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Impact of Ocean Acidification on carbonate production by large benthic foraminifera Marginopora vertebralis in coastal waters of Fiji

by

Roselyn Naidu

A thesis submitted in fulfillment of the requirements for the degree of Master of Science in Marine Science

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School of Marine Studies
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October, 2015
DECLARATION

Statement by Author

I, Roselyn Naidu, declare that this thesis is my own work and that, to the best of my knowledge, it contains no material previously published, or substantially overlapping with material submitted for the award of any other degree at any institution, except where due acknowledgement is made in the text.

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ABSTRACT

Increased CO₂ emissions into the atmosphere lead to increased concentrations of dissolved CO₂ in the ocean. A chemical reaction between the dissolved CO₂ and seawater produces HCO₃⁻, CO₃²⁻ and H⁺ ions. These H⁺ ions increase the acidity of seawater and decrease the pH. Increased acidity and decreased availability of CO₃²⁻ ion affect calcite and aragonite production by marine calcifiers in the ocean. Large benthic foraminifera, such as Marginopora vertebralis Quoy and Gaimard, 1830, produce calcite with high magnesium (Mg) content and have an important role in sand building in the Pacific Islands. Foraminifera also have the ability to integrate trace elements and isotopes from the seawater into their calcareous shells and serve as paleoceanographic archives. It is thus important to better understand the biomineralization processes in foraminifera to predict their calcification responses to ocean acidification.

To assess the response of a porcelainous benthic foraminifer with algal endo-symbionts to changing CO₂ levels, M. vertebralis were cultured at pH 7.5, pH 7.8 and pH 8.1 (ambient seawater). The fluorescent compound Calcein (40 μM) was added to the culture tanks to mark the calcite produced prior to the experiment. The specimens grown in the laboratory were analysed using Laser Ablation-Inductively Coupled Plasma Mass Spectroscopy (LA-ICPMS) and Electron Probe Micro Analyser (EPMA) to measure elemental compositions, providing data to calculate boron and strontium isotope ratios and Mg/Ca, Sr/Ca and S/Ca ratios. Shell growth, in terms of both radius and weight, decreased with decreasing pH, as did the boron isotope ratios. The Mg/Ca ratios decreased, while Sr/Ca ratios increased, with decreasing pH. The S/Ca ratio also increased with a decreasing pH. Isotopic ratios for B (δ¹⁰/¹¹B) and Sr (δ⁸⁸/⁸⁶Sr) both increased with decreasing pH. Since it is possible to calibrate the shell composition against the controlling factors, foraminiferal trace elements and isotopic ratios can provide researchers with vital and relevant proxies to investigate the physical, biological and chemical changes in the ocean.
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CHAPTER 1 INTRODUCTION

1.1 AIM

The primary goal of this thesis was to study the impact of declining pH (acidification) of seawater on calcification of Marginopora vertebralis Quoy and Gaimard, 1830, a reef-dwelling species of Foraminifera.

OBJECTIVES

- To experimentally determine the response of M. vertebralis to increased ocean acidification.
- To determine how selected elemental compositions and elemental ratios in shells of these foraminifera change in response to increased ocean acidification.
- To better understand how ocean acidification will affect calcification and calcification rates.

HYPOTHESIS

The hypothesis of the study is that the increased partial pressure of carbon dioxide ($pCO_2$) in the atmosphere leads to increased $pCO_2$ in the ocean, which causes increased acidification of seawater, thus decreasing the calcification rate and altering the shell chemistry in the large benthic foraminiferal species, Marginopora vertebralis.
1.2 INTRODUCTION

Ocean acidification (the decrease in pH, and subsequently in the carbonate ion concentration in seawater) in response to rising anthropogenic atmospheric $p$CO$_2$ is generally expected to reduce calcification of the main marine calcifiers, with potentially severe implications for coral-reef ecosystems (Kleypas et al., 1999). Algal symbiont-bearing, reef-dwelling foraminifera are one of the most important primary carbonate producers in coral reefs (Muller 1974; Smith and Wiebe 1977; Hallock, 1981). They mainly produce high-Mg calcite shells, whose solubility can exceed that of aragonite produced by corals, making them among the “first responders” in coral reefs to the decreasing carbonate (CO$_3^{2-}$) saturation state of seawater (Fujita, 2008). As acidity and sea temperature increase, the ocean’s actual ability to absorb atmospheric CO$_2$ is being reduced, thus exacerbating the rate of climate change. The rate at which the acidification of the ocean occurs depends mainly on the chemical exchange reactions with the atmosphere. Gaining knowledge of ocean conditions in the past enhances understanding of the present climate and also provides insight into future climate changes (Feely et al., 2009). “Proxies” (often assessed using foraminiferal shells) are used to develop methods to obtain information about oceanic changes.

Because $M$. vertebralis is an important source of carbonate sediments in Lauca Bay, Fiji, we assessed its potential responses to ocean acidification in a laboratory study of the effects of reduced pH on shell growth and shell chemistry using stable isotope composition and elemental ratios.

The structure of this thesis is to first present, background information on ocean chemistry, foraminiferal shells as indicators of ocean chemistry, and experimental methods utilized in previous studies of changes in ocean chemistry on calcareous organisms. Chapter 2 presents the methods and results of an experimental study of the influence of seawater pH on growth and calcification of $M$. vertebralis. Chapter 3 describes the carbonate chemistry of the cultured medium. Chapter 4 presents analyses of elemental ratios using EPMA (Electron Probe Micro Analyzer). Chapter 5 presents the results of analysis of elemental concentrations using LA-ICPMS (Laser Ablation
Inductively Coupled Plasma Mass Spectrometry). Chapter 6 presents a synthesis of the results from Chapters 2–5 and discussion of their significance, in context of previous studies and projection of future ocean acidification, concluding with suggestions for future studies.

1.3 BASICS OF OCEAN CHEMISTRY

Carbon dioxide is essential for life on Earth, including life in the ocean. The carbon cycle (Fig 1.1) is described as a biogeochemical cycle by which carbon is recycled and reused throughout the land, the oceans and the atmosphere. The major reservoirs of carbon include: the atmosphere, the terrestrial environment (including living organisms, fresh-water ecosystems and non-living organic matter), the ocean (dissolved inorganic carbon, marine biota, and non-living forms of organic carbon), the sediments and sedimentary rocks (including fossil fuels) and the Earth’s interior. The marine carbon cycle includes the production and recycling of organic material and carbonate sediments and rocks (CaCO$_3$).

Figure 1.1: Carbon cycle with different reservoirs where carbon is stored
(http://spark.ucar.edu/imagecontent/carbon-cycle-diagram-nasa)
Increasing atmospheric carbon dioxide is causing a significant decline in seawater pH as a result of the imbalance between the rate of CO₂ uptake into the ocean and the ocean’s ability to buffer the resulting increase in hydrogen ions (Caldeira and Wickett, 2003). Since the industrial revolution, seawater pH has declined by an average of 0.1 units (Kleypas et al., 2006; IPCC, 2013); pH is the negative logarithm of the hydrogen ion concentration (Fig 1.2). It has been estimated by IPCC (2013) that the pH will decrease from a current global average of approximately 8.1 to as low as 7.7 by 2100.

![Figure 1.2: The pH Scale (modified from Ophardt, 2003)](image)

### 1.3.1 Seawater Carbonate Chemistry

The carbonate system controls the acidity of seawater and acts as a regulator for the carbon cycle. The carbonate system of the ocean plays a key role in controlling the partial pressure of carbon dioxide in the atmosphere, which further helps in regulating the temperature of the globe.

Seawater pH is a key parameter that reflects the speciation of the carbonate system, nutrients and other major and trace-element species in the oceans. It is largely unknown if, or how, various organisms will adapt to the large-scale pH changes that are
anticipated over the next two to three centuries. At present it is not possible to determine how the ecosystem structure will change or how these ecosystem changes might influence future climate-feedback mechanisms. It is therefore important to develop new research strategies to better understand the long term vulnerabilities of sensitive marine organisms to these changes (Kleypas et al., 2006). Seawater acidification not only affects the ecosystem components that are CO$_2$ sensitive, but also affects those components that interact with them as epibionts, predators, preys and competitors (Langer et al., 2006).

Calcium carbonate production in the ocean is mostly biogenic. Photic zones have the highest biogenic calcification rates, while the highest dissolution rates occur in the deep ocean (Kleypas and Landon, 2006).

![Diagram of CO$_2$ absorption and chemical reactions](image)

Figure 1.3 The absorption of CO$_2$ and the chemical reactions that occur within the marine environment (Modified from Kleypas et al., 2006)
Four processes that are responsible for the uptake of atmospheric CO₂ into the ocean are: (i) air-sea exchange of CO₂ gas and formation of carbonic acid, which later dissociates to form bicarbonate and carbonate ions; (ii) production of CaCO₃ (calcification) and its dissolution; (iii) photosynthesis and respiration; and (iv) ocean circulation (Fig 1.3).

1.3.2 IMPORTANCE OF CARBON SINKS IN CHANGING CLIMATE

Processes that remove greenhouse gases from the atmosphere are called ‘sinks’ and processes that put greenhouse gases in the atmosphere are called ‘sources’.

Net emissions = \((\text{emissions from sources}) - (\text{removal by sinks})\)

Over the two decades of the 1980s and 1990s, only about half of the CO₂ released by human activity has remained in the atmosphere, with the oceans having taken up about 30% and the terrestrial biosphere 20% (Sabine et al., 2004). Similar partitioning of anthropogenic CO₂ is expected to continue with the result that the partial pressure of CO₂ \((p\text{CO}_2)\) dissolved in the surface ocean is likely to double its pre-industrial value within the next 50 years. Over the next millennium, the ocean will absorb about 90% of the anthropogenic CO₂ released to the atmosphere (Archer et al., 1989). Deep-ocean injection of CO₂ extracted from gases released from industrial and renewable energy (biogas) activities could potentially mitigate global warming by removing CO₂ from its sources and pumping it in the form of gel liquid into sea-floor depressions where it could dissolve over millennial time scales (Haugan and Drange, 1992).

1.3.3 CLIMATE CHANGE AND ITS IMPACTS

According to Nunn (2007), the people of the Pacific Island countries are likely to face climate changes that will threaten the sustainability of their livelihoods. This will occur through: a) increased climate variability such as amplification of the present ENSO pattern that sees El Niño occurring every 3–5 years with the likelihood that there will be less seasonal rain in the future; b) changes in climatic extremes, such as the recent increased incidence of intense tropical cyclones; c) temperature rise that will impact terrestrial ecosystem productivity and near shore ecosystems, particularly coral reefs; and d) sea level rise that will cause coastal inundations, shoreline erosion, groundwater
salination in low-lying areas, and allow more large amplitude waves to cross offshore reef barriers than at present.

The greenhouse effect causes the increase in surface temperature where the thermal radiation from the Earth’s surface is absorbed by atmospheric greenhouse gases (GHG) (water vapor, CO₂, N₂O, and CH₄) and is re-radiated in all directions. Consequently, further global warming is projected with an increase in global average temperature between 1.4°C – 5.8°C and rise in sea-level of 20–60 cm by the end of this century, 2100 (IPCC, 2013). Over the last 420,000 years, the global atmospheric concentration of CO₂ fluctuated naturally between 180 parts per million (ppm) and 280 ppm in glacial and interglacial conditions, respectively. In the last 200 years, since the industrial revolution, atmospheric CO₂ has increased dramatically, recently reaching 400 ppm (Fig 1.4). Both GHG emissions (CO₂) and the global mean temperature are consistent with the highest predictions rather than the middle of the range (IPCC, 2013). Thus some effects that, in 2000, were projected to occur in about 2020 are already being observed now.

Figure 1.4 CO₂ and temperature over the last 420,000 years (from IPCC Fourth Assessment Report, WG4, 2007). The current global concentrations of CO₂ in the atmosphere (~400 parts per million) are the highest in the last 420,000 years.
1.3.3.1 Contribution of atmospheric carbon dioxide to ocean acidification

Carbon dioxide (CO₂) is one of the most important gases in the atmosphere, affecting the radiative heat of the Earth as well as the calcium carbonate (CaCO₃) equilibrium of the oceans. As the amount of CO₂ dissolved in the ocean increases, the pH decreases, as does the availability of carbonate (CO₃²⁻) ions, which lowers the saturation state of the major carbonate minerals found in shells and skeletons of marine organisms. Tripling the pre-industrial atmospheric CO₂ concentration will cause a reduction in surface ocean pH that is almost three times greater than that experienced during transitions from glacial to interglacial periods. This decrease in available alkalinity is often termed “ocean acidification” because it leads to decreasing pH (Caldeira and Wickett, 2005).

Atmospheric CO₂ concentrations remained between 180 and 330 parts per million by volume (ppmv) for 650,000 years prior to the Industrial Revolution (Siegenthaler et al., 2005). Increased fossil-fuel burning associated with industrialization, as well as cement production and land-use changes associated with agricultural activities, are causing atmospheric CO₂ concentrations to rise at increasing rates (rates of increase have risen from 0.25% y⁻¹ in the 1860s to 0.75% y⁻¹ in recent years). Atmospheric CO₂ concentration is expected to continue to rise by about 1% y⁻¹ over the next few decades (Houghton, et al., 2001). The rate of current and projected CO₂ increase is about 100 times faster than what has occurred over the past 650,000 years, and the rise in atmospheric CO₂ levels is considered irreversible on human timescales (Dooney et al., 2009). Even with immediate action, the reaction time of the ocean will result in a declining pH in the oceans (Liu et al., 2010).

According to Steinacher et al. (2009), the issue of ocean acidification is linked with the saturation state of aragonite, which controls the growth of corals. Steinacher et al. found that the largest variation of pH occurs in the Arctic surface waters, where the hydrogen ion has increased 185% (ΔpH= -0.45) due to freshening and increased carbon uptake in response to sea-ice retreat.
1.3.3.2 Implications for ocean acidification and carbon sequestration

It is predicted that, if the current rate of CO$_2$ release into the atmosphere continues, by the year 2100 the pH of the surface ocean will be lowered by 0.4 units compared to the pH level at the start of the century, with a likely reduction of 0.7 units by the year 2250 (Caldeira and Wickett, 2003; IPCC 2013).

