) in mm and wet weight (m) in grams has been established for a variety of sea urchin species. For red sea urchins, Harrold and Reed (1985) empirically determined the following regression equation ($R^2 = 0.997$):

$$m = 0.0007\ d^{2.86}$$

Using this relationship, we converted the growth output of our demographic models to wet weights, in order to calculate the total biomass of potentially harvestable red sea urchins under current and future oceanic carbon dioxide concentrations.

RESULTS

Manipulation of pH and CO_2

As predicted, the pH of seawater decreased from ambient levels when bubbled with moderately higher concentrations of CO_2 (Fig. 3.2). When averaged across all measurements made during the 4-month experiment, the mean (±SE) pH of the high-CO_2 tanks (7.68 ± 0.008) was significantly lower than the control tanks (7.75±0.006) (*t*-test, $p < 0.05$).

Growth

All of the urchins in this study experienced positive growth rates throughout the course of the experiment, and no mortality was observed in either of the two treatments. Early in the experiment, there was no difference in the cumulative growth rate expressed as percent of starting mass (hereafter referred to as cumulative growth rate) of the urchins in the control and high-CO_2 tanks (Fig. 3.3). As time progressed, however, the cumulative growth rates of the urchins in the two experimental treatments began to diverge. After 4 months, the urchins exposed to higher CO_2/lower pH conditions had grown significantly less (nested ANOVA: CO_2 treatment effect $F_{1,19}$= 12.40, p=0.0055; tank nested within treatment $F_{9,19}$= 1.71, p=0.21) than those exposed to ambient CO_2 and pH conditions (relative growth rates were 26.45 ± 3.42 % (SE) and 40.46 ± 3.01 % (SE) respectively). That is, between month 0 and month 4, the urchins exposed to higher CO_2 levels grew only 68.28 ± 0.03% (SE) as much as the urchins in the control tanks, which represents an overall reduction in cumulative growth of 31.72%. The difference in growth rates was even more pronounced for the last 2 months of the experiment, where the growth of the urchins at lower pH was only 49.29 ± 0.04% (SE) that of the control urchins, or 50.71% less. Again, this difference was significant (nested ANOVA: CO_2 treatment effect $F_{1,19}$= 17.09, p=0.002; tank nested within treatment $F_{9,19}$= 1.47, p=0.28). For the juvenile sea urchins used in our experiment, the relationship between test diameter and wet mass was linear (y=0.5569x + 22.679) with an R^2 value of 0.9035. Expressed as a function of test diameter, calculated using the regression equation above, the average growth rate of red sea urchins (± SE) over the four-month period was 0.942 ± 0.085 mm/month for the control urchins and 0.574 ± 0.104 mm/month for the high-CO_2 exposed urchins.

Feeding Rate and Absorption Efficiency

Over the course of the experiment, there was no significant effect of CO_2 concentration on mean urchin feeding rate (ANCOVA, treatment effect: $F_{1,7}$= 0.1255, p=0.7336), but there was a significant effect of the size covariate (ANCOVA, size effect: $F_{1,7}$= 18.10, p=0.0038). The average feeding rate, in grams of kelp consumed/day, was consistently higher for larger urchins than for smaller urchins across the range of sizes measured (Fig. 3.4). When the non-significant effect of treatment was dropped from the analysis, size alone explained almost three quarters of the variation in feeding rate (linear regression: $F_{1,8}$= 20.90, p=0.0018, R^2 =0.723). Neither carbon dioxide concentration nor mean urchin size had an effect on absorption efficiency (ANCOVA, treatment effect: $F_{1,7}$= 0.1349, p=0.7242; size effect: $F_{1,7}$= 2.779, p=0.1394).

Demographic Modelling

The simple demographic models that we employed to simulate both kelp consumption rates and the biomass of harvestable urchins generated markedly different output values for the high-CO_2 scenarios relative to the control CO_2 scenario. Across the 20-year time span for which the models were run, both the instantaneous weekly feeding rates and harvestable urchin biomass (Figs 3.5a and b respectively) were consistently lower for the urchin cohorts modelled under the high-CO_2 scenarios than they were for urchins in the control CO_2 scenario. The only exception to this trend is in the urchin fishery model, where the high-CO_2 tails are slightly higher than the control for the last several years, due to the lag between the time it takes high-CO_2 urchins enter the fishery relative to the faster growing control urchins. However, by summing the weekly totals over the 20-year time span (effectively integrating the areas under the respective curves) we calculated that both the total biomass of kelp consumed and the biomass of urchins available to harvesters would decline significantly in a high-CO_2 world, even under the conservative scenario (Scenario A) (Table 3.2).

DISCUSSION

Our experiment has demonstrated that prolonged exposure to a 400 ppm increase in atmospheric CO_2 has a pronounced negative effect on the growth rate of juvenile red sea urchins. We calculated that the control group grew an average of 0.942 ± 0.085 mm/month (test diameter), which is comparable to the rates reported in other laboratory studies and in the

wild, while the urchins in the high-CO_2 tanks only grew an average of 0.574 ± 0.104 mm/month over the 4-month duration of this experiment. This represents a near 40% decrease in juvenile growth rates under a scenario of doubled atmospheric CO_2 levels. By applying our growth results to the simple demographic model for red sea urchin growth and mortality, we can estimate that the time required to reach reproductive size (50 mm; reproductive maturity is a function of size rather than age (Bernard and Miller 1973)) will increase from less than two years under present conditions to over three years at elevated CO_2 concentrations. Similarly, time to reach harvestable size (90 mm; DFO Canada 2001) is predicted to increase from five years to over eight years, with fewer urchins surviving to attain that size. Finally, kelp biomass consumed by urchins, and urchin biomass available to harvesters, both decline substantially in our elevated CO_2 scenarios (see Table 3.2 for values).

It is important to note that strong CO_2 effects only emerged during the second half of the experiment. A similar pattern was described by Shirayama and Thornton (2005) for the sea urchin *Echinometra mathaei*. It has recently been demonstrated that over the short-term, some animals are capable of maintaining or increasing calcification rates despite exposure to increased CO_2, which may explain the delayed growth effect observed here and elsewhere. Wood *et al*. (2008) reported an upregulation of metabolism and calcification in a brittle star exposed to reduced seawater pH, though this ability to compensate for increased seawater acidity came at the cost of reduced arm muscle mass, or muscle wastage. Wood *et al*.'s experiments were conducted over the short term (40 days), and the authors suggest that the use of the muscle as an energy source to maintain high rates of metabolism and calcification under hypercapnic conditions is unlikely to be sustainable over the long term. Ideally, we would have run our exposure experiment for a greater amount of time in order to capture the longer-term trend of growth rate reduction under higher CO_2, which we would expect to have increased with time as any potential compensatory mechanisms were exhausted. Thus, the output values for Scenario B, modelled using the last two months of growth data, may represent a more realistic outcome of long-term exposure to predicted future ocean conditions.

