HIGH SPATIAL-RESOLUTION ANALYSIS
OF TRACE ELEMENTS IN CORALS USING
LASER ABLATION ICP-MS

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The work presented in this thesis was carried out while I was a full-time student at the Research School of Earth Sciences, The Australian National University, between April 1994 and March 1999. Except where mentioned in the text, the research described here is my own. No part of this thesis has been submitted to any other university or similar institution.

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This research centres around the development and application of laser-ablation ICP-MS for quantitative analysis of B, Mg, Sr, Ba, and U in *Porites* coral skeletons. Methodology for laser ablation analysis of corals is not well established. A method has therefore been developed in which the laser beam is scanned continuously across the surface of both sample and standard to generate continuous profiles of trace element variation. The laser beam is masked to produce a long rectangular profile, which averages ablation across a range of structural features in the coral, while maintaining a high spatial resolution in the direction of the scan. A calcium silicate glass, made by fusing silica and coral powder, was used as a standard. This glass exhibited superior homogeneity, although matrix differences between it and the coral reduced accuracy and reproducibility.

At high spatial resolutions coral skeletons are extremely heterogeneous, with large trace element variations on sub-mm scales. While some of these variations may be attributed to contaminants, there is evidence to suggest that short-timescale physiological variations may be occurring. The factors influencing trace element concentrations in a coral are poorly understood, and a review is therefore presented that covers the inorganic chemistry of trace element coprecipitation, current theories regarding coral biomineralization, the physicochemical characteristics of biological precipitation from a supersaturated high pH microenvironment within the coral, the physiological factors affecting trace element transport through coral tissues to the calcifying environment, and the effects of gross polyp morphology on the distribution of elements within the skeleton. A second literature review summarises the observations of trace elements in coral skeletons, the mechanisms of incorporation, the fractionation relative to abiological aragonites, and the environmental and physiological parameters that are reported to influence elemental partitioning. These observations are compared with the theoretical factors, highlighting areas where current coral calcification models cannot adequately explain the observed trace element behaviour.

Measurements were made of a number of coral structural features at very high spatial resolutions. Patterns of trace element variation were not well correlated to visible structures within a coral, and did not appear to form clear 'horizons' of variation within a single corallite, or display clear symmetry across an end-section of a corallite. Several trace elements, however, were strongly correlated with each other, implying a consistent chemical fractionation. These interelement correlations could not all be accounted for by contaminant phases, suggesting a chemical variation in the calcifying fluid. Seasonal
variations in several trace elements displayed a similar correlation to fine-scale correlations, suggesting a mutual chemical link, yet fine-scale variations were too great to be accounted for by temperature or light intensity, and therefore neither of these parameters can be directly responsible for seasonal trends (although temperature may be mediating another chemical process).

The fully quantitative method was used to extract seasonal scale variations from a mid-reef coral, and these variations were calibrated against in-situ instrumental sea surface temperature (SST) records. Calibrations were in reasonable agreement with other calibrations reported in the literature, and LA-ICP-MS therefore has the potential to extract quantitative paleo-SST records from corals. The method is limited by the fine-scale variations which introduce uncertainty into the temperature reconstruction, and by the fact that the coral trace element record does not appear to be completely faithful to temperature, with other physiological or environmental factors influencing each trace element at high resolutions.

The fully quantitative method was also applied to analysis of an extended suite of trace elements in several coastal corals that are regularly subjected to fresh-water inundation during river floods. Barium, Y, La, and Ce were found to record river floods as enrichments in the coral skeleton, consistent with measurements made of a flood and coastal flood plume from the Tully River in North Queensland. Skeletal Ba can potentially provide quantitative discharge reconstructions; however, it is limited by a tendency to build up in the river catchments during dry seasons, and mobilise as a large pulse in the first river flood. Anomalous peaks in barium are also observed for some corals during spring. This phenomenon is difficult to explain, although a combination of environmental factors may trigger a physiological or ecological event that results in an enriched Ba phase being incorporated into the coral skeleton. Possibilities include coral spawning, or senescent Trichodesmium blooms.
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CHAPTER 1: INTRODUCTION

1.1 Introduction

1.1.1 Statement of Research

The aim of this research was to develop methodology for quantitative, high spatial-resolution analysis of trace elements (TEs) in Porites corals by laser-ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS), then to apply these methods to extract environmental information from skeletal trace element records, and to explore the chemistry and biochemistry of trace element deposition into coral skeleton.