1.4 FORAMINIFERA

1.4.1 Classification of Foraminifera

Foraminifera are testate protists, i.e., single-celled organisms that produce a shell. The name Foraminifera comes from “foramen”, which is the opening through the septum that separates each chamber (University College London, 2002). This opening gives this protozoan class its name.

Foraminifera have inhabited the oceans for more than 500 million years (Boudagher-Fadel, 2008). Their shells maybe one chamber, though many have shells that are divided into multiple chambers. These chambers are added during the growth of an individual (Sen Gupta, 2003). Foraminifera have historically been classified based on their shell structure. Three basic types of wall composition common amongst living foraminifera are: a) the agglutinated shell, which can either be composed of very small particles cemented together and have very even surface or be made up of larger particles and have an uneven surface; b) the hyaline perforate shell, made of interlocking crystals of calcite measured in micrometers, with glassy appearance and abundant pores; and c) porcelaneous imperforate shell, made of microscopic randomly oriented rod-shaped crystals of high magnesium calcite, resulting in a milky appearance (e.g., Loeblich and Tappan, 1964; Wetmore, 1995; Sen Gupta, 2003). Due to the abundance of well-preserved shells, foraminifera are very useful in analyzing recent and ancient marine environments (Murray, 2002).

Two other features that are important when it comes to classification of the Foraminifera are chamber arrangement and aperture style (University College London, 2002). The common types of chamber arrangements are unilocular (shells with only a single
chamber) and multilocular (shells with several chambers) (Fig 1.5). Among the multilocular arrangements there are the uniserial (the chambers are attached in a single straight line); biserial (the chamber are attached in straight line but in pairs); triserial (linear series in which chambers are attached in triplets); planispiral (the chambers take up a coil shape); trochospiral (the coiling of chambers takes up the shape of a spire); milioline (each chamber is added at an 180° angle from the previous chamber) (e.g., Loeblich and Tappan, 1964; Wetmore, 1995; Sen Gupta, 2003).

Figure 1.5 Principal types of chamber arrangement (University College London, 2002). 1, single chambered; 2, uniserial; 3, biserial; 4, triserial; 5, planispiral to biserial; 6, milioline; 7, planispiral evolute; 8, planispiral involute; 9, streptospiral; 10-11-12, trochospiral; 10, dorsal view; 11, edge view; 12, ventral view. (Loeblich and Tappan 1964).
Apertures link the external granuloreticulopodia (temporary projections of the cytoplasm that are used for locomotion and ingesting food) with the internal endoplasm (Sharma, 2007). Apertures are typically found in the walls of the final chamber (Fig 1.6). Since the aperture does not change position through ontogeny, each chamber is connected to the next by a single (foramen) or several opening (foramina) that were originally apertures (University College London, 2002).

Figure 1.6 Principal types of aperture (University College London, 2002). 1, open end of the tube; 2, terminal radiate; 3, terminal slit; 4, umbilical; 5, loop shaped; 6, interiomarginal; 7, interiomarginal multiple; 8, areal crbrate; 9, with phialine lip; 10, with bifid tooth; 11, with umbilical teeth; 12, with umbilical bulla. (Loeblich and Tappan 1964).
Fully grown individuals range from about 0.1 mm to almost 20 cm in size (Wetmore, 1999; Sen Gupta, 2003). A single individual may have one or many nuclei within its cell. Many of the largest living species in the warm subtropical seas have symbiotic relationships with algae. Most taxa, including those with algal symbionts, eat foods ranging from dissolved organic molecules, bacteria, diatoms and other single-celled phytoplankton, or small animals such as copepods (Wetmore, 1999).

Both planktonic and benthic species are sensitive to changes in food availability as well as to physical and chemical environmental parameters, such as salinity, nutrient content and temperature (Polyak et al., 2001). Because of this sensitivity, foraminifera are useful indicators of environmental change, both on the local and global scales.

Foraminifera are well known for their excellent fossil record which is used in biostratigraphic, paleoenvironmental, paleoceanographic and paleoclimatic reconstructions (Pawlowski et al., 2003). In biostratigraphy, foraminiferal species or assemblages are used to determine relative ages and to correlate sedimentary rock units across regions or even globally. For paleoenvironmental interpretations, fossil assemblages can indicate the environmental conditions under which sediments were deposited. Past surface and bottom water temperatures have been mapped from the measurements of stable oxygen isotopes in planktonic and benthic foraminiferal shells (Wetmore, 1999). Together with foraminifera, other marine organisms such as diatoms and testate amoebae can be used as sea level indicators (Allen, 2005). Gehrels (2001) used a multi-proxy method to obtain more precise estimates of sea level change. According to Lieberman (2000), ecological paleobiogeographic studies are an example of an area of future growth for this field of study. Paleocology and paleobiogeography can help the future scientists to understand how climate and ocean currents have changed in the past and how they may change in the future.

This study will focus on Marginopora vertebralis (Fig 1.7). This large benthic porcelaneous foraminiferal species lives in the shallowest ecological environment (i.e., the intertidal and shallow subtidal zone) and is an important producer of sediment in Fijian beaches (Sharma, 2007); its demise could have serious consequences for beach stability, especially under rising sea level.
The shells of foraminifera are preserved in the sediments after they die, allowing the analysis of the conditions in which they lived. *Marginopora vertebralis* is a shallow-water, tropical foraminifera species. Three stages of shell construction are evident in adult shells (Fig 1.8): the embryonic, orbitoid and reproductive chamber stages (Ross, 1972). This arrangement of chambers reveal three bands of coloration: a narrow, light yellowish-green band adjacent to the proloculus (initial chamber); a broader brownish-yellowish green band that represents most of the orbitoid vegetative chambers, and, if present, a cream-colored outer band of reproductive chambers (Ross, 1972). New chambers are typically added every four to five days. At the third stage of growth, 4–58 days after formation of the last vegetative chamber (Rottger, 1974), reproductive chambers are formed (Ross, 1972).
1.4.2 LIFE CYCLES OF FORAMINIFERA

Relatively little is known about how most species of Foraminifera live. The few species that have been studied show a wide range of behavior, diet and life cycles. Individuals of some species live for only a few weeks, while other species can live several years (Goldstein, 1999). Some infaunal benthic species burrow actively into sediments at speeds of up to 1 cm per hour, while some epifaunal species can attach themselves to the surface of rocks and marine plants (Capriulo, 1990).

Of the approximately 4,000 living species of Foraminifera, the life cycle of only 20 or so are known (University College London, 2002). However, the alternation of sexual and asexual generations is basic to the phylum (Goldstein, 2003) and this feature differentiates the Foraminifera from other members of the Class Granuloreticulosea (Murray, 1973; University College London, 2002).

Sexual reproduction, in which gametes are produced, results in the diploid generation. Because the gametes are very tiny, even when two fuse to produce a zygote, the resulting proloculus tends to be very small. Hence such individuals are called microspherics (University College London, 2002). When a diploid microspheric individual grows to reproductive size, it will undergo asexual multiple fission. Whether the offspring are haploid or diploid can vary by taxa and even environmental conditions (Leutenegger, 1977).

Figure 1.8: Growth stages of Marginopora vertebralis. A: embryonic, B: orbitoid and C: reproductive

0.7cm
The reproductive behavior of Foraminifera can involve several stages (Fig 1.9). The common mode of asexual reproduction occurs when an individual undergoes multiple fission, in which numerous offspring are produced. The offspring typically have a relatively large initial chamber (proloculus) and are referred to as the megalospheric generation. After reproducing, the parent shell becomes a dead sand particle. Whether those megalospheric individuals reproduce asexually or sexually can also vary with environmental conditions (Harney et al., 1998). Megalospheric individuals that reproduce asexually are known as the schizont generations.

Figure 1.9 Life cycles of Foraminifera. Diagram showing a generalized foraminifera life cycle note alteration between a haploid megalospheric form and a diploid microspheric form (Goldstein, 2003)
1.4.3 Distribution and Ecology of Foraminifera

Foraminifera found in marine environments have either a planktonic or benthic mode of life (Wetmore, 1999). According to Sen Gupta (2003), Foraminifera make up the most diverse group of shelled microorganisms in modern oceans. Out of approximately 4,000 known living species of foraminifera, 40 species are planktonic and the remaining ones are benthic (Wetmore, 1999). The shells of calcareous foraminifera act as a sink for calcium carbonate (Langer, 2008).

Species of foraminifera are adapted to specific environmental conditions (Wetmore, 1999). Some are plentiful only in the deep ocean, some are found only on coral reefs, and still other species live only in brackish estuaries or salt marshes along the shore (Wetmore, 1999). Murray (2006) stated that the distribution of foraminifera is determined by environmental variables, including depth, salinity, pH, sediment texture (grain size), temperature, and oxygen availability. The majority of foraminifera are classified as epifaunal, that is, are benthic and live at the surface of sediment or on algae or other substrate that project above the seafloor. Some specimens of foraminifera are infaunal and live below the surface, able to reach the deeper levels through bioturbation by macrofauna (Saffert et al., 1998).

The ecology of modern foraminifera is also controlled by feeding mode and food availability. Most foraminifera are heterotrophic and include micro-omnivores, which feed on small bacteria, algae, protists, invertebrates and dead particles. Most foraminifera feed by active searching or by suspension feeding in the case of sessile taxa. Some taxa, especially those living in warm waters, host algal symbionts in a relationship analogous to corals that host zooxanthellae. Many planktonic foraminifera host either dinoflagellates or cryptophytes, while benthic taxa can host dinoflagellates, diatoms, chlorophytes (green algae) or rhodophytes (red algae) (Hallock, 2003). Predators of foraminifera include worms, crustaceans, echinoderms and fish (Rottger, 1983). Different foraminiferal species have different temperature requirements. Moreover, the solubility of calcite is influenced by temperature (Erez, 2003).
1.4.4 Carbonate Production by Benthic Foraminifera

As noted in section 1.4.1, many foraminifera produce calcium carbonate shells, usually calcitic, though a few produce aragonite shells. Moreover, many agglutinated taxa produce calcite cements into which they incorporate sediment particles from the environment. Among those that produce calcite shells, there are two major modes of calcification, radial calcite and porcelaneous calcite (Sen Gupta, 1999; Erez, 2003).

The large benthic foraminifera that host with algal endo-symbionts are dynamic producers of carbonates in the coral reef environments (Hallock, 1981; Fujita, 2008), as they contribute an estimated 5% of the annual present-day carbonate production in the world’s reefs, an approximately 2.5% of the CaCO$_3$ produced in the oceans (Langer, 2008). The high carbonate productivity of the larger foraminifera is related to their endosymbiosis with microalgae (Lee, 1998).

Due to the abundance of the larger benthic foraminifera in shallow shelf environments of the West Pacific and East Indian Ocean (Hohenegger, 2006), they are major producers of sediments deposited in lagoonal habitats and on beaches (Langer and Lipps, 2003). Foraminifera contribute widely to the accumulation and stability of reefs and to the building of reef barriers (Lee et al., 1994). According to Langer (2008), the larger symbiont-bearing foraminifera are estimated to produce at least 130 million tonnes of CaCO$_3$/yr, whereas together with non-symbiont-bearing foraminifera, all benthic foraminifera are estimated to produce 200 million tonnes of CaCO$_3$/yr.

Based on population dynamics, calcium carbonate production by symbiont-bearing foraminifera can be calculated (Muller, 1974; Hallock 1981). Hohenegger (2006) also indicated that, besides the coastal erosion due to the natural forces like the abundant tropical cyclones in the West Pacific, there were also three human induced interferences that can destroy carbonate beaches. Firstly, the devastation of the production area (i.e., loss of habitat) by pollution or landfills; secondly, intensification of currents by coral mining or deepening for boat passages; and thirdly, the separation of production area, the reef crest, from the beach by breakwaters and groins that are inappropriate to stop the erosion. Nukubuco Reef Flat sedimentology showed that carbonate bioclasts originate
mostly from coral debris (65%), molluscan shells (15%; bivalves and gastropods), foraminifera (11%; mainly *Marginopora vertebralis*, some *Amphistegina* spp.) and in minor amounts from calcareous green algae (*Halimeda*), crustaceans, bryozoans, echinoderms and ostracods (Morris, 1998).

### 1.4.5 Previous studies of ocean acidification

#### 1.4.5.1 Previous studies of responses of foraminifera

Benthic foraminifera have been the subject of many experimental ocean-acidification studies compared to other calcifying marine protists (Bernhard *et al*., 2004; Dissard *et al*., 2009; Kuroyanagi *et al*., 2009; Dias *et al*., 2010; Dissard *et al*., 2010a; Fujita *et al*., 2011; Haynert *et al*., 2011; Glas *et al*., 2012; Macintyre-Wressnig *et al*., 2013; Knorr *et al*., 2015). The response parameters taken into account for ocean acidification studies (Table 1.1) were calcification, shell weight, size, growth, shell thickness, test diameter and community shift. The responses varied from no pH effect (Vogel and Uthicke, 2012; McIntyre-Wressnig, 2013) to decreases with lowered pH (Le Cadre *et al*., 2003; Russell *et al*., 2004; Kuroyanagi *et al*., 2009; Allison *et al*., 2010; Dissard *et al*., 2010a; Fujita *et al*., 2011; Sinutok *et al*., 2011; Uthicke and Fabricius, 2012; Haynert, 2013). An increase in growth was found in *M. vertebralis* by Vogel and Uthicke (2012) and in *Calcarina gaudichaudii* by Hikami *et al*. (2011). To date, no one has addressed the issue of ocean acidification on benthic foraminifera in Fiji.
Table 1.1: Ocean acidification studies and the response of lower pH on benthic foraminifera.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Response parameter</th>
<th>Response to low pH/elevated $\rho$CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le Cadre et al., 2003</td>
<td>Ammonia beccari</td>
<td>calcification</td>
<td>decrease</td>
</tr>
<tr>
<td>Russell et al., 2004</td>
<td>Marginopora kudakajimensis</td>
<td>shell weight</td>
<td>decrease</td>
</tr>
<tr>
<td>Kuroyanagi et al., 2009</td>
<td>Marginopora kudakajimensis</td>
<td>size, shell weight, growth</td>
<td>decrease</td>
</tr>
<tr>
<td>Allison et al., 2010</td>
<td>Elphidium williamsoni</td>
<td>shell thickness</td>
<td>decrease</td>
</tr>
<tr>
<td>Cigliano et al., 2010</td>
<td>Foraminiferal assemblage</td>
<td>community shift</td>
<td>changes</td>
</tr>
<tr>
<td>Dissard et al., 2010a</td>
<td>Ammonia tepida</td>
<td>weight</td>
<td>decrease</td>
</tr>
<tr>
<td>Haynert, 2011</td>
<td>Ammonia aomoriensis</td>
<td>test diameter</td>
<td>decrease</td>
</tr>
<tr>
<td>Fujita et al., 2011</td>
<td>Amphisorus hemprichii</td>
<td>shell weight</td>
<td>decrease</td>
</tr>
<tr>
<td>Hikami et al., 2011</td>
<td>Calcarina gaudichaudii</td>
<td>calcification</td>
<td>increase</td>
</tr>
<tr>
<td>Amphisorus kudakajimensis</td>
<td>calcification</td>
<td>decrease</td>
<td></td>
</tr>
<tr>
<td>Amphisorus hemprichii</td>
<td>calcification</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td>Sinutok et al., 2011</td>
<td>Marginopora vertebralis</td>
<td>calcification</td>
<td>decrease</td>
</tr>
<tr>
<td>Uthicke and Fabricius, 2012</td>
<td>Marginopora vertebralis</td>
<td>calcification</td>
<td>decrease</td>
</tr>
<tr>
<td>Vogel and Uthicke, 2012</td>
<td>Amphistegina radiata</td>
<td>growth</td>
<td>no effect</td>
</tr>
<tr>
<td>Heterostegina depressa</td>
<td>growth</td>
<td>no effect</td>
<td></td>
</tr>
<tr>
<td>Marginopora vertebralis</td>
<td>growth</td>
<td>increase</td>
<td></td>
</tr>
<tr>
<td>McIntyre-Wressnig, 2013</td>
<td>Amphistegina gibbosa</td>
<td>growth, fitness, survival</td>
<td>no effect</td>
</tr>
<tr>
<td>Knorr et al., 2015</td>
<td>Archaia angularis</td>
<td>growth</td>
<td>decrease</td>
</tr>
</tbody>
</table>