Our demographic model is simplified, and there are reasons to believe that its predictions may be conservative. For example, the impact of ocean acidification on urchin populations will be further exacerbated by the fact that the fecundity of sea urchins is exponentially related to body size (Paris and Pitelka 1962; Suchanek 1981; Tegner 1989), and

because sea urchins are commonly sperm limited (Levitan 1993, 1995). In addition, urchin fertilization success is positively correlated with the density of spawning conspecifics (Levitan 1991, 1992). Ocean acidification may therefore indirectly reduce fertilization and recruitment success both by reducing the number of urchins that reach reproductive maturity and by reducing the per capita number of gametes produced. Recent findings have also demonstrated more direct impacts of increased CO_2 on urchin fertilization success and rates of embryonic and larval development. While several authors report no effect (Byrne *et al*. 2009) or even a positive effect (Dupont and Thorndyke 2008) of decreased pH on fertilization and larval development for some urchin species, other studies have demonstrated that for many species of sea urchin, fertilization, cleavage and hatching rates, as well as larval size, survival and developmental speed all decrease with increased CO_2 (Kurihara and Shirayama 2004; Kurihara *et al*. 2004; Havenhand *et al*. 2008; Kurihara 2008). For species with planktonic larvae, where recruitment success is directly linked to larval survival (McEdward and Miner 2007), increased mortality or impaired growth of early life stages due to increased CO_2 could impact the long-term viability of the population (Clarke *et al*. 2009), even if adult organisms are not lethally affected (Legendre and Rivkin 2002).

This experiment is one of only a handful of published studies to examine the effects of a moderate pH drop on the feeding rate and absorption efficiency of an organism. Bamber (1987, 1990) examined the response of various marine bivalves to lowered pH and found that feeding activity was suppressed when pH was less than 7.5 and inhibited below a pH of 7.0. In a study with the green sea urchin, *Strongylocentrotus droebachiensis*, both feed intake rates and feed conversion efficiency were significantly reduced in urchins exposed to high levels of CO_2 (where resultant pH was 6.98) (Siikavuopio *et al*. 2007). We had therefore predicted that our experimental increase in dissolved CO_2, although smaller in magnitude than in previous studies, would also cause a reduction in the feeding rate and assimilation efficiency of juvenile red sea urchins. However, after four months of exposure, we found no difference in either the feeding rate or absorption efficiency of our experimental urchins. It was recently reported that for CO_2 values ranging between 700-2000 ppm, there was no effect of hypercapnia on two species of marine bivalve (Gazeau *et al*. 2007). In an experiment similar to ours, Kurihara *et al*. (2008) also found no effect of elevated CO_2 levels on the feeding rate of a common marine shrimp species, *Palaemon pacificus*, even though these were reared in seawater equilibrated

with air containing 1000 and 1900 ppm CO_2 for 30 and 15 weeks respectively. Thus it appears that although extremely high levels of CO_2 often cause reduced metabolic and feeding activity in marine invertebrate species, the more moderate increases in CO_2 predicted of the next century may not generate the same direct effects on a species' ecological performance.

Although elevated CO_2 did not result in a drop in individual urchin feeding rates, this does not mean that there will be no change to the rate of urchin grazing in kelp forests if the IPCC IS92a scenario (~740 ppm CO_2 in 2100) is realized (IPCC 2001). Our model suggests that urchins in a high CO_2 world will spend more time in smaller size classes, and we found a pronounced size effect on urchin grazing rates, independent of treatment. Because smaller urchins consume less than larger urchins, it is reasonable to hypothesize that under a future scenario of doubled atmospheric CO_2, populations of sea urchins will consume less kelp, per capita, than they do today by virtue of their taking longer to reach larger sizes with no concomitant increase in longevity.

The relationship between sea urchins and kelp is well studied. Numerous published examples of trophic cascades in kelp forest communities have illustrated that in the absence of control, populations of sea urchins are capable of overgrazing kelp beds (e.g., Estes and Palmisano 1974; Estes *et al*. 1989). When this occurs kelp forest communities are reduced to urchin-dominated barrens. Conversely, a reduction in urchin grazing (as mediated by an increase in sea otter predation, for example) can result in the regeneration and expansion of kelp beds, which in turn increases the structural and species diversity of the community (Duggins 1980). The model estimates generated in the present study indicate that ocean acidification may indirectly reduce the per capita grazing pressure of this keystone species, which could affect the kelp forest communities that they inhabit (Estes *et al*. 1989). By reducing the grazing effect of sea urchins, ocean acidification has the potential to moderate the importance of top-down biological control of benthic biodiversity in these important marine ecosystems (see also Widdicombe and Spicer 2008).

We acknowledge that our model simulations are highly simplified, and that exact estimates of how changes in ocean pH will affect urchin population dynamics will require further investigation of other life-history related parameters like fecundity, larval survival and recruitment success, as well as density-dependent effects and other non-linearities. In particular, the size and time-dependence of the impact of higher CO_2 on urchin growth would

require longer term data sets and a more advanced modelling approach to be better constrained. Despite the uncertainties in our model, our predictions provide a very basic estimate of future changes that are likely qualitatively accurate if not quantitatively exact. It is, however, important to note one final reason that even our less conservative model scenario may underestimate future CO_2-mediated reductions in urchin growth and by extension urchin grazing and population growth rates. The average pH in our control tanks, while lower than the supposed value of 8.1 for ocean pH, is consistent with recent field measurements taken off the western continental shelf of North America, which suggest the decline in sea-surface pH is occurring at a faster rate than previously predicted (Wootton *et al*. 2008; Feely *et al*. 2008). The pH in our high-CO_2 tanks is reflective of the values predicted by recent oceanographic models (ie. pH < 7.7 when pCO_2 reaches approximately 800ppm (Caldeira and Wickett 2003)), though the ultimate reduction in pH that we achieved between treatments was somewhat less than what has been reported in other studies where seawater was subjected to similarly increased CO_2 levels. This might suggest that the nominal concentration of dissolved carbon dioxide in our "high-CO_2" treatment was actually less than the assumed 780 ppm, or that the CO_2 concentration in our control treatment was somewhat higher than 380 ppm (possibly due to biological activity in the closed tanks, with a similar outcome to respiration in a tidepool for example). This being the case, the reduction in growth rates reported here, even at the 16-week mark, would underestimate the response of sea urchins under the projected scenario of atmospheric CO_2 levels reaching nearly 800 ppm by the end of the century.