1.1.2 Why Investigate Corals?

As corals grow they deposit an aragonitic skeleton, into which trace elements from the surrounding sea water are coprecipitated. The skeletal trace element concentrations are controlled by a number of factors, including the composition and temperature of the sea water. Corals therefore have the potential to act as an archive of the chemical and physical environment in which they are growing.

Corals deposit their calcareous skeletons at a relatively uniform rate, extending by around 1 - 2 cm a year in the case of Porites (Veron, 1986). The density of the skeleton they deposit varies seasonally, generating annual density bands that can be clearly seen in X-ray photographs of coral slices. Coral density bands can therefore be used to assign an accurate chronology to the coral, in much the same way that counting rings can be used to tell how old a tree is. In addition, the presence of other skeletal markers, such as fluorescent flood bands (Barnes and Taylor, 1998; Boto and Isdale, 1985; Scoffin et al., 1989; Susic et al., 1991), can be used to independently verify the chronology (Isdale and Parker, 1995). Corals therefore combine the dating accuracy of tree rings with the same potential to act as geochemical archives that is offered by sediment cores.

Individual Porites colonies have been known to grow continuously for up to 1,000 years (Isdale and Parker, 1995; Veron, 1986). The long records that these corals may contain span a critical period of recent human history, covering the dramatic increases in human population, the rise of industrialisation, and the arrival of European settlers to the
Chapter 1

southern Pacific. Corals are therefore well suited for providing high fidelity climate records with which to test anthropogenic influences on the global and local environments, such as global warming, pollution, and the impact of high-intensity agriculture along tropical coastlines. Being aragonite, coral skeletons preserve readily, and fossil corals are providing vital information on global climate from as far back as the penultimate deglaciation (McCulloch et al., 1999).

Coral records are all the more valuable because of the lack of other paleo-recorders in tropical regions (e.g. tree rings and ice-cores). The tropics, however, are an important environment, with most of the world's fresh water and sediments entering the oceans at tropical latitudes (Chester, 1990; Holland, 1978), and the warm waters of the western Pacific significantly influencing global climate.

While corals have been the subject of a considerable amount of research over the last decade, there remain many areas of coral geochemistry still to explore; including new chemical tracers, new locations and environments, and a greater understanding of the biochemical and biological processes influencing trace element coprecipitation.

1.1.3 Why High Resolution?

It is common for corals to be analysed at fairly low spatial resolutions (quarterly or annually Lea et al., 1989; Shen and Boyle, 1988; Shen et al., 1992; Shen and Dunbar, 1995; Shen and Sanford, 1990), partly because of the amount of work involved in analysing long coral records, and partly because of the practical limitations of sampling and measuring very small amounts of coral. As analytical and sampling methods have improved with time, it has been possible to analyse corals at increasingly finer resolutions, until a coral can now be analysed at close to daily resolution using microbeam methods (Allison, 1996b; Allison and Tudhope, 1992; Hart and Cohen, 1996).

There are three main reasons to analyse corals at very high spatial resolutions. Firstly, a number of interesting environmental processes occur on sub-monthly timescales. Examples include individual climatic events such as cyclones, intrusions of upwelled water, river floods, single discharges of pollutants, algal blooms, etc. Many of these events have durations of days to weeks only. In order to characterise such short-lived phenomena in a coral record, it is necessary to sample at a resolution that is significantly higher than the event.