1.4.5.2 Culture setups for ocean acidification studies

A variety of culture methods are reported to have been used for experimental ocean acidification studies by these different authors (Table 1.1).
Findlay et al. (2008) developed a flow-through tidal microcosm system that allows the investigator to simultaneously manipulate temperature and CO$_2$ to stimulate a realistic intertidal scenario. The objective of the study was to investigate the survival, growth and development of larvae, juveniles and adult intertidal organisms. The concentration of CO$_2$ flowing into the microcosms in that study was manipulated through an air to CO$_2$ gas mixing system.

Several studies have used high precision \( p\text{CO}_2 \) control systems to investigate the effect of ongoing ocean acidification on the calcification of foraminifera. Living foraminifera from three genera (i.e., Baculogypsina, Calcarina, and Amphisorus), were subjected to seawater at five different \( p\text{CO}_2 \) levels, within a range of 300-1000ppm (Hikami et al., 2011). The \( p\text{CO}_2 \) levels were adjusted and kept constant by bubbling CO$_2$ gas with an ALCAL system. During the culturing period of 12 weeks, Hikami et al. kept cultured individuals under constant seawater temperatures, light intensity and photoperiod. Their results suggested that ongoing ocean acidification will be favorable for some hyaline taxa of symbiont-bearing reef foraminifera under intermediate levels of \( p\text{CO}_2 \) (600 and 800ppm), but unfavorable for both hyaline and porcelaneous taxa at higher \( p\text{CO}_2 \) levels (>1000ppm).

Glas et al. (2012) added CO$_2$ enriched air into a semi-closed circulation system of filtered natural seawater, where the CO$_2$ enriched air was humidified via a system of Erlenmeyer flasks and bubbled into an aerated reservoir tank, connected to the incubation chambers, which contained the organisms. Macintyre-Wressnig et al. (2013) maintained elevated atmospheric \( p\text{CO}_2 \) in incubators using a feedback-controlled infrared CO$_2$-sensor, together with a CO$_2$ inlet tube that was inserted through a hole in the incubators side wall to ensure fast mixing of gases. A recent study by Knorr et al. (2015) tested the response of Archaicas angulatus to simulated ocean acidification by applying an automated pH controlled CO$_2$\(_{(g)}\)-injection method.
For this study, methods were adapted from the culturing setup used by Widdicombe et al. (2009), where the CO₂ supplied to the seawater was monitored via a pH controller and the water circulation was provided using a peristaltic pump.

1.4.5.3 Measuring growth (Weighing method and Calcein marking)

There are several ways to measure growth in foraminifera. A common method includes weighing each specimen or a group of specimens at the start of an experiment, and at the end of the treatment period (e.g., Hallock et al., 1986). Another useful method is to mark or label the specimens prior to the experiment. For example, incorporation of the fluorescent dye Calcein (Bis [N,Nbis(carboxymethyl)aminomethyl]-fluorescein) into the skeleton, allows distinguishing pre-existing shell from chambers added during the experimental treatment (Bernhard et al., 2004; De Nooijer et al., 2007; Dissard et al., 2009). The new chambers can then be analyzed for elemental concentrations and ratios using EPMA and LA ICP-MS.

1.4.5.4 Elemental concentrations & ratios

Foraminiferal shells have been studied for records of Mg, Sr, Cd, Ba, F, Li, V, U and other elements and their isotopes in a wide variety of environmental and paleoceanographic studies (Hester and Boyle, 1982; Delaney and Boyle, 1986; Rosenthal et al., 1997). Several trace elements in the benthic foraminiferal calcite have been shown to be influenced by the carbonate ion concentration, either because of dissolution or changing saturation (McCorkle et al., 1995; Elderfield et al., 1996; Marchito et al., 2005).

Foraminifera have been cultured at different pH levels with constant temperature, where together with Mg/Ca and Sr/Ca ratios, other ratios such as B/Ca, Cd/Ca, Ba/Ca, Nd/Ca and U/Ca were also studied. The proxy using foraminiferal Cd/Ca is particularly important. Cadmium concentrations in overlying waters are preserved in benthic foraminifera (Hester and Boyle, 1982). According to Marechal-Abram et al. (2004); the shell of a foraminifer can record the average dissolved cadmium concentration during the whole lifetime of an individual. The boron isotopes in foraminiferal shells are known to reflect seawater pH and for this reason they are used to reconstruct paleo-seawater pH.
(Hönisch et al., 2009). Barium cycles like a nutrient but its regeneration occurs deeper than the organic matter, which results in close correlation between organic matter and alkalinity in today’s ocean (Lea, 1993). The Ba/Ca ratio in foraminifera has been used as the paleo-tracer of alkalinity and water masses (Lea and Boyle, 1990b). The U/Ca ratio of benthic foraminifera also links with the carbonate-system parameters such as pH and CO$_3^{2-}$ (Russell et al., 2004) and is a possible alternative to the B/Ca ratio to reconstruct seawater CO$_3^{2-}$.

1.5 STUDY GOALS

As increasing amount of carbon dioxide in the atmosphere are taken up by the ocean, calcification rates in large benthic foraminifera are likely to decrease, and such changes will also affect trace element composition of resulting shells. Therefore, the main goals of this study are to determine experimentally the effects of acidified seawater on Marginopora vertebralis calcification and potential dissolution, as well as the effects on their shell chemistry.
CHAPTER 2 GROWTH OF *Marginopora vertebralis* IN DIFFERENT PH TREATMENTS

2.1 INTRODUCTION

This chapter describes the design and implementation of a culture experiment with a goal of determining growth rates of *Marginopora vertebralis* under three different pH treatments. The results from the two methods of assessing growth are presented, as are ancillary observations of behavior and visual characteristics of the experimental specimens.

2.2 METHODOLOGY

2.2.1 SAMPLE COLLECTION

The samples of *M. vertebralis* were collected from Makuluva and Nukubuco reefs on the Suva Barrier Reef (Fig 2.1). The location of the collection site was determined and plotted using GPS data. Salinity and temperature of the sample collection area were assessed using a YSI85 meter. The maximum diameter (d) of the specimens used in this study ranged from $0.4 \leq d \leq 1.4$ cm.

The foraminifera and associated sediments were collected using hand scoops and immediately placed in an aerated aquarium with filtered seawater (0.45µm), where they were kept overnight. The next day, *M. vertebralis* specimens had crawled upward and attached themselves to the sides of the aquarium and therefore could be easily identified and removed, based on the methods of Sharma (2007).
Figure 2.1: (A) Map of Laucala Bay; (B) The mapping of the site of collection of Marginopora vertebralis; (C) Algal flat habitat between Sandbank Island and Nukubuco Reef; and (D) M. vertebralis attached to algae and coral (arrow). Scale bars (A) 5km; (B) 0.06km (Source: SOPAC, 2012; Sharma, 2007); (D) 1 cm.
2.2.2 EXPERIMENTAL SET-UP

Natural seawater (salinity 33.0 psu), contained in large (35 L) reservoir tanks, was acidified by means of a bubbling system supplying CO$_2$ gas. The gas, saturated with water vapor (to limit evaporation) was injected through the water as very fine bubbles allowing the gas to rapidly dissolve.

The seawater pH in the reservoirs was monitored using a flat-surface, combination pH electrode (YSI Environmental pH 100). Once the pH reached the required level, the supply of CO$_2$ was halted via an automated feedback relay system (Accu-Max pH controller). As the acidified water was pumped from the reservoir to supply the experimental tanks, the overflow water was returned to the reservoir. Whenever the pH increased, CO$_2$ bubbling was triggered to ensure that the desired pH level was maintained. Each reservoir contained a CO$_2$ Reactor, an air pump and a water pump. The reservoirs were refilled with natural seawater (pH=8.1) every 10 days from a separate 300 m$^3$ seawater tank, causing the pH in the reservoir tank to increase. This increase triggered the supply of CO$_2$ to be restarted and CO$_2$ continued to bubble through the water until the pH was reduced to the required level. Using this method it was possible to supply large quantities of CO$_2$ acidified seawater with a consistent pH.

Two seawater acidification reservoir tanks (pH 7.8, pH 7.5) and one control (ambient pH 8.1) were maintained. From each tank, water was supplied to the corresponding three small tanks (25 x 10 x 15 cm) where the *M. vertebralis* specimens were cultured.

The experimental setup model in Figure 2.2 shows how the connections were made from the reservoirs to the connecting tanks and vice versa. The CO$_2$ system is plugged with pH controllers (adjusted at pH 7.8 and 7.5). The CO$_2$ is released with the help of the bubble counter, check valve and CO$_2$ injector. The pH controller regulates and stabilizes the pH in the reservoirs and the peristaltic pump helps in the circulation of seawater, creating an ocean-like environment.

Nine plastic tanks (3 treatments with 3 replicate each) were placed in a room (ambient temperature ~ 27.5° C) and connected to a pH controller system (Fig 2.2). Each tank was individually supplied with seawater at a rate of approximately 3.3 ml min$^{-1}$ using a
peristaltic pump. The *M. vertebralis* specimens were randomly assigned to one of the three pH treatment levels (8.1 [ambient seawater], 7.8, and 7.5). The treatments were maintained under a 12-hour light and 12-hour dark cycle using a T5 Aquarium lamp with a light intensity of 42.5 watts m$^{-2}$.

Acidification of the seawater did not begin until 24 hours after the *M. vertebralis* specimens were placed in their treatment tanks. Seawater pH was reduced gradually over a period of two days and the experiment started when the final water chemistry for each treatment was reached.

The experiment ran for 11 weeks, during which time, the pH of the water supplied to the tanks was monitored daily via a pH controller (Accu Max I). Samples for Total Alkalinity were taken at the beginning of the experiment and then once per week throughout the duration of the experiment. The values given by the pH electrodes in the reservoir tanks were cross-checked every week against values measured by a regularly calibrated pH meter (InLab 413SG, Mettler-Teledo).
Figure 2.2 Experimental set-up model
2.2.3 MEASURING GROWTH

2.2.3.1 Fluorescent Marker

Calcein (-bis [N, N-bis (carboxymethyl) aminomethyl]-fluorescein) was used to fluorescently label existing chambers in *M. vertebralis*. Calcein makes newly formed chambers distinguishable when the specimens are viewed under a confocal microscope (Dissard *et al.*, 2009).

*Marginopora vertebralis* in each treatment and replicates were placed in a solution at 40 μM of Calcein for approximately 48 hours to allow the incorporation of the green fluorescent cell marker (Fig 2.3). Once Calcein is incorporated inside the cells, Calcein AM (a nonfluorescent molecule) is hydrolyzed by endogenous esterase into the highly negatively charged green fluorescent Calcein. The fluorescent Calcein is retained in the cytoplasm in living cells but is not incorporated into the subsequently added chambers (Papadopoulous *et al.*, 1994). At the end of the experiment, this technique helps to distinguish between the pre-existing and the newly grown calcite (Bernhard *et al.*, 2004; Dissard *et al.*, 2009).

![Figure 2.3 M. vertebralis treated in 40 μmol/L of Calcein](image)

A confocal microscope (Nikon ECLIPSE TE2000-U) was used to create sharp images of specimens that would otherwise appear blurred when viewed with a conventional
microscope. The software used was EZC 3.91. Imaging is achieved by excluding most of the light from the specimen that is not from the microscope’s focal plane. The image has less haze and better contrast than that of a conventional fluorescent microscope and shows a thin cross-section of the specimen. Thus, in addition to allowing better observation of fine details, it is possible to build three-dimensional (3D) reconstructions of a volume of the specimen by assembling a series of thin slices taken along the vertical axis.

In confocal microscopy, there is never a complete image of the specimen because at any instant only one point is observed. The Calcein in the specimen is excited by the laser light and fluoresces. The green fluorescent light is de-scanned by the same mirrors that are used to scan the blue excitation light from the laser and then passes through the dichroic mirror. Thereafter, it is focused onto the pinhole. The light that makes it through the pinhole is measured by a detector such as a photomultiplier tube (Fig 2.4).

Growth of the selected specimens was assessed using confocal imagery. The thickness of the calcite shell added after Calcein incubation revealed growth that occurred during
experimental treatments. The selected specimen was moved around under the confocal microscope and the edges which showed growth was marked. Using the ruler property from the EZC1 3.91 software, the increase in radius was noted in µm. The mean increases in radius at each treatment for each replicate was recorded.