Ocean acidification is already underway, and for a shallow-water calcifying species like the red sea urchin, it may prove to be the most serious consequence of the current period of human-induced climate change. At the level of individuals, while climatically relevant increases in CO_2 may not necessarily have direct impacts on feeding rates or performance, this change is causing measurable reductions in the rates of calcification and growth in a large suite of marine organisms. Among species like the sea urchin where an individual's size dictates its fecundity and more importantly time to first reproduction, reductions in individual growth rates will have far-reaching population-level consequences, and may ultimately impact their abundance and distribution. In addition, because size is also often correlated with feeding rates among invertebrates, reductions in the growth rates of consumers may indirectly reduce the feeding or grazing pressure on their prey. For species that have a large impact on the

biodiversity of their surrounding environments --by way of strong top-down control in the case of sea urchins-- ocean acidification may also have cascading effects on entire communities. Thus, while laboratory experiments which measure the response of individual organisms under conditions of decreased seawater pH are useful and necessary, the use of models is critical to predict the larger-scale and longer-term impacts of ocean acidification at population, community and perhaps even ecosystem levels.

ACKNOWLEDGEMENTS

We thank the dive team at the Bamfield Marine Sciences Centre for their assistance with animal collections, Wen-Ling Liao for her help with the experimental set-up, and Cathy Pfister, Brad Anholt, Laura White and two anonymous reviewers for discussion and comment on previous drafts of this manuscript. This study was supported by grants from the Natural Sciences and Engineering Research Council of Canada, the Canada Foundation for Innovation, and the British Columbia Knowledge Development Fund.

Table 3.1: Values for parameters used in demographic models for the growth and survival of single cohorts of red sea urchins (*Strongylocentrotus franciscanus*) over a 20-year time span under both current and future CO_2 scenarios. All growth and morality rates were converted to weekly values.

Model Parameter	Value	Standard Deviation	Reference
L	122 mm	10 mm	Morgan *et al*. 2000
K (control)	0.28 $year^{-1}$	0 $year^{-1}$	Morgan *et al*. 2000
Growth rate reduction factor (Scenario A)*	0.68	0.3	This study
Growth rate reduction factor (Scenario B)*	0.49	0.43	This study
Z	0.1 $year^{-1}$	0.064 $year^{-1}$	Smith *et al*. 1998
F	0.3 $year^{-1}$	NA	Smith *et al*. 1998
Initial cohort size (t=0)	100 individuals	NA	N/A
Initial urchin size (t=0)	0.50 mm	NA	Ebert 2001
Minimum legal harvest size (Canada)	90 mm	NA	DFO 2001

* experimentally determined value for the relative reduction in sea urchin growth rate upon exposure to ~780 ppm CO_2 . Multiplied by K (control) to generate K (high CO_2) in the model.

Table 3.2: Output values (±SE) for the 20-year kelp consumption and urchin fishery models run for individual cohorts of 100 red sea urchins (*Strongylocentrotus franciscanus*) under current and future atmospheric CO_2 levels. Future scenarios were calculated by applying our experimental estimates of the relative reduction in urchin growth rates under a "high CO_2" scenario to the demographic model of urchin growth and survival.

Output Parameter	Control CO_2 Scenario (Current pCO_2 value ~380ppm)	High CO_2 Scenario A (Future pCO_2 value ~780ppm)	High CO_2 Scenario B (Future pCO_2 value ~780ppm)
Time to reproductive size (50 mm)[a]	1.89±0.02 years	2.86±0.12 years	3.31±0.18 years
Time to legal harvest size (90 mm)[b]	4.97±0.10 years	7.41±0.33 years	8.58±0.55 years
Total biomass of kelp consumed[c]	2760±140 kg	2030±140 kg	1210±150 kg
Biomass of 20-year catch[d]	19.70±0.85 kg	13.60±1.00 kg	7.05±1.00 kg

[a] H=89.16, df=2, $p < 0.0001$; [b] H=74.82, df=*2*, $p < 0.0001$; [c] H=36.96, df=2, $p < 0.0001$; [d] H=47.55, df=2, $p < 0.0001$

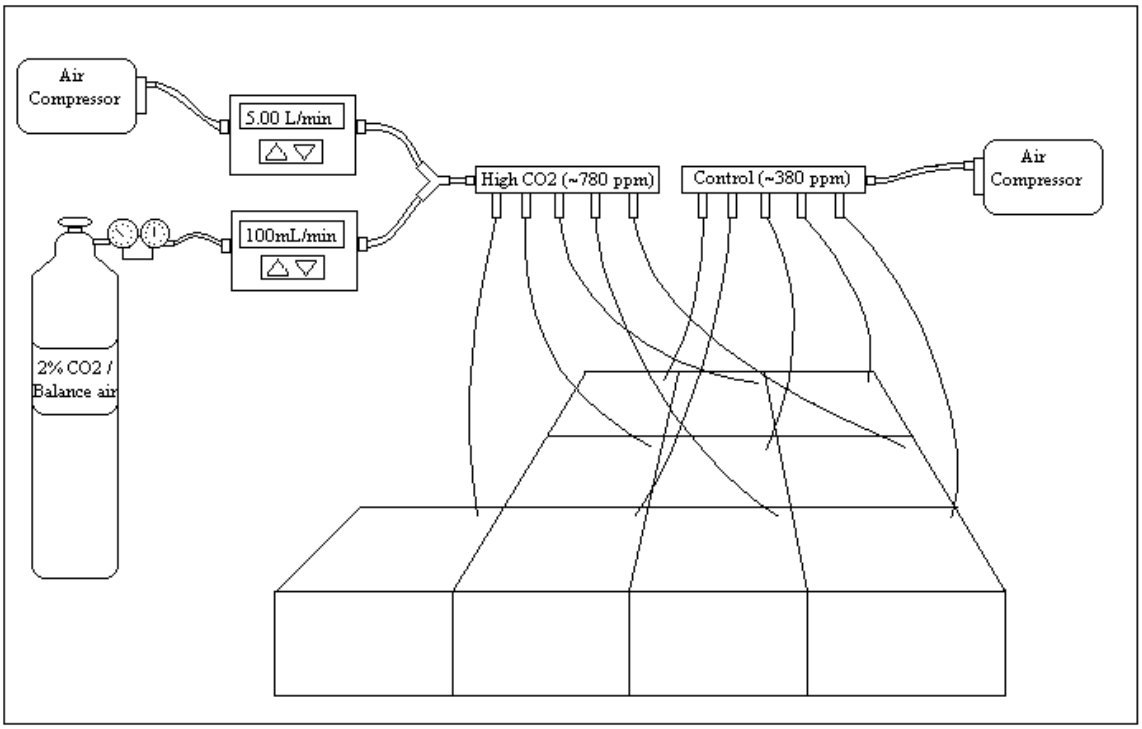

Figure 3.1: Schematic of experimental set up used to manipulate CO_2 levels and reduce pH in the seawater tanks. See text for explanation.