The second reason to analyse skeletons at high resolution is to examine physiological events in the coral. Physiology is believed to have an influence on the trace element composition of the aragonite deposited by a coral (e.g. de Villiers et al., 1995; de Villiers et al., 1994); however, there is still much debate over the magnitude of such effects and how exactly physiology influences the skeletal chemistry. A number of critical
physiological processes occur on sub-monthly timescales, including diurnal variations in calcification rate, feeding, stress events, bleaching, and spawning. Examining coral skeletons at high resolutions may help to resolve these effects.

Finally, the bulk composition of coral skeletons may be significantly affected by the presence of finely dispersed micro-scale non-aragonitic contaminant phases, such as microborings, organic inclusions, centres of calcification and embedded particulates (Allison, 1996b; Allison and Tudhope, 1992). By examining skeletons at high resolution, it might be possible to determine which of these phases have the potential to affect coral trace element records.

1.1.4 Why Laser Ablation ICP-MS?

Laser ablation ICP-MS is a relatively new technique, which had yet to be applied to the analysis of corals. The equipment used for laser micro-sampling is capable of focusing an ultraviolet laser beam onto a spot as small as 20 μm in diameter; when coupled with a quadrupole ICP-MS, the technique can easily provide sensitive multi-element analysis of coral skeletons at daily resolution.

Laser ablation has a number of advantages over other micro-beam techniques such as ion and electron microprobe. Because the laser system uses long focal-length optics, ablation occurs with almost equal efficiency over depths of up to 1 mm, and there is consequently no need to impregnate coral samples with epoxy to block holes and pores within the structure. Sample preparation is therefore minimal, and the sampling is essentially non-destructive, allowing later milling of the coral for analysis by wet-chemical techniques. The laser can be masked to produce a number of shapes and sizes of laser spot, and ablation intensities can be varied, making LA-ICP-MS more flexible than other competing techniques. By slowly shifting the coral beneath the laser it is possible to 'scan' the sample surface, providing a continuous profile of trace element concentration with distance. Finally, the technique is very rapid compared with other methods, with 5 cm of coral taking approximately 1½ hours to analyse.

1.2 Summary of Research Presented

1.2.1 Part 1: Method Development

Chapters 2 - 4 of this thesis describe the development and testing of a fully quantitative method for analysis of B, Mg, Sr, Ba, and U in corals by LA-ICP-MS.
Why Fully Quantitative?

Trace elements coprecipitate with Ca into aragonite in proportion to the TE/Ca ratio in the precipitating solution. By measuring TE/Ca ratios in coralline aragonite it is therefore possible to quantitatively estimate the composition of the seawater in which the coral was growing (e.g. Lea et al., 1989; Shen et al., 1987; Shen and Sanford, 1990). A number of these trace elements show a temperature dependent partitioning. Accurate TE/Ca measurements can therefore be used as the basis of a quantitative sea-surface temperature (SST) reconstruction.

Trace elements that have been reported as having a temperature dependence include Sr, U, and Mg (Min et al., 1995; Mitsuguchi et al., 1996; Shen et al., 1996; Shen and Dunbar, 1995; Smith et al., 1979). For Sr, the variation in water temperature between summer and winter produces variations in the Sr/Ca ratio of only a few percent, and LA-ICP-MS is unlikely to ever be precise enough for useful SST reconstruction. Both U and Mg vary by up to 30%, and if an analytical method can be developed that results in accuracies and precisions better than about 5%, then it can be used to extract high fidelity, high-resolution SST information from coral skeletons.

Obtaining profiles of relative trace element variation is a simple matter using LA-ICP-MS. Quantifying those variations, however, is more difficult, requiring the synthesis of suitable standard materials, and the adoption of strategies to minimise and correct for fluctuations in ICP-MS sensitivity. While this adds complexity to the analytical method, the capacity to provide fully quantitative analysis of trace elements significantly expands the applications and potential of the technique.

Summary of Chapters

Chapter 2 describes the routine methodology used for much of the research presented in this thesis. This includes descriptions of coral sampling strategies, sample preparation, equipment, analyses of materials by both solution and LA-ICP-MS, and data processing. The development of the LA-ICP-MS method is covered in detail, and a number of the analytical problems encountered during this process are discussed.