2.2.3.2 Weighing Method

The 25 *M. vertebralis* specimens in each treatment were weighed once each week as a group using an analytical balance (Libror-Aex 200g). The specimens were collected using hand scoops, pat dried using tissue, and wet weights of the group of specimens from each replicate of each treatment were determined.
2.3 RESULTS

We cultured 225 *M. vertebralis* individuals in all, at three different pH values: pH 7.5 (n=75), 7.8 (n=75), and control (pH ~8.1; n=75). The *M. vertebralis* cultured at pH 7.5 showed a 429 µm mean increase in radius and at pH treatment of 7.8 the mean increase in radius was 441 µm. The increase in radius was nearly the same for pH treatment of 7.5 and 7.8. The *M. vertebralis* cultured at pH 8.1 showed 1024 µm mean increase in radius, which was more than doubled the increase noted for pH 7.5 and 7.8. The mean increases in radius (n=25) at the end of each treatment for each replicate are presented in Table 2.1. Examples of measurement for each treatment are shown in Figures 2.5 to 2.7.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Replicate</th>
<th>Mean increase in radius (µm)</th>
<th>SD</th>
<th>n</th>
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<tbody>
<tr>
<td>7.5</td>
<td>R1</td>
<td>471</td>
<td>102</td>
<td>25</td>
</tr>
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<td></td>
<td>R2</td>
<td>489</td>
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<td>25</td>
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<tr>
<td></td>
<td>R3</td>
<td>326</td>
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<td>7.8</td>
<td>R1</td>
<td>476</td>
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</tr>
<tr>
<td></td>
<td>R2</td>
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<td></td>
<td>R3</td>
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<td>R1</td>
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<td></td>
<td>R2</td>
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<tr>
<td></td>
<td>R3</td>
<td>1267</td>
<td>306</td>
<td>25</td>
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Table 2.1: Mean increase in radius of shells of specimens in each replicate of each treatment after 11 weeks in culture.
Figure 2.5. Confocal microscopy image (A1) of a specimen from pH treatment 7.5. The growth measured for A₁ was 561 μm and 724 μm.

Figure 2.6. Confocal microscopy image (B1) of a specimen from pH treatment 7.8. The growth measured for B₁ was 746 μm and 1000 μm.
Figure 2.7 Confocal microscopy images (C₁) of specimens from two replicate tanks of pH treatment 8.1. The growth measured for C₁ was 1400 μm and 934μm.
Weekly weights of the 25 \textit{M. vertebralis} specimens from each replicate of each treatment are plotted in Figure 2.8. Linear regression parameter for each dataset for each replicate is provided in Table 2.2, along with the total change in weight.

The \textit{M. vertebralis} cultured at pH 7.5 showed very little growth, averaging only 0.0227 g/week and pH treatment 7.8 showed only slightly more growth, 0.0334 g/week. The specimens cultured at pH 8.1 grew an average of 0.0658 g/week (Fig 2.8).
The relative weight gain (%) of 25 individuals of *M. vertebralis* at each pH treatment for a period of 11 weeks was calculated and graphed (Fig 2.9). Individuals treated at pH 7.5 had an average percentage growth of 3.24 % whereas individuals treated at pH 7.8 had a
percentage growth of 4.24 %. At pH 8.1; the highest percentage growth of 8.39 % was noted.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Replicate</th>
<th>Mean increase in radius (μm) per individual</th>
<th>Mean increase in weight (mg) per individual</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>R1</td>
<td>471</td>
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<td>Mean ± SD</td>
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<tr>
<td></td>
<td>Mean ± SD</td>
<td>441 ± 64.1</td>
<td>14.5 ± 3.71</td>
</tr>
<tr>
<td>8.1</td>
<td>R1</td>
<td>1019</td>
<td>24.3</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>785</td>
<td>27.3</td>
</tr>
<tr>
<td></td>
<td>R3</td>
<td>1267</td>
<td>35.4</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>1024 ± 241</td>
<td>30.0 ± 5.72</td>
</tr>
</tbody>
</table>

The mean shell radius of cultured individuals measured after 11 weeks of culture and the mean shell weight measured every week of all cultured specimens of *Marginopora vertebralis* are presented in Table 2.3. The result in Figure 2.10 indicates that 1) shell size increased
with time in all pH treatments and 2) pH of the medium significantly influenced the increase in shell radius through a culture period (Table 2.3). The results in Table 2.3, revealed notably less growth at the lowest pH condition both with respect to shell radius and shell weight (Fig 2.10).

<table>
<thead>
<tr>
<th>pH</th>
<th>Treatment</th>
<th>Growth rate (mg day⁻¹)</th>
<th>Growth rate (mg month⁻¹)</th>
<th>Growth rate (g month⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>R₁</td>
<td>1.96</td>
<td>58.8</td>
<td>0.0588</td>
</tr>
<tr>
<td></td>
<td>R₂</td>
<td>3.98</td>
<td>119</td>
<td>0.119</td>
</tr>
<tr>
<td></td>
<td>R₃</td>
<td>4</td>
<td>120</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>3.31 ± 1.17</td>
<td>99.4 ± 35.2</td>
<td>0.0994 ± 0.035</td>
</tr>
<tr>
<td>7.8</td>
<td>R₁</td>
<td>6.08</td>
<td>182</td>
<td>0.182</td>
</tr>
<tr>
<td></td>
<td>R₂</td>
<td>4.25</td>
<td>128</td>
<td>0.128</td>
</tr>
<tr>
<td></td>
<td>R₃</td>
<td>3.8</td>
<td>114</td>
<td>0.114</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>4.71 ± 1.21</td>
<td>141 ± 36.2</td>
<td>0.141 ± 0.036</td>
</tr>
<tr>
<td>8.1</td>
<td>R₁</td>
<td>7.88</td>
<td>236</td>
<td>0.236</td>
</tr>
<tr>
<td></td>
<td>R₂</td>
<td>8.38</td>
<td>251</td>
<td>0.251</td>
</tr>
<tr>
<td></td>
<td>R₃</td>
<td>11.5</td>
<td>344</td>
<td>0.344</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>9.25 ± 1.95</td>
<td>277 ± 58.5</td>
<td>0.277 ± 0.058</td>
</tr>
</tbody>
</table>

For comparison with previous studies, growth rates were calculated per day and per month (Table 2.4). The 25 individuals of *M. vertebralis* together grew an average of 0.0994 g/month at pH 7.5, grew an average of 0.141 g/month at pH 7.8 and an average of 0.277 g/month at pH 8.1.
2.3.1 Other observations

At the end of the experiment, most specimens contained pale pink cytoplasm, which indicated that they were alive throughout the entire experimental period.

*Marginopora vertebralis* has a disc shape. Individuals can roll on their edge and climb the tank wall using their reticulopodia (Fig 2.11). It was observed that they disperse and move more towards light.

Several specimens were observed to reproduce asexually. When an individual is ready for reproduction, it changes its color to white with a pale purple ring in the middle. During reproduction (Fig 2.12), the parent shell becomes white as the endoplasm containing the dinoflagellate symbionts is incorporated into the megalospheric offspring. All the replicate tanks had a mixture of adult and juvenile *M. vertebralis* but reproduction was only noted at the pH treatment of 8.1.
Figure 2.12 Reproduction of *Marginopora vertebralis* at pH treatment 8.1 (black arrow)
2.4 DISCUSSION

The microscopic observations of *Marginopora vertebralis* specimens documented that the precipitation of calcite did not occur evenly over the organic membrane of the developing chamber during their calcification process. Calcification began at many small sites on the membrane rather than in a uniform manner. After a few hours, the patchy calcite interconnects and then thickens the chamber wall.

Erez (2003) stated that reticulopodia in foraminifera are important for food gathering, movement, shell building, respiration and extraction of waste materials. Thus, the reticulopodia in foraminifera enable them to interact with their surrounding (Goldstein, 2003). The shells of foraminifera are structured in such a way as to allow for effective communication of cytoplasm between the intra-shell endoplasm and the outside environment (Funnel et al., 1986; Hottinger et al., 1993 and Erez, 2003).

Sharma (2007) collected *M. vertebralis* from Laucala Bay and cultured them at ambient pH of seawater (pH 8.1). The results revealed that a group of *M. vertebralis* larger than or equal to a diameter of 1 cm grew at 0.13 g/month while *M. vertebralis* specimen larger than 0.5 cm and smaller than or equal to a diameter of 0.7 cm grew at 0.0063 g/month. In this study smaller and larger individuals were mixed (Table 2.4), 25 individuals of *M. vertebralis* together grew an average of 0.0994 g/month at pH 7.5, an average of 0.141 g/month at pH 7.8 and an average of 0.277 g/month at pH 8.1. Based on the reports of Hallock (1981) for *Amphistegina* spp. and *Calcarina* spp. and Smith and Wiebe (1977) for *Marginopora vertebralis*, foraminifera are probably the major CaCO₃ producers in the low calcifying zones, as both species can produce sediment at rates of 0.5 kg CaCO₃ m⁻² yr⁻¹.

Seawater with lower pH levels had a significant effect on the shell growth of *M. vertebralis*. Individuals treated at pH 7.5 grew an average of 3.2% whereas individuals treated at pH 7.8 grew by 4.2% (Fig 2. 9), well below the 8.4 % growth at pH 8.1.

The smaller individuals of *M. vertebralis* showed a growth rate of 7.7% of their initial body weight while the larger organisms showed a growth rate of 1.9% of their initial body weight (Sharma, 2007). Sharma (2007) concluded from her study that the
The approximate rate of sediment production from the three *M. vertebralis* colonies from the Sandbank Island was 36 kg of sediments each month and 431 kg of sediments each year, at an average of 0.13 kg/m²/yr. If the ocean acidification continues at predicted rates, the sediment production rates by Sharma (2007) could be well overstated.

The results from this study showed that the rate of increase in shell weight of *M. vertebralis* is more sensitive to changing pH (Fig 2.8). Furthermore, the relationship between shell radius and shell weight is different among the three pH conditions (Fig 2.10). Our results are in agreement with Sinutok *et al.* (2011) and Uthicke and Fabricius (2012), who also reported a decrease in *M. vertebralis* calcification in response to lower pH. Thus, in an increasingly acidified ocean, the organisms may need more energy to produce the same amount of calcite, which in turn will reduce the gross calcite production by calcifying foraminifera (de Nooijer *et al.*, 2009; Knorr *et al.*, 2015). Significant reductions in the growth rates of *M. vertebralis* were recorded in response to the decreases in the seawater pH. This sensitivity to ocean acidification indicates that *M. vertebralis* might not cope well with further increases in atmospheric and oceanic *p*CO₂.
CHAPTER 3 CARBONATE CHEMISTRY OF CULTURE MEDIUM

3.1 INTRODUCTION
3.1.1 PURPOSE

The purpose of Chapter 3 of this thesis is to present the methods used to measure and calculate carbonate species, and to report the resulting data on water chemistries to which Marginopora vertebralis experienced during the experiment presented in Chapter 2.

3.1.2 INORGANIC CARBON IN SEAWATER

The ocean processes CO₂ in multifaceted ways. Three inorganic forms of dissolved carbon dioxide occur in seawater: free aqueous carbon dioxide (CO₂ (aq)), bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) ions (Zeebe, 2011). Due to the influx of anthropogenic CO₂, the carbonate system of the world’s ocean is changing rapidly (Orr et al., 2005; Guinotte and Fabry, 2008).

The inorganic carbon dioxide system in the oceans can be characterized using any two of the four measurable parameters, pH, total alkalinity (TA), fugacity of carbon dioxide (fCO₂) and the total inorganic carbon dioxide (TCO₂), given accompanying measurements of temperature (°C), salinity (psu) and pressure (atm). Dissociation constants of carbonic acid are also needed to calculate the components of the CO₂ system from these measurements.

The equilibrium of gaseous and aqueous CO₂ when air-sea gas exchange occurs can be represented as:

\[ \text{CO}_2 \ (g) \leftrightarrow \text{CO}_2 \ (aq) \]  \hspace{1cm} (3.1)

Carbon dioxide in the atmosphere mixes with seawater to produce carbonic acid (H₂CO₃), which readily dissociates into hydrogen (H⁺) and bicarbonate (HCO₃⁻) ions:

\[ \text{CO}_2 \ (aq) + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+ \]  \hspace{1cm} (3.2)
The bicarbonate ions can further dissociate, resulting in a hydrogen ion and a carbonate ion:

\[
\text{HCO}_3^- \rightleftharpoons \text{H}^+ + \text{CO}_3^{2-}
\]  
(3.3)

Foraminifera require both Ca\(^{2+}\) and CO\(_3^{2-}\) ions to produce calcium carbonate shells (CaCO\(_3\)(s)):

\[
\text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3(s)
\]  
(3.4)

When dissolution occurs, the calcium carbonate dissolves into calcium ions and carbonate ions:

\[
\text{CaCO}_3(s) \rightarrow \text{Ca}^{2+} + \text{CO}_3^{2-} \]  
(3.5)

The rate of dissolution depends on pH, temperature, salinity, pressure and the concentration of carbonate ions in seawater. Hence, the addition of carbon dioxide to seawater increases the proportions of hydrogen ions, carbonic acid, and bicarbonate and lowers the proportion of carbonate ions. The generation of protons [H\(^+\)] during the carbon dioxide dissociation steps increases seawater acidity and causes a decrease of the oceanic pH (Cao and Caldeira, 2008; Doney et al., 2009; Doney and Feely, 2011; Neuer et al., 2014).

The decline in oceanic pH affects the proportions of the three inorganic carbon species: CO\(_2\), HCO\(_3^-\), and CO\(_3^{2-}\), which are in equilibrium with each other. The sum of all dissolved inorganic carbon (CT):

\[
\text{C}_T = [\text{CO}_2] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}]
\]  
(3.6)

According to Riebesell et al. (2010), Total Alkalinity (AT) can be represented as:

\[
\text{A}_T = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B(OH)}_4^-] + [\text{OH}^-] - [\text{H}^+] + \text{minor components}
\]

Total Alkalinity can be determined by the titration of seawater with a strong acid. The major component of this buffering system is the carbonate alkalinity (AC):

\[
\text{A}_C = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}]
\]  
(3.7)

The dissociation constants \(K_1\) and \(K_2\) are defined as:

\[
K_1 = [\text{H}^+] [\text{HCO}_3^-] / [\text{CO}_2]
\]  
(3.8)

\[
K_2 = [\text{H}^+] [\text{CO}_3^{2-}] / [\text{HCO}_3^-]
\]  
(3.9)
where the square brackets are used to denote the concentration in mol kg$^{-1}$ of the species in seawater.