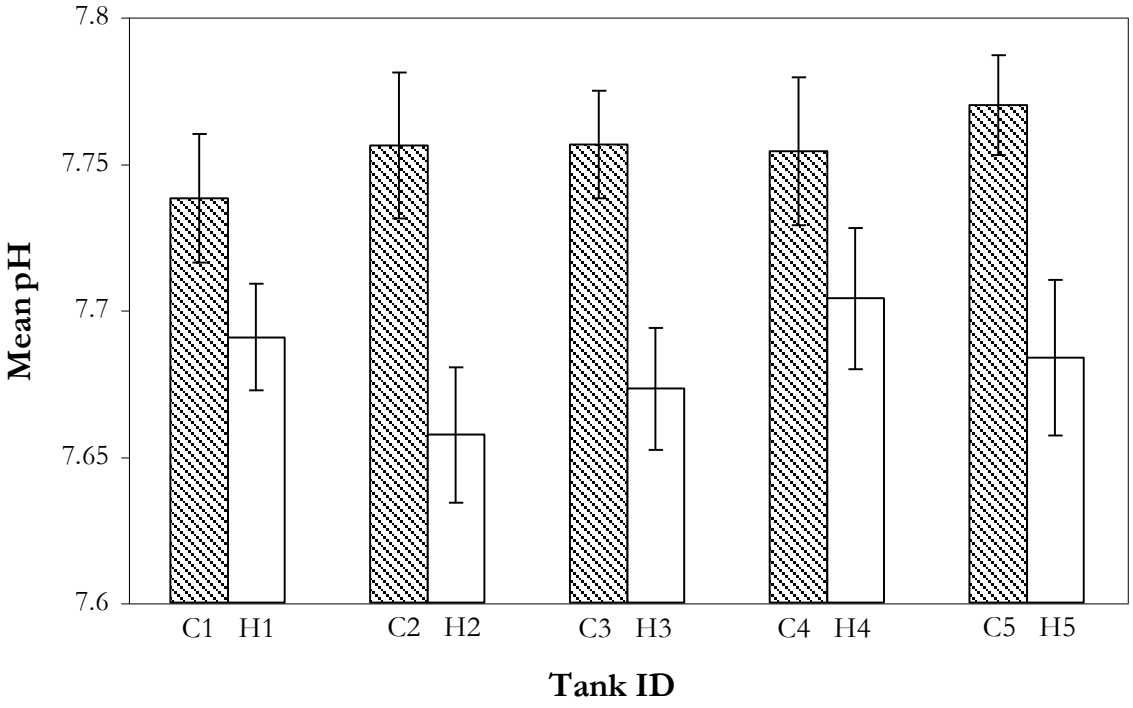

Figure 3.2: Mean pH of seawater in the control (cross-hatched bars) and high-CO_2 (white bars) tanks, calculated as the average of all measurements made over the 4-month duration of the experiment. Error bars represent the standard error of the means.

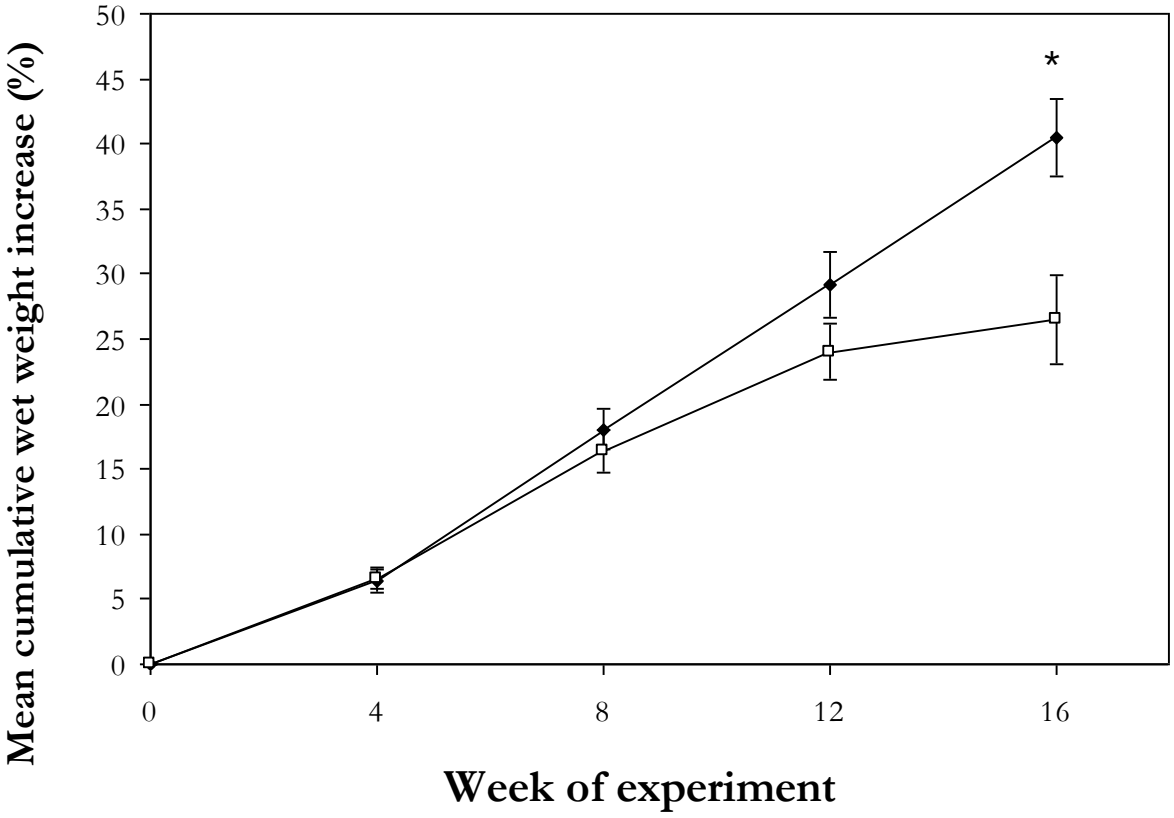

Figure 3.3: Mean change in the cumulative growth rate (expressed as percent of starting mass) of sea urchins (*Strongylocentrotus franciscanus*) grown under control (♦) and high-CO_2 (□) conditions, derived from wet weight measurements taken at monthly intervals throughout the duration of the 4-month exposure experiment. Error bars are the standard error of the means, and asterisks indicate significant differences (nested ANOVA: CO_2 treatment effect $F_{1,17}$= 10.2, p=0.0109; tank nested within treatment $F_{7,17}$= 2.00, p=0.164).