Chapter 3 presents the search for a suitable standard for coral analysis by laser ablation. Theoretical aspects of standardisation, and the properties of an ideal coral standard are discussed. The synthesis, calibration, and analysis of several candidate reference materials is described, and their potential as coral standards are evaluated.

Chapter 4 presents a detailed evaluation of the final analytical method, focusing on the performance of a coral-composition CaSiO₃ glass standard. The method is evaluated with respect to sensitivity, precision, reproducibility, and accuracy, and the components of variance contributing to precision and reproducibility are calculated.
1.2.2 Part 2: Coral Calcification

Chapters 5 - 8 of this thesis investigate the inorganic, biological, physical and environmental factors that control trace element coprecipitation into skeletal aragonite.

Why Study Calcification?

Until relatively recently, research into trace elements in coral skeletons has largely been observational and empirical: researchers have noted correlations between a trace element and an environmental parameter in one coral and used this property to reconstruct environmental signals from analyses of other corals. Although some attempts have been made to rationalise these correlations in terms of calcification chemistry and physiology, few completely satisfactory explanations have emerged; corals are often assumed to passively incorporate trace elements, or are treated as 'black boxes'. While this approach may be justified for some elements that appear to respond purely to temperature, there is a growing body of evidence suggesting that trace element deposition may be significantly affected by physiological and chemical processes occurring within the coral polyp.

In the past, coral geochemists have not needed a detailed understanding of the physiology and biology of calcification. It is critical to the future of coral environmental records that some of these underlying principles are understood. Coral science is now at a point where research needs to be more interdisciplinary; synthesising chemical, environmental, and biological aspects of coral trace element chemistry into a broader holistic understanding of calcification and trace element records.

Summary of Chapters

Chapter 5 is a research essay asking the question 'How are trace elements taken from seawater and precipitated into coral skeleton?'. It covers inorganic trace element coprecipitation theory, biochemical models of calcification, physiological models of ion transport to the calcifying surface, calcification biology, and the geometrical controls these impose on the distribution of trace elements within the coral structure. This chapter also highlights areas where important background knowledge is lacking, and where current models fail to explain some observations. A simple conceptual summary of the main ion transport pathways to the skeleton is presented, including the physiological and biochemical factors that may influence calcification, together with the implications these have for trace element deposition into the coral skeleton.

Chapter 6 is a systematic review of B, Mg, Sr, Ba, and U in coral skeletons. It covers the postulated mechanistics and kinetics of coprecipitation for each element, and the environmental and biological factors that influence their concentrations in the skeleton.
This literature survey highlights areas of debate, areas where understanding is poor, and observations that are inconsistent with the generally accepted physiological and inorganic mechanisms.

Chapter 7 presents the results of very high (daily) resolution laser analyses of individual skeletal structures in a coral from Davies Reef. It examines the spatial distribution of the major trace elements relative to structural features in the skeleton, and the correlations of the trace elements to each other. Attempts are made to rationalise these observations with the growth and physiological processes reviewed in the previous two chapters, and to help constrain the various conflicting models.

Chapter 8 focuses on the cyclic seasonal-scale trace element variations in a coral from Davies Reef, correlating them with in-situ instrumental sea surface temperature measurements. The geochemical thermometers for B, Mg, Sr, and U are tentatively calibrated, treating the temperature correlations empirically. Deviations from pure temperature behaviour are examined as evidence for other confounding environmental or physiological processes.

1.2.3 Part 3: Case Study: Coastal Corals

Chapters 9 and 10 of this thesis present a case study of the chemistry of corals growing close to the Queensland coast, with a particular emphasis on the geochemical records of river floods in their skeletons.

Why Coastal Corals?