The International Panel on Climate Change (IPCC) predicted future scenarios, indicating that the atmospheric CO$_2$ will increase to concentrations as much as 970 ppm by the year 2100 (IPCC, 2013). An increasing number of field and laboratory studies have indicated negative impacts of increased seawater carbon dioxide and related changes in the carbonate chemistry on marine calcifying organisms, such as corals and foraminifera (Kleypas et al., 1999; Riebesell et al., 2000).
The 12 major constituents of seawater are listed in Table 3.0. These major components have relatively long residence times (ocean mixing time of ~1000 years) in the ocean (Chester and Jickells, 2012).

<table>
<thead>
<tr>
<th>Component</th>
<th>Average conc [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O</td>
<td>54,880,000</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>559,000</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>480,000</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>54,100</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>28,900</td>
</tr>
<tr>
<td>K$^+$</td>
<td>10,500</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>10,500</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>1,890</td>
</tr>
<tr>
<td>Br$^-$</td>
<td>863</td>
</tr>
<tr>
<td>CO$_3^{2-}$</td>
<td>189</td>
</tr>
<tr>
<td>Sr$^{2+}$</td>
<td>84</td>
</tr>
<tr>
<td>B(OH)$_4^-$</td>
<td>70</td>
</tr>
</tbody>
</table>

Trace elements are minor components of the seawater (less than 1 µmol/L) with shorter residence times in the ocean than the major constituents (Chester and Jickells, 2012).
3.1.3 Benthic foraminifera under ocean acidification

Based on the absence of calcareous benthic foraminifera in acidic seawater associated with natural seafloor venting of carbon dioxide in a variety of shallow-water locations, Uthicke et al. (2013) concluded that, if ocean acidification continues at predicted rates, many benthic foraminifera will be extinct by 2100. However, Pettit et al. (2013) did not find a significant response in the benthic foraminiferal assemblages to reduced $p$CO$_2$ in deeper shelf and slope settings.

Similarly, laboratory experiments examining responses of calcareous foraminifera to lower pH and elevated $p$CO$_2$ have shown significant differences among different taxa and even within the same species (Doo et al., 2014). The majority of studies have shown that pH lower than 7.8 can have deleterious effects on calcareous benthic foraminifera (Kuroyanagi et al., 2009; Dias et al., 2010; Fujita et al., 2011; Vogel & Uthicke, 2012; McIntyre-Wressnig et al., 2013; Knorr et al., 2015). These effects include reduced calcification (Kuroyanagi et al., 2009; Moy et al., 2009; Haynert et al., 2011) and decreased growth rates (Manno et al., 2012; Reymond et al., 2013), indicating the decline in carbonate sediment production by foraminifera as ocean acidification proceeds (Dias et al., 2010; Knorr et al., 2015). On the other hand, Fujita et al. (2011) and Vogel & Uthicke (2012) reported that Marginopora vertebralis, in laboratory experiments, responded to increased $p$CO$_2$ with increased rates of calcification.
3.2 METHODOLOGY

3.2.1 DETERMINATION OF TOTAL ALKALINITY IN SEAWATER

To determine the carbonate chemistry in the culture treatments described in Chapter 2, the following procedures and calculations were carried out based on best practices.

Borosilicate bottles, used to collect the water samples for alkalinity measurements, were rinsed with concentrated HCl, followed by at least five rinses with deionised water. Seawater samples were collected from the experimental aquaria. Borosilicate bottles were fully filled and tightly capped. Analysis was carried out within 6 hours of sample collection.

To standardize acid used in titrations, 10 ml aliquot of 0.01M sodium tetraborate was placed into a 250 ml conical flask and a few drops of mixed indicator were added. The aliquot was titrated with 0.01 M HCl until the end point was reached (color changed from green to purple). The titre was recorded for the calculation of HCl concentration:

\[
\text{Conc of HCl} = \frac{\text{Vol. of Borax} \times \text{Mass of Borax} \times 1000 \times 2}{\text{Vol. of titre} \times 381.37 \times 100}
\]

To determine the total alkalinity \((A_T)\) of a sample, 50 ml of the water sample were pipetted into a 250ml Erlenmeyer flask and few drops of phenolphthalein indicator were added. Once the solution color turned pink, it was titrated with 0.01M HCl until the color disappeared and the volume of added acid was recorded. Then 2-3 drops of mixed bromocrescol green/methyl red indicator were added to the same solution and titrated with standardized 0.01M HCl to a pink color. Then \(A_T\) was calculated using this formula:

\[
\text{Total Alkalinity, } A_T \text{ (mg/L)} = \frac{A \times M \times 50,000}{\text{Sample volume}}
\]

Where \(A = \text{Volume of standard HCl used (ml)}\)

\(M = \text{Molarity of HCl}\)
The pH of the two acidification treatments described in Chapter 2 were adjusted and maintained at 7.5 and 7.8 using a pH controller system w/electrode (Accu-Max, Ultralife Reef Products). Since pH 8.1 was the pH of ambient seawater, carbon dioxide was not injected into the reservoir or the replicate tanks. The pH was cross checked using a pH meter (YSI Environmental pH 100).

3.2.2 Analysis using CO2SYS

The MATLAB program CO2SYS (single-input mode directly adapted from Lewis and Wallace, 1998) was used to calculate and present seawater parameters where the salinity was constant at 35 psu and temperature was constant at 27.5°C. The input temperature and pressure were from measurements performed in the laboratory. There are four measurable parameters of the aquatic carbon dioxide system: pH, $p\text{CO}_2$, total dissolved inorganic carbon (DIC) and total alkalinity (TA). The measured pH and alkalinity values were entered into the program. The equilibrium constants from Mehrbach et al. (1973) as refit by Lueker et al. (2000) on a total scale were used. The measurements made by Mehrbach et al. (1973) were made on real seawater.

Input variables (input conditions):

- Salinity (35 psu), Temperature (27.5°C) and Pressure (1 atm).
- Total Si (optional) and Total Phosphate (optional). If left empty, the total Si and total P concentrations are assumed to be zero in the calculations.
- Two (2) known CO2 parameters (TA, pH).

Output (for both “input” and “output” conditions):

- The other CO2 parameters ($p\text{CO}_2$, total dissolved inorganic carbon [DIC].
- Contributions to the alkalinity.
- Carbonate speciation
3.3 RESULTS

In each aquarium analyzed, 25 individuals of *M. vertebralis* were placed as it is difficult to assess the growth per individual in terms of shell weight and calcification. There were little uncertainties which influence the calcification estimates of *M. vertebralis*, but it does not affect the concluding result that calcification decreases as a function of decreasing \([\text{CO}_3^{2-}]\) and the final weight observations.

Seawater chemistry contrasted strongly between the 2 treatments and control, with mean values ranging from 8.1 to 7.5 pH units and 309 to 1717 matm \(p\text{CO}_2\), respectively. Mean total alkalinity was 2277 \(\mu\text{mol kg}^{-1}\) \((n=11, \text{SD}=101)\). The alkalinity values were between 2176 and 2408 \(\mu\text{mol kg}^{-1}\), a range we used together with the pH measurements from all aquaria (treatments) to calculate \(p\text{CO}_2\) concentrations (Table 3.1).

| Table 3.1 Carbonate system parameters pertinent to the experiment. The input parameters of temperature (T), salinity (S), total alkalinity (A\(_T\)), and the total scale pH (pH\(_T\)) were measured directly. The treatment and control values are mean of measurements \((n=11)\) taken over the course of the experiment. The remaining values were calculated using Lewis and Wallace (1998). The equilibrium constants from Mehrbach *et al.* (1973) as refit by Lueker *et al.* (2000) on total scale were used. Temperature (T) is in °C, salinity (S) is in psu, partial pressure of CO\(_2\) in seawater \((p\text{CO}_2)\) is in milliatmospheres (matm); \([\text{OH}^-]\) is given in mmolkg\(^{-1}\), \(A_T, [\text{HCO}_3^-]\) and \([\text{CO}_3^{2-}]\) is given in mmolkg\(^{-1}\)SW. |
|---|---|---|---|---|---|---|---|---|
| T °C | S psu | pH\(_T\) | A\(_T\) mmolkg\(^{-1}\)SW | \(p\text{CO}_2\) matm | \([\text{OH}^-]\) mmolkg\(^{-1}\) | \([\text{HCO}_3^-]\) mmolkg\(^{-1}\)SW | \([\text{CO}_3^{2-}]\) mmolkg\(^{-1}\)SW |
| 8.1 control | 27.5 | 35 | 8.1 \((0.05)\) | 2176 | 309.1 | 9.66 | 1577.3 | 240.6 |
| 7.8 treatment | 27.5 | 35 | 7.8 \((0.05)\) | 2247 | 738.5 | 4.84 | 1891.4 | 144.6 |
| 7.5 treatment | 27.5 | 35 | 7.5 \((0.05)\) | 2408 | 1717 | 4.8 | 2203.5 | 84.5 |
The pH measurements showed a good correlation to the long term averages of $p$CO$_2$ for the two treatments and control (Table 3.2, Fig 3.1, $R^2=0.95$).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Replicate</th>
<th>Mean $p$CO$_2$ (matm)</th>
<th>Mean [CO$_3^{2-}$] (mmol kg$^{-1}$ SW)</th>
<th>Mean growth (g)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>$R_1$</td>
<td>1717</td>
<td>84.5</td>
<td>0.1513</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>$R_2$</td>
<td>1716.3</td>
<td>84.5</td>
<td>0.3063</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>$R_3$</td>
<td>1716.5</td>
<td>84.5</td>
<td>0.3081</td>
<td>11</td>
</tr>
<tr>
<td>7.8</td>
<td>$R_2$</td>
<td>738.5</td>
<td>144.6</td>
<td>0.4681</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>$R_3$</td>
<td>738.6</td>
<td>144.6</td>
<td>0.2929</td>
<td>11</td>
</tr>
<tr>
<td>8.1</td>
<td>$R_1$</td>
<td>308.3</td>
<td>240.6</td>
<td>0.6066</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>$R_2$</td>
<td>309.1</td>
<td>240.6</td>
<td>0.6835</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>$R_3$</td>
<td>308.6</td>
<td>240.6</td>
<td>0.8838</td>
<td>11</td>
</tr>
</tbody>
</table>

Figure 3.1: $p$CO$_2$ in seawater of two pH treatments (7.5 and 7.8) and control (8.1).
The shell growth in *M. vertebralis* was negatively affected by elevated $pCO_2$ (Fig 3.2) and was positively related to increased $CO_3^{2-}$ (Fig 3.3).

Figure 3.2: Mean increase in shell weight of 25 *M. vertebralis*, grouped by pH level (n=11) at three $pCO_2$ levels.

Figure 3.3: Mean shell weight (mg) of 25 *M. vertebralis*, grouped by treatment (n=11) against $CO_3^{2-}$ (mmol kg$^{-1}$ SW).
3.4 DISCUSSION

Every component in the carbonate system is interlinked; therefore, any changes in any single component of the carbonate system will lead to changes in other components. The burning of fossil fuels, deforestation, industrialization, and other land-use changes are contributing to the increasing atmospheric CO₂ levels which directly lead to decreasing ocean pH through the air-sea gas exchange.

Foraminiferal species have specific ecological niches and populations respond quickly to environmental changes (Hallock et al., 2003). Experimental evidence in the past two decades has shown that foraminifera respond to the changes in seawater temperature and chemistry (Erez and Luz 1982, 1983; Lea and Spero 1992, 1994; Russel et al., 1994; Lea et al., 1999). The results of the laboratory work in this study indicate that, at the rate at which ocean acidification is currently occurring, there will likely be intense biological consequences for ocean ecosystems within the coming decades and centuries, particularly those organisms that calcify. The results also are consistent with the predicted decrease in calcification in foraminifera over a similar increase in pCO₂ (Bijma et al., 1999; Le Cadre et al., 2003; Hikami et al., 2011; Sinutok et al., 2011; Uthicke and Fabricius, 2012).

Calcification rates are largely related to the amount of available carbonate [CO₃²⁻] ions in the water. A recent study by Dissard et al. (2010a) focused on shell weight and shell size measurements of Ammonia tepida specimens, combined with the analysis of Mg/Ca and Sr/Ca in shells, cultured under the different pCO₂ conditions, temperatures and salinities. The results indicated that the shell weight decreased with decreasing [CO₃²⁻]. The results obtained in this research for M. vertebralis from Laucala Bay are consistent with those of Dissard et al. (2010a), and also can be compared to other studies of M. vertebralis (Sinutok et al., 2011; Uthicke and Fabricius, 2012), which also found that calcification of M. vertebralis decreased at higher pCO₂ levels.

Environmental records of past atmospheric CO₂ levels and ocean pH imply that projections of future atmospheric CO₂ levels are higher than those of the past 45 million years. In the very near future, the larger foraminifera, including M. vertebralis, will
therefore be exposed to very different ocean chemistry than has existed for millions of years, and reductions in calcification rates are likely. If foraminifera must increase the rates to active ion transport to maintain a fixed difference between seawater and their calcifying vesicles, calcification at a lower pH will in turn decrease the carbonate available for calcite precipitation (de Nooijer et al., 2009). The foraminifera will need to spend more energy to produce the same amount of calcite and therefore, it would be less likely that calcite precipitation will occur in an acidified ocean.