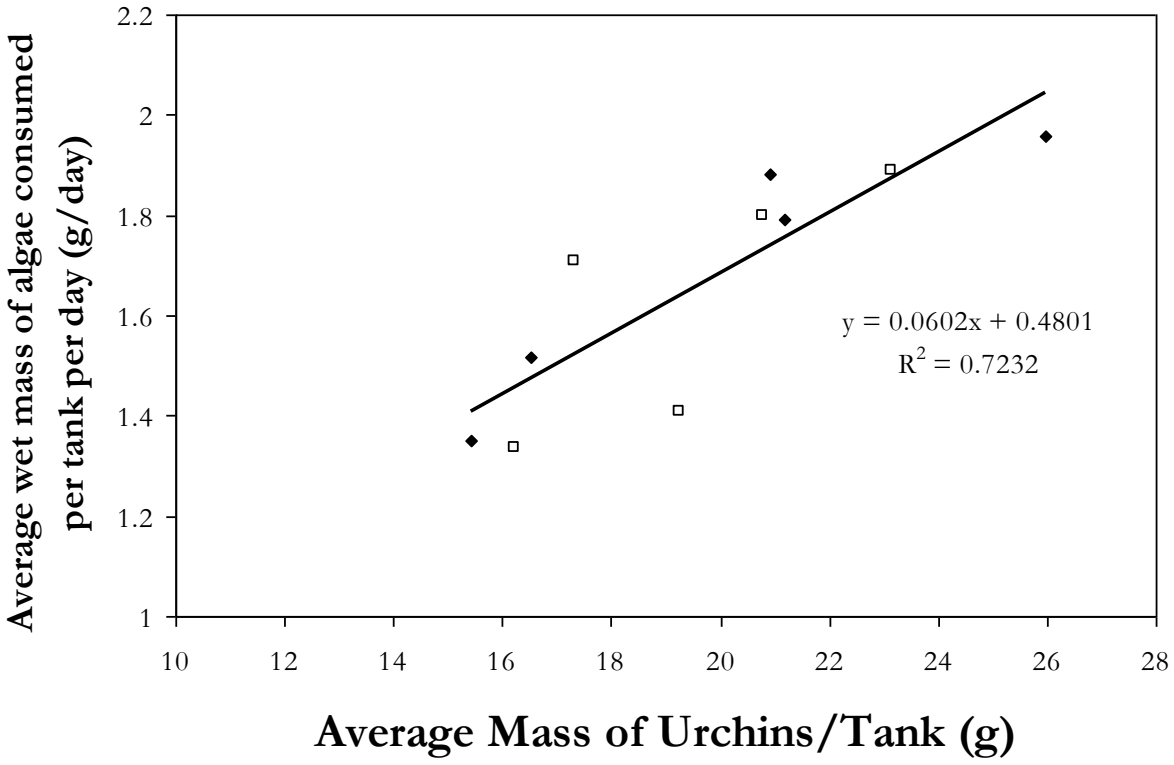

Figure 3.4: Relationship between the wet mass of juvenile sea urchins (average mass of the two urchins in each tank) and the daily average wet mass of giant kelp that they consumed. For the size class of urchins used in this experiment, the relationship was linear and was independent of whether urchins were exposed to control (♦) or high-CO_2 (□) conditions (see text for details).

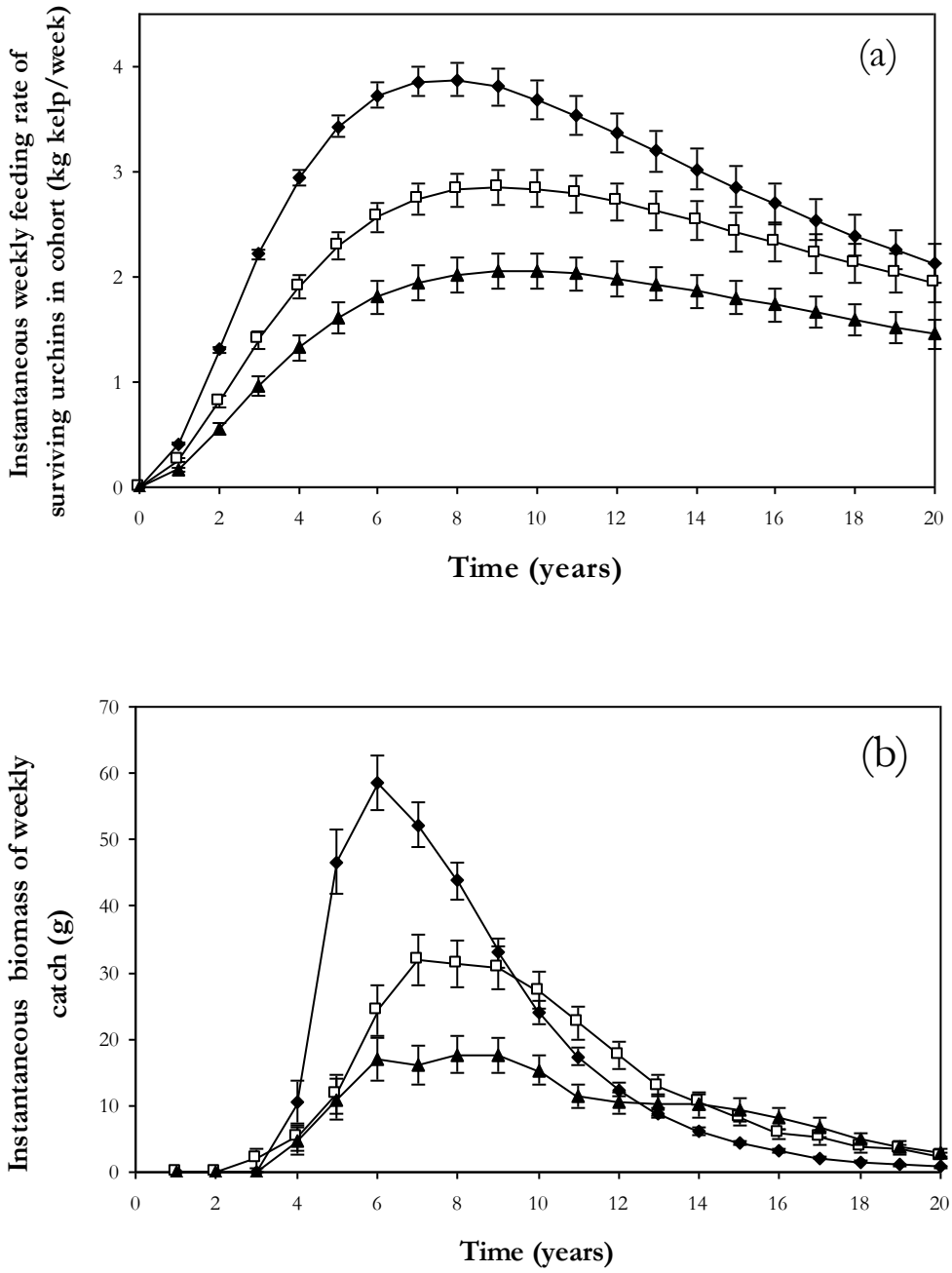

Figure 3.5: Graphical representations of (a) the kelp consumption model and (b) the harvestable urchin biomass model, showing the instantaneous weekly feeding rates of surviving urchins and the biomass of the weekly harvestable urchin catch, respectively, under current (♦) and future CO_2 scenarios A (□) and B (▲). Both models represent applications of a demographic matrix model which calculated the growth and survival of single cohorts of 100 newly settled urchins at weekly intervals over a 20-year time span, using published