What remains to be determined is whether *M. vertebralis* exposed to elevated $p$CO$_2$ for an entire life cycle can acclimate or if long-term exposure will result in decreased calcification rates, as has been observed for other benthic foraminifera (Dissard et al., 2010a; Fujita et al., 2011; Haynert et al., 2011; Knorr et al., 2015). Moreover, the combined effects of temperature, nutrient availability and CO$_3^{2-}$ need to be studied in order to estimate the impact of oceanic environmental changes on *M. vertebralis* calcite production.
CHAPTER 4 ELEMENTAL RATIOS USING EPMA (ELECTRON PROBE MICRO-ANALYSER)

4.1 PURPOSE/INTRODUCTION

Magnesium/calcium ratios, strontium/calcium ratios and sulfur/calcium ratios were determined in the shells of the benthic foraminifer, Marginopora vertebralis, which were collected from Laucala Bay, Suva, Fiji and cultured in the laboratory under three different pH levels. The goal of these analyses was to determine whether Mg, Sr and S in the shells could be used as a proxy measurement for the pH of seawater in which the M. vertebralis grew.
4.2 METHODOLOGY

4.2.1 EMBEDDING OF SPECIMENS

Specimens of *M. vertebralis* from culture experiments described in Chapters 2 and 3 were randomly selected from each treatment for analysis of magnesium/calcium ratios, strontium/calcium ratios and other parameters using an electron probe micro-analyzer (EPMA). These specimens were washed four times with double distilled water. For each wash, the specimens were placed on a MRC lab rotator (model: 2100A) for five minutes. Later the specimens were soaked overnight in sodium hypochlorite at a ratio of 1:10 (1ml of sodium hypochlorite to 10ml of double distilled water). The specimens were again washed four times with double distilled water to remove traces of sodium hypochlorite. Afterwards, the specimens were placed in petri dishes and dried for 90 minutes in an oven at 50°C.

After removal from the oven, two specimens from each of the three pH treatments (7.5, 7.8, 8.1) were observed under a confocal microscope to mark the areas of growth (Fig 4.1), then one quarter of the specimen was cut as shown in Figure 4.2.

Each quarter specimen was embedded (Epoxy Resin 1: 0.7 Epoxy Hardener), using the following protocol (Fig 4.3). First, Teflon tubes (1” diameter) were greased and the specimen area that was to be viewed was placed upside down in the tube. The epoxy mixture was prepared by adding 2.6 ml of epoxy hardener to 4 ml of epoxy resin and mixing slowly and gently until it turned from milky to clear. The epoxy mixture was then poured into each Teflon tube, covering the specimens.
Figure 4.1 Growth was measured in RNAR$_2$-5 (pH 7.5); RNBR$_3$-2 (pH 7.8); RNCR$_1$-1 (pH 8.1) using a confocal microscope (upper row of images). The growth areas were marked, cut, embedded with epoxy mixture, then imaged and analyzed with EPMA (lower row of images).
After one hour, the Teflon tubes containing the epoxy mixture were placed in a vacuum jar until no bubbles were seen in the mixture. All samples were left in an open room for 12 hours to air dry and solidify. The embedded and cured specimens were then polished on a series of diamond grit papers, starting from 60 µm followed by 30, 12, 9, 3 and 1 micron until the test was exposed. This critical step before analysis is important so that surface imperfections would not interfere with electron beam-sample interactions. After polishing, the embedded samples were placed in an ultrasonic cleaner for one minute. Then they were coated with gold by means of evaporative deposition to prevent electrical charging of the sample and observed under the EPMA.

![Diagram showing layers](image)

**Figure 4.2** How the specimens were cut for embedding using epoxy mixture and then for viewing under EMPA; note that the outer layer in this case are the chambers added during the experiment.
The EPMA, informally called electron microprobe, is a microbeam instrument used primarily for non-destructive chemical analysis of minute solid samples. The process is fundamentally the same as SEM (Scanning Electron Microscopy), with the added capability of chemical analysis (Bonetto et al. 2001). The EPMA bombards the specimen with a beam of accelerated electrons, which are focused on the surface of a specimen using a series of electromagnetic lenses, and these energetic electrons produce characteristic X-rays within nine cubic microns of the specimen. The detection limits differ for each element, and are affected by the overall composition of a sample and the analytical conditions. For most elements, the detection limits for the wavelength dispersive spectrometers (WDS) is between 50 and 500 parts per million (ppm). With an added ability to detect elements down to the level of 1%, EPMA is well-suited to the analysis of heterogeneous specimens. The instrument used in this study (Fig 4.4) was an Electron Probe Micro-Analyzer (jeol, jxa -8600 superprobes).

Figure 4.3: Preparation of sample for EPMA: (A) The edges where growth has occurred were cut for analyses. (B) Epoxy mixture (Epoxy Resin1: 0.7 Epoxy Hardener) was prepared. (C) The specimens were embedded with epoxy mixture. (D) Epoxy blocks with embedded specimens were ground using a series of diamond paper to expose the shell of *M. vertebralis* for subsequent analysis.
Figure 4.4: A typical arrangement of a probe lab (Picture taken at the Freddy and Nadine Herrmann Institute of Earth Sciences, The Hebrew University of Jerusalem (Givat Ram/ Israel).
4.3 RESULTS

4.3.1 IMAGES FROM EPMA (ELECTRON PROBE MICRO ANALYZER)

Shell growth or dissolution was measured by observing the number of chambers formed or dissolved after the specimens were marked using Calcien. In Figure 4.5, the areas circled in red provide examples of calcification or dissolution of *M. vertebrealis* chambers. Images A₁ & A₂ are the EPMA images of specimens from the 7.5 pH treatment, where there is little or no growth of new chambers, instead dissolution in chambers is observed. Images B₁ & B₂ are of specimens from the 7.8 pH treatment, again, where minimal growth of new chambers and some signs of dissolution are observed. Images C₁ & C₂ are from the 8.1 pH treatment, where new growth of chambers is seen; the newly grown chambers are very close together.
Figure 4.5: Growth or dissolution was determined by observing the number of chambers formed or dissolved. Areas marked with a red circle in A1, A2, B1, B2, C1, C2 display the calcification or dissolution of M. vertebralis chambers. Images A1 & A2 are the EPMA images of specimens from the 7.5 pH treatment (sample name: RNAR2-1; RNAR2-5) where there was little or no growth of new chambers, instead dissolution in chambers can be observed. Images B1 & B2 are the EPMA images of specimens from the 7.8 pH treatment (sample name: RNBR2-1; RNBR2-2), again where little growth of new chambers and some signs of dissolution was observed. Image C1 & C2 are the EPMA images of specimens from the 8.1 pH treatment (sample name: RNCR2-1; RNCR2-1) where new growth of chambers can be seen; newly grown chambers are very close together.
4.3.2 EPMA Data Analysis

Two specimens from each pH treatment were imaged using EPMA and an outline of inner, middle and outer areas was drawn to locate points for analysis (Fig. 4.6, 4.7.4.8). Using the images of the two specimens from each pH treatment (Fig 4.5), areas for analysis were marked for each (Fig 4.6–4.8). An outline of inner, middle and experimental growth areas for analysis was drawn as indicated by the blue lines in each figure, and the red triangles indicate the points analyzed. Spectral data were generated from each point. Examples of spectral data from one analysis point for each treatment are shown in Figure 4.9. Relative concentrations of Mg, S, Ca, and Sr were extracted from each spectral analysis, and averaged for each layer for each specimen examined (Table 4.1). From those data, Mg/Ca and S/Ca ratios were calculated; the Sr/Ca ratios were not calculated because Sr was close to or below detection limits in most foraminifer specimens.
Figure 4.6: Outline of areas of each specimen cultured at pH 7.5 that was analyzed and points of analysis of RNAR3-1 (a) and RNAR2-5 (b)
Figure 4.7: Outline of areas of each specimen cultured at pH 7.8 that was analyzed and points of analysis of RNBR2-1 (a) and RNBR3-2 (b).
Figure 4.8: Outline of areas of each specimen cultured at pH 8.1 that was analyzed and points of analysis of RNCR3-1 (a) and RNCR1-1 (b)
Figure 4.9: Peaks generated by EPMA while analyzing *M. vertebralis* cultured at (a) pH 7.5; (b) pH 7.8; (c) pH 8.1. The accelerating voltage was 16KeV with a takeoff angle of 40°. The analysis time for the generation of peaks was 100 seconds.
Table 4.1 Relative concentrations of Mg, S, Ca, P, Na and Sr extracted from each spectral analysis, and averaged for each layer for each specimen examined. From these data, Mg/Ca and S/Ca ratios were calculated.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Specimen #</th>
<th>Layer</th>
<th>Mg</th>
<th>S</th>
<th>Ca</th>
<th>P</th>
<th>Na</th>
<th>Sr</th>
<th>Mg/Ca</th>
<th>S/Ca</th>
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<tr>
<td>7.5</td>
<td>RNAR3-1</td>
<td>Inner</td>
<td>8.18</td>
<td>0.9</td>
<td>90.5</td>
<td>0.062</td>
<td>0.29</td>
<td>0.00</td>
<td>0.0903</td>
<td>0.00994</td>
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<td></td>
<td></td>
<td>Middle</td>
<td>6.67</td>
<td>1.08</td>
<td>91.9</td>
<td>0.14</td>
<td>0.16</td>
<td>0.00</td>
<td>0.0725</td>
<td>0.0117</td>
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<td>Outer</td>
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<td>0.853</td>
<td>91.6</td>
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<td>0.283</td>
<td>0.00</td>
<td>0.0779</td>
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<td>RNAR2-5</td>
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<td>0.19</td>
<td>22.3</td>
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<td>0.12</td>
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<td>7.34</td>
<td>0.95</td>
<td>91.3</td>
<td>0.00</td>
<td>0.37</td>
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<td>0.2</td>
<td>22.5</td>
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<td>0.1</td>
<td>0.01</td>
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<td>0.153</td>
<td>0.0367</td>
<td>0.114</td>
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<td>RNCR1-1</td>
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<td>30.9</td>
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<td>0.125</td>
<td>0.00</td>
<td>0.113</td>
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<td>0.005</td>
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<td>30.5</td>
<td>0.05</td>
<td>0.147</td>
<td>0.01</td>
<td>0.0908</td>
<td>0.00907</td>
</tr>
</tbody>
</table>

4.3.3 Variations in elemental ratios in relation to \([\text{CO}_3^{2-}]\)

The Mg/Ca and S/Ca ratios from the points analyzed in the outer layer of each specimen (Table 4.1), which represented growth in culture, were compared with the carbonate concentrations in the culture media \([\text{CO}_3^{2-}]\) in Figure 4.9. The Mg/Ca ratios were directly correlated with \([\text{CO}_3^{2-}]\), while the S/Ca ratios were inversely correlated.
Figure 4.10 Mean Mg/Ca (mmol/mol) against $\text{CO}_3^{2-}$ (a) and mean S/Ca (mmol/mol) against $\text{CO}_3^{2-}$ (b). The Mg/Ca ratio (mmol/mol) increased with increasing $\text{CO}_3^{2-}$ with a $R^2$ value of 1. The opposite trend was observed for S/Ca ratio (mmol/mol) against with a $R^2$ value of 0.685.
4.4 DISCUSSION

Elemental ratios of foraminiferal shells have become a vital tool in estimating past oceanic conditions (Martin et al., 1999; Russell et al., 2004; Dissard et al., 2009). The magnesium/calcium (Mg/Ca) ratio in the shells of foraminifera is widely used as a paleo-temperature proxy in paleoceanography because the magnesium content of calcite is a function of its precipitation temperature (Elderfield et al., 2006). However, most studies have considered responses of hyaline taxa, especially low Mg-calcite species, while relatively few have looked at the high Mg-calcite porcelaneous taxa. Major differences in Mg/Ca ratios among lineages can represent different biomineralization pathways that developed during the evolution of the Foraminifera (Bentov and Erez, 2006).

In this study, micro-distributions of selected elements in the shells of the porcelaneous species, Marginopora vertebralis, cultured at three pH ($p$CO$_2$) levels, were examined to determine if water chemistry influenced incorporation of those elements from seawater. Prior to the experiment, M. vertebralis specimens were marked with Calcein, which helped in distinguishing the unstained areas of growth using confocal microscopy. According to Dissard et al. (2009), the fluorescent marker Calcein does not affect Mg and Sr incorporation into foraminiferal calcite.

The elemental measurements were designed to examine three different stages of growth of the specimens: inner (embryonic), middle (juvenile, pre-treatment) and experimental growth. In theory, the inner and middle stages should depict the natural environment in which the M. vertebralis were living before they were collected for the experiment. The unstained outer chambers were added during the experimental treatments. Indeed, experimental results from this study showed that the Mg/Ca ratios in the outer layers were lower in the lower seawater pH treatments (Table 4.1, Fig 4.10). Moreover, the specimens cultured at pH 7.5 and 7.8 exhibited minimal growth (see Chapter 2) and even some dissolution (Fig 4.5). Thus, the Mg/Ca differences across the layers may have been diminished by dissolution. Engel (2010) found that Amphisorus hemprichii that were partly dissolved by acidic waters exhibited a detectable decline in Mg/Ca ratios, which she predicted because Mg is more soluble than Ca at reduced pH.
The results of the present study on the response of *M. vertebralis* in relation to increasing ocean acidity are in contrast to a study by Lea *et al.* (1999) on two species of planktonic foraminifera, *Globigerina bulloides* and *Orbulina universa*, which indicated that seawater pH has secondary influence on the shell Mg/Ca ratio and that a decrease in the Mg/Ca ratio was noted with an increase in pH.

Studies have proposed that benthic foraminiferal Sr/Ca ratio decreases with increasing calcification rate (Elderfield *et al.*, 1996), increasing post-depositional dissolution (McCorkle *et al.*, 1995), and increasing pressure (Rosenthal *et al.*, 1997). Higher pH appeared to increase shell Sr/Ca through the kinetic influence of calcification (Lea *et al.*, 1999). However, it was not possible to show the relationship of Sr to pH or [CO$_3^{2-}$] as the Sr concentrations in *M. vertebralis* were too low to be measured by the instrument (Jeol, Jxa -8600 Superprobes) used in this study.

Erez (1994) cultured the benthic foraminifera *Amphistegina lobifera* at the salinity of 35‰ and seawater pH adjusted between 7.9 and 8.4, with results showing a linear relationship between the sulfate content and pH, with higher S/Ca ratio at lower pH. The cultured *M. vertebralis* from the present study support the expected inverse relationship of foraminiferal S/Ca ratio with pH or [CO$_3^{2-}$] shown by Erez (1994).