demographic parameters for red sea urchins in the wild and our experimentally determined values for the overall relative reduction in growth rates of urchins exposed to 780 ppm CO_2 after 4 months (Scenario A), and for the more pronounced reduction observed between months 2 and 4 of the experiment (Scenario B). Error bars are the standard error of the means of 99 jack-knife iterations of each model, for which variance was calculated using the available estimates of variation for the model input parameters.

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CHAPTER 4: GENERAL CONCLUSIONS

SUMMARY: SIGNIFICANCE OF THE FINDINGS

The body of experimental work presented in this thesis has generated several important findings in the growing field of ocean acidification studies. When I began work on this thesis in the fall of 2006, there were relatively few published studies in the field. Of these, many were poorly or pseudo-replicated, examined only growth or survival of individual organisms, and often involved manipulating CO_2 concentrations to levels far exceeding those predicted of the next several centuries (IPCC 2001). Having here designed well-replicated exposure experiments using climatically relevant CO_2 levels, I was able to demonstrate not only individual-level growth effects for two different species of marine invertebrate, but also effects on relative rates of shell dissolution and deposition in a temperate marine mollusc. In addition, I used data collected at the individual level for an echinoderm species of both ecological and economic importance to upscale to the population level, and in so doing generated useful predictions about potential indirect impacts of elevated CO_2 on kelp forest resources and sea urchin fishery harvests.

The results of the study detailed in Chapter 2 provide insight into a potential mechanism that could explain the widely observed phenomenon of reduced growth rates in calcifying organisms exposed to elevated CO_2 levels. Specifically, they suggest that shell dissolution rates may play a more important role in dictating overall changes in the mass of calcium carbonate skeletons upon exposure to elevated CO_2 than shell deposition rates. This finding has the potential to change the way that researchers in the field define, measure and report changes in calcification and growth for calcifying organisms in CO_2 manipulation experiments. It is generally assumed that increases in CO_2 reduce calcification rates among shelled invertebrates. However, the results of this study indicate that at least for a temperate marine mollusc, while the overall mass of shell declined with increasing CO_2, after correcting for shell dissolution, the rate of shell deposition did not. This could represent the means to unify the apparently contrasting results of earlier studies, wherein several authors have recently reported increased calcification rates with increasing CO_2 despite the fact that the majority of authors report decreased calcification rates under similar conditions. As we have shown, it is critical to quantify the rates of both of the processes that dictate overall changes in shell mass

in order to properly report or discuss changes in biogenic calcification (ie. shell deposition) or shell dissolution rates. Clearly, the community of ocean acidification scientists will need to establish proper semantics in order to ensure consistency and allow for useful comparisons to be drawn among different studies.

What makes the work described in Chapter 3 particularly novel is the application of the experimentally determined estimates of the future CO_2-mediated reduction in urchin growth to a simple demographic matrix model in order to assess how the size distribution and survivorship of red sea urchin cohorts will change with time as CO_2 levels increase. Most critically, it was found that slower growth rates in urchins translate to a substantial delay in age of first reproduction and to reduced per-capita kelp grazing rates, which could have significant consequences for urchin population dynamics and kelp forest communities, respectively. This study highlights how reductions in individual growth can indirectly impact important population-level attributes such as time to first reproduction and reproductive output, as well as a species' interaction strength with respect to its prey species. The modelling approach taken here could easily be applied to other species, provided that the relative reductions in growth rates under various CO_2 concentrations are first established using well designed exposure experiments.

ADDITIONAL EXPERIMENTS AND SUGGESTIONS FOR FUTURE RESEARCH

In addition to the two studies detailed in this thesis, I conducted a number of other experimental studies which were not included here, but are worth briefly mentioning if not for the significance of their findings, then for their potential to serve as starting points or guides for future research. Among these was an examination of urchin shell chemistry after prolonged exposure to elevated CO_2, a study of the potential impact of increased CO_2 on urchin thermal tolerance, and an attempted assessment of the impact of elevated CO_2 on the growth and palatability of a common green algal species.

Impact of Elevated CO_2 on the Mg:Ca of Red Sea Urchin Tests

At the end of the 4-month exposure experiment with juvenile red sea urchins, all individuals were sacrificed and their hard parts separated from their soft tissue. I was interested in determining whether long-term exposure to elevated CO_2 and reduced pH would alter the

elemental composition of the urchin tests—specifically whether there was a difference in the relative abundance of Mg ions within the calcite crystal lattice of the control and the high-CO_2 urchin tests. Given that the relative solubility of calcium carbonate is directly proportional to the % of Mg incorporated into the mineral (Davis *et al*. 2000), and that the Mg/Ca of urchin tests and spines is known to vary spatially within individuals (Magdans and Gies 2004), I was curious to see if prolonged exposure to reduced calcite saturation states would cause a shift in urchin carbonate mineralogy towards less soluble, lower % Mg tests. Unfortunately, the cost of elemental analysis of the test material limited the number of samples that were sent out for analysis to just 5 from the control group and 5 from the high CO_2 group. Based on the results from this small sample size, we did not find any significant difference in the Mg/Ca ratio between treatments (ANOVA: $F_{1,8}$=1.81, p = 0.22), although there was a significantly higher molar % of both Mg (ANOVA: $F_{1,8}$=7.09, p = 0.03) and Ca (ANOVA: $F_{1,8}$=12.97, p = 0.009), and a lower percentage of volatilized material (ANOVA: $F_{1,9}$=10.47, p = 0.012), in the high treatment tests relative the controls. These results imply that there may have been a lower proportion of organic matrix in the high CO_2 shells, though the implications of these findings are unclear, and would require further elemental analysis to resolve.