The results from this study revealed that porcelaneous high Mg-calcite foraminifera may respond differently from the hyaline low-Mg calcite foraminifera (such as *M. vertebralis*) with respect to environmental pH. The hyaline taxa actively concentrate Ca$^{2+}$ in vacuoles (Erez, 2003), and may actively remove Mg$^{2+}$ (Mewes *et al.*, 2014). The porcelaneous taxa appear to concentrate seawater. Their increase in shell Mg/Ca ratios with increased temperature is well-documented (Raja *et al.*, 2005) and may be associated with increased carbonate saturation, which would be consistent with the current results that show Mg/Ca ratios declining as [CO$_3^{2-}$] and pH declined.
CHAPTER 5 ELEMENTAL CONCENTRATIONS USING LA-ICPMS

5.1 INTRODUCTION

5.1.1 PURPOSE

The present study focused on changes in physical proxies as the seawater chemistry is altered, to see the response of shell chemistry of Marginopora vertebralis to a decrease in pH or [CO$_3^{2-}$]. B/Ca, Mg/Ca, Sr/Ca ratios, $^{10}$B/$^{11}$B (denoted $\delta^{11}$B) and $^{88}$Sr/$^{86}$Sr (denoted $\delta^{88/86}$Sr) from cultured specimens described in Chapters 2 and 3 were examined. Although the dominant parameter that has been studied as the cause of variation in Mg/Ca ratios in benthic foraminiferal shells is temperature (Rosenthal et al., 1997; Barker et al., 2003; Russell et al., 2004), this study focused on the effects of lower seawater pH on Mg/Ca ratios in M. vertebralis, while keeping the temperature constant.

5.1.2 BACKGROUND

Large climatic changes have occurred in the near geological past (Pleistocene, early Holocene). The sea surface and deep-sea records of stable isotopes in foraminiferal shells, as well as those of major and trace elements, provide proxy information for paleotemperatures, paleocirculation and paleochemistry of the oceans (Emiliani, 1955; Broecker and Peng, 1989, 1982; Boyle, 1988; Bijma et al., 1999). Such paleoceanographic information is essential for the tests and calibrations of global circulation models trying to predict the response of the atmosphere-ocean system to changes such as increase in atmospheric CO$_2$ (Archer and Maier-Reimer, 1994).

To interpret past environments through the use of proxies, it is necessary to associate these proxies with specific environmental conditions. This is done by measuring these proxies under different conditions in living specimens. To identify past oceanic environments, analyses of the elemental compositions of foraminiferal shells are widely used (Martin et al., 1999; Russel et al., 2004).

Nutrient proxies (Cd and Ba), physical proxies (Mg, Sr and $^{10}$B/$^{11}$B) and chemical proxies (Li, U, V, $^{87}$Sr/$^{86}$Sr and $^{142}$Nd/$^{146}$Nd) are three groups of elemental proxies that
are expressed as a trace element/Ca ratio (Lea, 1999). Chemical proxies include those elements whose variability in shells is primarily controlled by changes in the ocean carbonate chemistry, while nutrient proxies provide information on seawater nutrients. Physical proxies include those elements whose variability is primarily affected by physical factors such as pressure, temperature, salinity and pH. Both Sr and B have important isotope systems that can be assessed using foraminiferal shells (Lea, 2003). Strontium has four isotopes, 84 (~0.56), 86 (~9.87), 87 (~7.04), and 88 (~82.53), having the isotopes of 84, 86, 88 as stable isotopes, in contrast to 87 on geological time scales (Veizer, 1989). Foraminifera actively precipitate their shells, affecting the chemistry and structure of shell calcite. For example, calcification rate increases with rising pH, which increases the incorporation of Sr into calcite (Morse and Bender, 1990). As described by Pingitore (1986), there are two general mechanisms for trace element incorporation in foraminiferal calcite: direct solid solution (e.g., trace element substitutes directly for Ca\(^{2+}\) in the calcite structure) and trapping (e.g., trace elements occurring as adsorbed ions).

5.2 METHODOLOGY

Experimental specimens of *M. vertebraulis* were maintained under constant temperature, pressure and salinity at three different pH levels (7.5, 7.8 and 8.1, as described in Chapters 2 and 3). After Calcein marking, calcite deposited under experimental conditions was easily identified using confocal microscopy (as described in Chapter 4) and analyzed for isotopes of Ca, B, Mg, and Sr using Laser Ablation ICP-MS. Factors that are known to influence life activities in foraminifera, such as light, temperature, salinity and pH, were carefully controlled during culture experiments. Temperature and pH were monitored every 2\(^{nd}\) day to ensure stable conditions.

For analysis with LA-ICPMS, two specimens were examined per treatment. Twelve points were ablated per specimen along the growth marked area (see Fig 5.1). To prepare specimens for analysis, they were soaked in 3-7% NaOCl solution for 30 mins to remove the organic matter (Dissard *et al.*, 2010b). Specimens were removed from the NaOCl solution as soon as they bleached completely. A stereomicroscope (low magnification)
was used to observe the specimens during the cleaning process. Immediately after removal from NaOCl solution, the samples were rinsed with deionised water to ensure complete elimination of reagent, as recommended by Dissard et al. (2010b).

The newly formed chambers of *M. vertebralis* (identified using confocal microscopy) were ablated using a laser ablation system (Figs. 5.2, 5.3) inside an ablation chamber flushed with helium (similar procedure as used by Dissard et al., 2010b).

Elemental concentrations were determined using the UP193-FX Excimer laser ablation system (Fig 5.4) at the laboratory in Freddy and Nadine Herrmann Institute of Earth Sciences, The Hebrew University of Jerusalem (Givat Ram/Israel). Analyses were calibrated against JCp-1 (coral), JCT-1 (clam) and Arag. AK Standards. Samples and standard solutions were systematically adjusted to 100 ppm Ca through dilution to (1) avoid dominant Ca signal increasing the salt deposition on cones and affecting the ICP-MS stability, and (2) adjust the Ca concentrations being introduced in the ICP-QMS to control Ca matrix effects. To compensate for signal derivation, standards (JCp-1, and JCT-1 and Arag. AK) were run on every five and ten samples, respectively. Instrumental calibration was achieved using standard solution for each element (Harding et al., 2006) and by routinely measuring carbonate standards (JCp-1, JCT-1 and Arag. AK; Inoue et al., 2004). The UP193 Laser Ablation System coupled effectively and transferred its photon energy directly to the sample matrix for accurate and precise measurement of trace elements and isotopic compositions (Table 5.1). The samples were bombarded via

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Figure 5.1 Example of *M. vertebralis* specimen (a) before (b) after analysis with LA-ICPMS. The arrows show the path of analysis.
UP193 Laser Ablation System for polishing and the distance cleaned was 600 microns. Before ablating the sample, the background of each standard was measured, this took 40 seconds.

<table>
<thead>
<tr>
<th>Table 5.1: Laser Ablation ICP-MS operating parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard</strong></td>
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<td>Repetition frequency</td>
</tr>
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</tr>
<tr>
<td>Power/energy input</td>
</tr>
<tr>
<td>70%</td>
</tr>
<tr>
<td>Ablation pattern:</td>
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<tr>
<td>Line, 50 μm spot</td>
</tr>
<tr>
<td>Scan speed</td>
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<tr>
<td>3 μm/sec</td>
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</table>

Figure 5.2 Shows a laser - ablation inductively - coupled - plasma mass - spectrometry (LA-ICP-MS) Instrument Control setup (image captured from the computer screen while preparing ICP-MS for analyses of *M. vertebralis*).
Figure 5.3 Typical transient LA-ICP-MS signal from different elements while analyzing for elemental concentration in *Marginopora vertebralis* (in Freddy and Nadine Herrmann Institute of Earth Sciences, The Hebrew University of Jerusalem (Givat Ram/ Israel)).

Figure 5.4 Shows UP193-FX Excimer laser ablation system in Freddy and Nadine Herrmann Institute of Earth Sciences, The Hebrew University of Jerusalem (Givat Ram/ Israel)
5.2.1 Laser Ablation ICP-MS Analysis with SILLS

The Laser Ablation ICP-MS results were analyzed with SILLS (Signal Integration for Laboratory Laser Systems) software version: V 1.1.0. SILLS is a data processing package based on MATLAB (Figs. 5.5, 5.6).

Spike elimination (based on Grubbs test) was conducted by SILLS (Fig 5.7) where the outliers were detected and replaced with calculated and suggested values obtained from the analyses of M. vertebralis using ICP-MS.
After the standards and unknowns were loaded in SILLS, calculations were carried out and calibration graphs were plotted (Fig 5.8). Then Element/Ca^{2+} ratios were calculated, and means and standard errors were calculated and recorded.

**Figure 5.8 Calibration Graphs window**

**PLOT 1: RELATIVE SENSITIVITY:** The graph in the top-left compares the sensitivity of any two elements, i.e. plots cps/ppm for isotope A (eg 88Sr) vs. cps/ppm for isotope B (eg 43Ca). A separate line is shown for each standard measured. The relative sensitivity of different isotopes was compared by selecting them from the drop-down lists.

**PLOT 2: DRIFT IN RELATIVE SENSITIVITY:** The graph in the top-right shows the relative sensitivity of isotope A (eg 88Sr) and isotope B (eg 43Ca) as a function of time (as specified by the user). White dots represented the standards and yellow dots represented the unknowns. The slope is the least-squares regression line through the standard data, with each standard weighted equally.

**PLOT 3:** The table comprises of the standard names and the time each analysis. The values of relative sensitivities of element/Ca were calculated using SILLS.

**PLOT 4: % DRIFT IN RELATIVE SENSITIVITY:** The bar graph in the bottom-right shows the percentage drift in each isotope (based on the calculated drift between the first and last measured standard).
The $^{88}\text{Sr}/^{86}\text{Sr}$ and $^{11}\text{B}/^{10}\text{B}$ values were calculated for the *M. vertebralis* samples to study how these stable isotope systems may be affected by increasing acidity of seawater. To be consistent with notations for strontium isotopes, as reported by Fietzke and Eisenhauer (2006) and Krabbenhöft *et al.* (2010), and boron isotopes (Sanyal *et al.*, 1995; Rollion-Bard and Erez, 2010), the results are expressed in terms of the $\delta$ notation:

$$\delta^{11/10}\text{B} = \left\{\left[\frac{(^{11}\text{B}/^{10}\text{B})_{\text{sample}}}{(^{11}\text{B}/^{10}\text{B})_{\text{standard}}}\right] - 1\right\} \times 1000$$

and

$$\delta^{88/86}\text{Sr} = \left\{\left[\frac{(^{88}\text{Sr}/^{86}\text{Sr})_{\text{sample}}}{(^{88}\text{Sr}/^{86}\text{Sr})_{\text{NBS987}}}\right] \times 1000 - 1000\right\}.$$
5.3 RESULTS

The mean values of the elemental composition for two specimens (12 ablation points per specimen) of *M. vertebralis*, assessed in shell produced under each pH treatment, are presented in Table 5.2, along with the ratios for standard associated with those analyses. The resulting element/Ca ratios, calculated from the data in Table 5.2, are summarized in Table 5.3. Because the standards are for aragonite and low-Mg calcite, their values for Mg$^{24}$/Ca$^{43}$ and for Mg$^{26}$/Ca$^{43}$ are substantially lower than those for the experimental data, across treatments.

<table>
<thead>
<tr>
<th>pH 7.5</th>
<th>10 B</th>
<th>11 B</th>
<th>24 Mg</th>
<th>26 Mg</th>
<th>43 Ca</th>
<th>46 Ca</th>
<th>86 Sr</th>
<th>88 Sr</th>
</tr>
</thead>
<tbody>
<tr>
<td>standard</td>
<td>126.7</td>
<td>299.2</td>
<td>254.17</td>
<td>275.84</td>
<td>664.18</td>
<td>985</td>
<td>478</td>
<td>46.7</td>
</tr>
<tr>
<td>sample</td>
<td>75</td>
<td>136.1</td>
<td>183.33</td>
<td>165.74</td>
<td>283.34</td>
<td>460.2</td>
<td>259</td>
<td>45.4</td>
</tr>
<tr>
<td>pH 7.8</td>
<td>standard</td>
<td>118.7</td>
<td>385</td>
<td>198.9</td>
<td>279.12</td>
<td>349.46</td>
<td>803.3</td>
<td>2123</td>
</tr>
<tr>
<td>sample</td>
<td>72.73</td>
<td>159.1</td>
<td>176.37</td>
<td>114.55</td>
<td>148.18</td>
<td>358.2</td>
<td>296</td>
<td>36.4</td>
</tr>
<tr>
<td>pH 8.1 (control)</td>
<td>standard</td>
<td>73.42</td>
<td>311.4</td>
<td>120.25</td>
<td>154.43</td>
<td>98.735</td>
<td>260.8</td>
<td>624</td>
</tr>
<tr>
<td>sample</td>
<td>65.09</td>
<td>223.6</td>
<td>114.15</td>
<td>121.7</td>
<td>99.057</td>
<td>218.9</td>
<td>539</td>
<td>25.5</td>
</tr>
</tbody>
</table>
Table 5.3. Mean values of Element/Ca ratios of *M. vertebralis* in shell produced under each pH treatment. The values are in mmol mol$^{-1}$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$^{10}$B/$^{43}$Ca</th>
<th>$^{11}$B/$^{43}$Ca</th>
<th>$^{24}$Mg/$^{43}$Ca</th>
<th>$^{26}$Mg/$^{43}$Ca</th>
<th>$^{86}$Sr/$^{43}$Ca</th>
<th>$^{88}$Sr/$^{43}$Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.5</td>
<td>ratio mean</td>
<td>0.000259</td>
<td>0.00156</td>
<td>21.03</td>
<td>3.06</td>
<td>0.641</td>
</tr>
<tr>
<td></td>
<td>standard error</td>
<td>0.000026</td>
<td>0.0000548</td>
<td>0.0523</td>
<td>0.00821</td>
<td>0.00179</td>
</tr>
<tr>
<td>pH 7.8</td>
<td>ratio mean</td>
<td>0.000272</td>
<td>0.00149</td>
<td>22.6</td>
<td>3.29</td>
<td>0.586</td>
</tr>
<tr>
<td></td>
<td>standard error</td>
<td>0.000012</td>
<td>0.0000257</td>
<td>0.0769</td>
<td>0.0109</td>
<td>0.00193</td>
</tr>
<tr>
<td>pH 8.1</td>
<td>(control) ratio</td>
<td>0.000377</td>
<td>0.00217</td>
<td>24.4</td>
<td>3.52</td>
<td>0.532</td>
</tr>
<tr>
<td></td>
<td>standard error</td>
<td>0.000009</td>
<td>0.0000265</td>
<td>0.0502</td>
<td>0.00751</td>
<td>0.00125</td>
</tr>
</tbody>
</table>

The mean $^{10}$/Ca$^{43}$ and $^{11}$/Ca$^{43}$ ratios for shell produced under each pH treatment are presented in Figure 5.8, revealing linear increases in ratios with increasing pH. The slopes for the two regressions indicate a roughly 5 times faster increase in the $^{11}$/Ca$^{43}$ ratios as compared with the $^{10}$/Ca$^{43}$ ratio. The Mg$^{24}$/Ca$^{43}$ and Mg$^{26}$/Ca$^{43}$ ratios similarly revealed increases with increasing pH (Fig 5.9), with the ratio for Mg$^{24}$/Ca$^{43}$ increasing roughly 7 times faster than that for Mg$^{26}$/Ca$^{43}$. The Sr/Ca ratios (Fig 5.10) in the shells produced in the experimental treatments revealed negative correlations with pH, with the ratio for the heavier isotope (Sr$^{88}$/Ca$^{43}$) declining nearly 10 times faster than the ratio for the lighter isotope (Sr$^{86}$/Ca$^{43}$). Given these fractionation differences between isotopes of B, Mg and Sr (Figs 5.8–5.10), changes in isotopic ratios with pH were calculated for B ($\delta^{10/11}$B) and Sr ($\delta^{88/86}$Sr); both decreased with increasing pH (Table 5.4, Figs 5.11 and 5.12).
Fig 5.8: Mean B/Ca plotted against pH of culture media. B/Ca data represent means of all measurement for specimens from that treatment.