In two independent studies, Mg/Ca ratios were found to decrease with decreasing pH in the calcite tests of two different species of foraminifera (Lea *et al*. 1999; Russell *et al*. 2004), while a more recent study on a third species showed no change in Mg/Ca with changes in the carbonate ion concentration of the culture medium (Dissard *et al*. 2009). While the results of our analyses may be consistent with the latter study, a larger sample size, as well as additional CO_2 treatment levels would be required before making any conclusions about the effect of increased CO_2 on the proportion of Mg incorporated into urchin skeletal structures. Had I been able to resolve these issues, there could have been some very interesting ecological implications from such a study. Namely, while the incorporation of Mg ions within calcite crystals increases the inherent solubility of the biomineral, it also increases its strength and resistance to breakage due to the distortion of the crystal lattice that results when smaller Mg ions are substituted in place of Ca ions within the crystal structure, which impedes cleavage along the lattice. For this reason, the bases of urchin spines typically contain a higher proportion of Mg ions than the much more commonly broken tips (Magdans and Gies 2004). That is, there appears to have been selection to increase the Mg:Ca ratio of calcite structures

that would be most energetically expensive to re-grow upon breakage, despite the trade off of these structures having a lower saturation state in seawater. However, if chronic exposure to elevated CO_2 and lower calcite saturation states leads to selection against more soluble, higher Mg calcite skeletal structures, the resulting spines, tests and teeth of urchins would be weaker, and more susceptible to predation from test-crushing fish, for example. Further investigations of shell chemical properties under elevated CO_2 conditions are certainly warranted, as very little information currently exists for organisms other than planktonic foraminiferans.

Impact of CO_2 Acclimation Concentration on the Critical Thermal Maxima of Juvenile Red Sea Urchins

I ran a second exposure experiment with juvenile red sea urchins in order to assess whether acclimation to different CO_2 concentrations would alter their behavioural response to thermal stress. Given that marine organisms will have to contend with changes in multiple climate parameters over the coming century which will include both decreased pH and increased temperatures, a study of this kind seemed both timely and relevant. Assuming that prolonged exposure to elevated CO_2 levels could impose physiological stress upon urchins, it was predicted that their ability to tolerate additional stress in the form of elevated seawater temperature would be compromised, as manifested by lower critical thermal maxima. 10 juvenile urchins were acclimated to each of three seawater CO_2 concentrations including current CO_2 levels (380ppm), 2X current levels (780ppm) and 4X current levels (1580ppm) for a period of 1 week. After acclimation, urchins were exposed to rising thermal stress, using aquarium heaters to induce to a rapid and steady increase in temperature (0.4ºC/min) from 13ºC to 34ºC. Observing the urchins closely during the temperature ramp, I recorded the temperatures at which each urchin exhibited a progressive series of behavioural responses as defined and characterized for this species at increasing temperature increments by Hernandez *et al*. (2004); retraction and decreased movement of tube feet, decreased spine movement, no movement of any kind and death. According to that 2004 study, the CTMax of urchins corresponded with the temperature at which the sea urchins stopped moving altogether.

I found no effect of acclimation CO_2 concentration on the CTMax of juvenile sea urchins as defined by observable behavioural responses to thermal stress. This suggests that exposure to moderate increases in seawater CO_2 concentrations may not impair the ability of urchins to respond to increases in seawater temperature. The results of the previous study with

red sea urchins, which demonstrated no effect of CO_2 on feeding rates or assimilation efficiency, also indicate that a climatically relevant increase in CO_2 may not impact the feeding behaviour or digestive processes of urchins. This leads to a tentative hypothesis that the primary effect of ocean acidification on species like the red sea urchin will be reduced calcification and/or growth rates owing to decreased calcium carbonate saturation states, rather than metabolic depression or physiological stress.

While there was no effect of CO_2 on the behavioural response of juvenile red sea urchins to thermal stress, there was an effect of urchin size (diameter) on the CTMax measured in this way. Using least squares regression, the relationship between urchin CTMax (°C) and diameter (D) was found to be significant ($F_{1,29} = 6.83$, $p = 0.014$, $R^2 = 0.20$) and linear for the small size range (21-52 mm) of urchins used in this study ie.

$$\text{CTMax} = 30.89 + 0.021\ \text{D}$$

That is, the critical thermal maximum, defined as the "thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from a condition that will promptly lead to its death when heated [further]"(Cox 1974) decreases with decreasing urchin size. This result alone is not all that surprising given that it would take longer for the deep body tissues of larger animals to heat up to environmental temperatures than it would for smaller individuals. However, when considered alongside our results from the previous urchin study in which higher CO_2 concentrations led to slower urchin growth rates, this finding suggests that ocean acidification could indirectly effect the ability of sea urchins to tolerate thermal stress by virtue of it taking longer for urchins to reach larger, more heat tolerant sizes. Such potential impacts may prove detrimental when organisms are exposed to natural heating cycles such as those that may occur in tidepools, or when a layer of warm water overlaying cooler waters descends onto an animal as the tide goes out.

Owing to the rather subjective definitions of CTMax employed in this study, it is difficult to draw any strong conclusions from the results. For this reason, the study was not prepared for publication. In retrospect, it would have been much more appropriate to employ molecular genetic techniques to quantify the expression levels of heat shock proteins, known to be differentially induced by organisms during periods of physiological stress, thermal or

otherwise. Employing gene expression assays in this way, O'Donnell *et al*. (2009) successfully demonstrated that red sea urchin larvae raised under elevated CO_2 conditions (540 and 970 ppm) suffered a reduced ability to mount a physiological response to acute temperature stress relative to those raise under control CO_2 conditions.

Because the changes in ocean chemistry induced by increased anthropogenic atmospheric CO_2 concentrations are expected to occur in concert with increased sea surface temperatures in many areas, experimental studies designed to investigate the potential synergistic effects of elevated CO_2 and temperature on the physiology, growth, behaviour and survival of marine species will be key to enhancing our understanding of the response of different species to changes in multiple climate parameters.

Impact of Elevated CO_2 on the Growth and Palatability of a Common Marine Algal Species

In the summer of 2007, I set-up an outdoor CO_2 exposure experiment consisting of a complex series of header tanks into which air containing two different levels of CO_2 was bubbled and through which a continuous supply of seawater was slowly introduced. Individual thalli of the common green algae *Ulva lactuca* were preweighed and glued by their holdfasts to small rocks and were then placed in the experimental tanks supplied seawater with differing nominal pH from the header tanks. The purpose of this experiment was to culture the algae under 2 different CO_2 levels and assess any changes in growth rates (as measured by changes in blotted wet mass) and palatability (as measured by feeding preference trials with a common generalist grazer, *Littorina scutulata*) after 5 weeks of exposure.