Fig 5.9: Mean Mg/Ca plotted against pH of culture media. Mg/Ca data represent means of all measurement for specimens from that treatment.
Table 5.4 Mean values of the Sr and B isotopic compositions of *M. vertebralis* cultured at three different pH treatments. The values are expressed as ‰. The number of analyses on *M. vertebralis* from each treatment was 12.

<table>
<thead>
<tr>
<th>pH treatment</th>
<th>$\delta^{11}B$</th>
<th>$\delta^{88/86}Sr$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH treatment 7.5</td>
<td>0.551</td>
<td>0.17</td>
<td>12</td>
</tr>
<tr>
<td>pH treatment 7.8</td>
<td>0.457</td>
<td>0.12</td>
<td>12</td>
</tr>
<tr>
<td>pH treatment 8.1 (control)</td>
<td>0.291</td>
<td>0.05</td>
<td>12</td>
</tr>
</tbody>
</table>

Fig 5.10: Mean Sr/Ca plotted against pH of culture media. Sr/Ca data represent means of all measurement for specimens from that treatment.

Fig 5.11: The $\delta^{11}B$ of *M. vertebralis* increases with increasing seawater acidity.
Fig 5.12: The $\delta^{88/86}$Sr of M. vertebrales increases with increasing seawater acidity.
Foraminifera are organisms that precipitate their shells by complex physiological processes that are biologically controlled (Lowenstam and Weiner 1989). As a consequence, most paleoceanographic proxies, such as isotopic and elemental ratios in shells, are affected to some degree by these processes during the lives of the organisms (e.g., foraminifera, coccolithophores, and corals). Such influences are called “vital effects” and are influenced by respiration, symbiont photosynthesis and biomineralization processes (Dissard et al., 2010a). Moreover, as noted in Chapter 4, major differences in Mg/Ca ratios between lineages can represent different biomineralization pathways that developed during the evolution of the Foraminifera (Bentov and Erez, 2006). The porcelaneous Miliolida produce Mg-calcite relatively close to equilibrium with seawater, while many (but not all) hyaline Rotalida and Globigerinida produce low Mg-calcite of generally less than 4 micromol Mg/mol Ca.

The use of controlled laboratory cultures can provide insights into the controls on foraminiferal shell chemistry (Filipsson, 2008). Differences in the rates of calcification are often used to explain the vital effects on isotopes and trace elements (Boyle, 1995; Elderfield et al., 1996), as the effects of these physiological processes are observed in stable isotopes, as well as concentrations of major and trace elements (Erez, 1978; Erez and Honjo, 1981; Boyle, 1995; Spero et al., 1997; Weiner and Dove, 2003). Trace elements are incorporated directly from seawater during shell precipitation, reflecting both seawater composition and the physical and the biological conditions present during precipitation (Sen Gupta, 2003).

The Mg/Ca ratio varies also within single individual foraminiferal shells (Toyofuku and Kitazato, 2005) and through chamber walls (Eggins et al., 2004; Sadekov et al., 2005; Kunioka et al., 2006). Different parts of a shell also can have different concentrations of trace elements (Eggins et al., 2003), reflecting physiological and environmental differences that occur during the life of the specimen. This is particularly true in larger benthic foraminifera such as M. vertebralis that can live for a year or more, so calcify under a seasonal range of conditions. Moreover, even specimens grown under identical
conditions have revealed individual differences in both Mg and Sr composition (Reichart et al., 2003; Dissard et al., 2010a, b). Thus, natural variability in Mg/Ca ratios can be expected in the results such as those reported here.

Interest in using shell the Mg/Ca ratios as a proxy of sea surface temperature (SST) intensified in the 1990’s. In benthic foraminifera, Mg/Ca ranges from 0.5 to more than 10 mmol/mol had been observed, with the highest values in shells from the shallowest (warmest) sites (Rathburn and Decker, 1997; Rosenthal et al., 1997b). Moreover, it has long been known that the porcelaneous foraminifera produce Mg-calcite, and can incorporate more than 20 mmol Mg per mol Ca (Rathburn and Decker, 1997). In the present study utilizing the porcelaneous, tropical benthic foraminifera, *M. vertebralis*, mean Mg/Ca ranged from 21.0 to 24.4 mmol/mol (Table 5.2), with the highest values in shells from the 8.1 pH treatment (control).

Elderfield et al. (2006) presented the carbonate ion hypothesis, which proposed that lower \([\text{CO}_3^{2-}]\) results in lower Mg/Ca in benthic foraminifera. The results obtained in this study for *M. vertebralis* are consistent with this hypothesis. Mean Mg/Ca ratios, from multiple spot measurements on the experimental *M. vertebralis*, confirm that seawater acidification (lower pH) is an important factor controlling Mg uptake into porcelaneous foraminiferal shells (Fig 5.9). As noted in Chapter 4, the results for *M. vertebralis* are in contrast to results reported by Lea et al. (1999) on two species of planktonic foraminifera, *Globigerina bulloides* and *Orbulina universa*, who reported that seawater pH had secondary influence on the shell Mg/Ca ratio and that a decrease in the Mg/Ca ratio was noted with an increase in pH.

During the deposition of calcium carbonate, the amount of magnesium (Mg) incorporated into the mineral matrix can also alter the properties of the shell (Lin and Dexter, 1988; Kuffner et al., 2007; Engel et al., 2015; Knorr et al., 2015). Skeletons with significant amounts of magnesium incorporated into the matrix (greater than 12%) are more soluble, so the presence of this mineral can negatively impact shell durability (Kuffner et al., 2007; Ferguson et al., 2008). As seawater pH declines in the future as
atmospheric and oceanic $pCO_2$ increase, the Mg/Ca ratios in calcite shells will decrease, actually reducing solubility, and increasing strength of high Mg-calcite shells.

The Sr/Ca ratio is commonly measured along with Mg/Ca ratio in foraminifera. The Sr/Ca ratios in *M. vertebralis* shells grown under controlled pH showed a decrease with increasing pH (Fig 5.10).

An 80-million-year record of shell Sr concentrations has indicated that oceanic Sr concentrations have changed significantly, recording processes such as tectonic uplift and continental erosion (Delaney and Boyle, 1986). Moreover, studies have found that, during glacial advances, benthic foraminiferal Sr/Ca ratios were elevated by 3-6% and the changes varied among species and locations (Martin *et al*., 1999; Shen *et al*., 2001). Because the seawater Sr/Ca ratio remained near constant on glacial-interglacial timescales during the late Pleistocene, Yu *et al*., (2014) suggested that Sr/Ca in deep-sea benthic foraminifera may be used as a secondary proxy for deep water changes in $[CO_3^{2-}]$. Studies on benthic foraminifera have demonstrated that there is a linear decrease in benthic shell Sr with water depth suggesting that pressure is the most likely cause of change with depth in the oceans (Elderfield *et al*., 1996; Rosenthal *et al*., 1997b).

The Sr/Ca ratio is also used as a proxy for sea-level fluctuations. Lower Sr concentrations in seawater reflect high rates of deposition of aragonite on continental shelves (Schlanger, 1988) since Sr preferentially substitutes into the aragonite crystal lattices. The $^{87}$Sr/$^{86}$Sr ratio of the oceans, as recorded in foraminifera shells and bulk deep-sea carbonates, has risen from about 0.7082 (24 Mya) to 0.7091 at present (Lea, 2003). Raymo and Ruddiman (1992) hypothesized that the cause of this increase is due to the changes in the isotopic composition and flux of Sr from rivers draining uplifted continental areas, which includes rocks with high $^{87}$Sr/$^{86}$Sr. The $\delta^{88/86}$Sr of water masses can provide additional knowledge about ocean circulation and biomineralization processes (Fietzke and Eisenhauer, 2006).

The two isotopes of strontium recorded in this study in cultured *M. vertebralis* shells were $^{86}$Sr and $^{88}$Sr. Given the projected decline in ocean pH by 2100 (8.1 to 7.5), the $^{88}$Sr/$^{86}$Sr ratio in *M. vertebralis* could increase from 0.05 to 0.17 (Table 5.4, Fig 5.12).
Another set of useful geochemical proxies in environmental reconstructions are the boron isotopes, which are used to determine pH changes in the ocean water (Sanyal and Bijma, 1999), using B as a pH proxy on the isotopic composition of skeletal carbonates (Rollion-Bard and Erez, 2010). The ratio of \(^{10}\text{B}\) to \(^{11}\text{B}\) does not vary within the ocean (Lea, 2003), but boron exists as a mixture of the two species, borate \([\text{B(OH)}_4^-]\) and boric acid \([\text{B(OH)}_3^-]\), the proportions of which are pH dependent. Boron isotopic measurements of marine biogenic carbonate provide a tool for estimating paleo-pH of seawater because in modern oceans, borate is proportional to pH (Sanyal et al.; 2001; Yu et al., 2007, Kaczmarek, 2015). Both the \(^{10}\text{B}/\text{Ca}\) and \(^{11}\text{B}/\text{Ca}\) ratios increased linearly with increasing pH (Fig 5.8), though the \(^{11}\text{B}/\text{Ca}\) ratio increased faster. As a consequence, the boron isotope ratio, \(^{10}\text{B}/^{11}\text{B}\), in cultured specimens of \(M.\ vertebralis\) decreased with increasing pH (Fig 5.11).

As noted in Chapter 4 and demonstrated again in this chapter, porcelaneous Mg-calcite foraminifera may respond differently from the hyaline low-Mg calcite foraminifera with respect to environmental pH. The hyaline taxa actively concentrate Ca\(^{2+}\) in calcifying vacuoles (Erez, 2003), and may actively remove Mg\(^{2+}\) (Mewes et al., 2014). The porcelaneous taxa do not, so their well-documented increases in shell Mg/Ca ratios with increased temperature (Raja et al., 2005) may be associated with the increased carbonate saturation, which would be consistent with the results from this study that show Mg/Ca ratios declining as [CO\(_3\)^{2-}\] and pH declined.
CONCLUSIONS

The experimental results from the present study showed that calcification and growth rate of *Marginopora vertebralis* decreased with decreasing pH, and the shell weight was closely related to changing pH between 7.5 and 8.1 (NBS scale). This tendency of the growth rate of *M. vertebralis* to decrease with decreasing pH is consistent with the results reported for many other calcifying marine organisms. In addition, *M. vertebralis* showed a decrease growth in response to a change in pH from 8.1 to 7.5, in par with other organisms that also display a decrease in growth due to the elevated *pCO₂* at around this pH range. The results also showed that the calcification and growth rates were similar between pH 7.5 and 7.8, and substantially less than observed at pH 8.1 (control).

The laboratory experiment showed that calcification of *M. vertebralis* will likely decrease in response to the projected (IPCC, 2013) future increases in atmospheric CO₂. This study also supports the observation that CO₃²⁻ is the limiting factor in *M. vertebralis* calcification in relation to increasing *pCO₂*.

The calcification rate of *M. vertebralis* will decline substantially if pH drops to 7.5 or lower; such conditions may not allow the survival of this species unless it has time to adapt over several generations.

*Marginopora vertebralis* is a major contributor to sediment for Fijian beaches and its diminished calcification rate could have serious consequence on the beach stability, especially as sea level rises. When calcification rates of the major calcifers in the ocean, including foraminifera, decline, the result is a small negative feedback mechanism to global CO₂ increase (Bentov *et al.*, 2009).

EPMA-corrected and LA-ICP-MS data are used to compare the Mg/Ca, Sr/Ca, Sr and B isotope ratios in *M. vertebralis* shells produced under three pH levels. Comparison of Mg/Ca and Sr/Ca compositions obtained by both methods against pH suggests that
Mg/Ca increases and Sr/Ca decreases with increasing pH. These results clearly indicate that if ocean acidification (lower pH and [CO$_3^{2-}$]) continues, the Mg/Ca ratio will decrease and Sr/Ca ratio will increase in _M. vertebralis_. Similarly, $\delta^{11}$B and $\delta^{88/86}$Sr ratios increased with increasing ocean acidity.

Since it is possible to calibrate the shell composition against the controlling factors, foraminiferal trace elements can provide researchers with vital and relevant proxies to investigate the physical, biological and chemical changes in the ocean.

6.1 FUTURE RESEARCH TOPICS

- Future studies may seek to culture other important species of foraminifera around Fiji and compare their response to increasing ocean acidity.
- Future studies could look at other parameters that may affect the inclusion of major, minor and other trace elements in the _M. vertebralis_ shells.
- Modeling and documentation of carbonate production differences in Foraminifera in a historical context, along with present and future projections will be vital to determine coastal protection and conservation strategies.
- Further developmental work in high purity sample preparation should be done to improve the analytical reproducibility in the isotope analysis of benthic foraminiferal shells, where each element can be examined in more detail.
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