It is commonly held that, unlike terrestrial plants, most marine algae will not benefit from CO_2 enrichment since they use bicarbonate ions rather than CO_2 as a substrate for photosynthesis (Gattuso and Buddemeier 2000) when submerged. I wanted to test this hypothesis by measuring relative growth rates of the algae grown under current and a doubling of current CO_2 concentrations. Furthermore, the production of a variety of algal secondary metabolites, which often serve as chemical defenses to deter grazers, can be induced by changes in seawater chemistry including salinity and nutrient concentration (Connan *et al*. 2004). I was therefore interested to see whether changes in seawater pH could similarly alter the palatability of the algae for a common generalist grazer. Had I found differences in feeding

preference between the algae from different culture conditions, I would then have quantified the levels of herbivore deterrent compounds in those algal samples.

Despite my best efforts to correct for them, the experiment suffered from a number of complications that rendered any of the findings unpublishable. Not only did it prove extremely difficult to culture the chosen species of algae in the experimental tanks (individuals were found to decay and break over time, which confounded the growth measurements) but more importantly, it was found that the pH in the experimental tanks containing *Ulva* experienced dramatic temporal changes independent of CO_2 treatment owing to the respiration and photosynthesis of the algae itself. While unsuccessful, this study highlighted the ability of marine plants to induce large changes in the CO_2 concentrations of their surrounding media when the volume of seawater they inhabit is small, as is the case in tide pools, for example. It would therefore prove interesting and relevant to investigate the adaptive mechanisms employed by common tide pool species which allow them to tolerate and compensate for frequent, though short-term, changes in pH which are commonly much greater than any predicted of surface seawater for the next several centuries.

In the months following this experiment, a paper detailing a study very similar to the one that I had conducted was published which demonstrated the ability of elevated CO_2 to induce phlorotannin production in two species of kelp, which subsequently reduced the amount of high-CO_2 exposed tissue eaten by an herbivorous snail (Swanson and Fox 2007). To date this represents one of the very few studies have sought to examine the effects of ocean acidification on the chemical ecology of marine macroalgae. This line of experimental work, if successfully implemented, is extremely relevant to enhancing our understanding of whether changes in seawater [CO_2]/pH could potentially alter chemical defence-mediated plant-herbivore interactions. As the results of the Swanson and Fox paper suggest, higher CO_2 conditions in the future could differentially benefit certain algal species, with the potential for shifts in the abundance and distribution of species within benthic macroalgal communities.

ADVICE FOR THE SUCCESSFUL IMPLEMENTATION OF CO_2 MANIPULATION STUDIES

Having designed and conducted several extensive CO_2 manipulation experiments throughout the course of my thesis work, and having had to overcome a number of obstacles in

their set-up and maintenance, there are several critical aspects that I would suggest that any future students in the field consider before initiating their own similar studies.

First, when attempting to measure the effect of increased CO_2 on growth or calcification rates, the duration of exposure is often the most important aspect of the experimental design. For certain species, such as some corals, it has been reported that decreases in calcification rates in response to changes in the carbonate system are immediate and reversible (Marubini and Atkinson 1999) and are not significantly different between short-term (ie. days) and long-term (ie. months) exposure to elevated CO_2 (Langdon *et al*. 2000). This might suggest that the duration of exposure experiments is inconsequential. However, the ability to detect potential differences in calcification or growth rates between experimental treatments will depend on the size and relative growth rates of the experimental organism, as well as on the precision and accuracy of the instruments used to measure this effect.

In the experiment with *Nucella*, we were able to detect differences in shell weight changes over an exposure period of several days, owing both to the precision of the weighing protocol employed and to the fact that a) shell mass makes up a significant proportion of total mass in this species and b) this species deposits shell at an easily measurable rate. In contrast, for the experiment with juvenile sea urchins, it took more than three months before a significant growth effect of elevated CO_2 was observed. Though this may have in part been due to the inability to attain high-precision measurements when weighing such large animals (and therefore to detect small differences in growth), it may also reflect the potential of certain calcifiers to maintain high rates of calcification despite being exposed to increases in CO_2 (Wood *et al*. 2008) at least over the short-term. Clearly, if growth is the effect being measured in such experiments, it is important to consider both the characteristics of the experimental species and the available instrumentation in order to determine the appropriate length of time for the exposure experiment.

Secondly, because the physicochemical characteristics of seawater dictate calcium carbonate deposition and dissolution rates, it is critical to carefully measure as many seawater properties as possible in all of the experimental tanks and treatments, as often as possible. When I began work in this field, very few published studies existed, and it was a challenge even for me to design and initiate a working experimental set up for the manipulation of CO_2 levels in seawater. Since then, hundreds of CO_2 manipulation studies have been published,

prompting the recent release of several reports detailing guidelines and standards to ensure the successful implementation and consistent reporting of such studies (e.g., Dickson *et al*. 2007; Riebesell *et al*. 2009). These reports highlight the importance of a fully constrained and accurate characterization of the inorganic carbon system in seawater when conducting CO_2 manipulations. This requires the proper measurement of at least two of the CO_2 related parameters in seawater: total dissolved inorganic carbon (C_T), total alkalinity (A_T), the concentration of dissolved CO_2 [CO_2], and the hydrogen ion concentration [H+] (as calculated from pH). As long as at least two of these components are defined, and the required solubility and equilibrium constants for the chemical species of interest at a given temperature and pressure are known, a mass balance approach can be taken to calculate the remaining components of the system. The primary weakness of the experimental work presented in this thesis is that by measuring only pH, I was not able to fully characterize the carbonate chemistry of the seawater in my CO_2 manipulation experiments. This is obviously an area upon which improvements could be made in the future.

CONCLUSIONS

Through extensive literature review and the undertaking of a diversity of experimental studies over the course of my graduate work, I have developed a broad yet thorough understanding of the wide range of potential impacts of ocean acidification on marine organisms and communities. From an examination of calcification and growth effects in marine invertebrates, to impacts on feeding behaviour and thermal tolerance, to experiments assessing potential impacts on shell chemistry and the relative contributions of calcium carbonate dissolution and deposition in dictating observed reductions in shell mass, to scaled-up model predictions of population and community level changes, my investigations have led me on a well-rounded exploration of some of the more critical consequences of ocean acidification.

While several of the exposure experiments that I conducted were unsuccessful, the questions that I was attempting to answer in designing them were certainly valid and, for the most part, still have yet to be answered. This body of work comprises but a small chapter in the multi-volume collection of studies currently being undertaken by scientists worldwide who are frantically attempting to asses and predict the potential havoc that recent rapid changes to the

long-established fundamental carbonate chemistry of the sea surface could wreak on ocean ecosystems. It is hoped that the experimental findings described herein will shed some light on our understanding of how marine organisms, communities and ecosystems will respond to such changes in the centuries to come.

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