Most of the coral-based trace element records that have been published are derived from colonies that are growing in mid-oceanic environments (e.g. Galapagos Islands Lea et al., 1989; Linn et al., 1990; Shen and Sanford, 1990). This is not surprising, as oceanic corals are removed from the complicating geochemical influences of coastal zones and continental margins. Until recently, relatively little interest had been shown in corals growing close to shore because of the 'messy' trace element signals that they produce.

There is no doubt that the coastal zone is a complex and geochemically dynamic environment, influenced by continental weathering, upwelling, turbidity, sediment chemistry, and fresh water inputs (Chester, 1990). This zone, however, represents the major link between the land and the ocean. The chemistry that occurs here has a significant impact on the chemistry of the global oceans. Although well studied, there are still areas where current understanding of coastal processes is poor. Trace element records from near-shore corals may therefore hold important information about the chemistry and variability of the coastal environment.
Introduction

Because so many processes affect the coastal waters, corals have the potential to hold information on a diverse range of chemical processes (e.g. sediment chemistry, weathering, and transport), physiological processes (e.g. bleaching), and climatic processes (e.g. rainfall and river discharge). Of major interest is the potential for corals to record information on the impact of European settlement and agricultural practices on the coastal environment. Intensive agriculture is responsible for increases in deforestation, soil erosion, sedimentation, and nutrient levels, while large human population centres are associated with discharge of industrial and urban effluents. Because of the long lifetime of individual colonies, corals may provide high fidelity proxy records of water quality prior to instrumental records.

Why River Floods?

River discharge is the major link between the mainland and the coastal waters. In tropical latitudes where Porites corals are found, the rainfall is generally dominated by monsoonal and cyclonic weather patterns in summer (Wolanski, 1994). This tends to lead to a long dry season during winter when tropical rivers hardly flow, followed by a short intense rainy season characterised by several extreme river floods. These large floods are responsible for delivering most of the annual load of sediment, dissolved material and fresh water to the coastal zone, hence they can significantly influence the chemistry of this environment.

Major river floods are difficult to predict. They are also short in duration, with river levels dropping significantly within a few days, and coastal flood plumes mostly dispersed after several weeks (Wolanski and Jones, 1981). This makes them hard to sample, especially as most of the dissolved and particulate material is mobilised during the early rising flood waters. Corals growing in the coastal zone are ideally placed to act as proxy recorders of river discharge, and the formation of distinct fluorescent lines in their skeletons during flood events aids in the identification and extraction of river-flood records.

Summary of Chapters

Chapter 9 presents water sample analyses from a river flood in North Queensland in 1996. Fresh water and saline flood plume waters were collected as profiles in space and time, and analysed for a range of trace elements to gain insight into the behaviour and transport of trace elements into the coastal regions. The implications of these results for coral records of river floods are discussed.

Chapter 10 presents analyses of an extended set of trace elements from 4 coastal corals that experience discharge from 3 different Queensland rivers, and demonstrates the utility of multi-trace element analysis in a set of corals. This chapter focuses on flood-
plume signals, and the anomalous behaviour of barium in two of the corals. Possible environmental and physiological causes of the barium anomalies are discussed.
CHAPTER 2: ANALYTICAL METHOD

2.1 Coral Sample Preparation

2.1.1 Collection

Coral Drilling

Corals analysed during the course of this research were obtained from the Australian Institute of Marine Science (AIMS) collection. These were drilled in the early 1980s by P. Isdale, or collected by D. Barnes and J. Lough. For details of the AIMS drilling equipment, refer to Isdale and Daniel (1989). The corals collected from Davies Reef and the Whitsundays were drilled by a team from the Research School of Earth Sciences (RSES) in 1993 and 1995 using drilling equipment designed and built at the department.

The RSES drill is driven using pressurised seawater pumped by a Honda powered 'Davey' twin impeller high-pressure fire-fighting pump. This allows one pump to be used for powering the drill and flushing the core barrel, and eliminates the need for a second hose to return hydraulic fluid back to the surface. A significant advantage of this system is the lack of oil-based hydraulic fluids or lubricants which may contaminate both the sample and the environment.

Components are largely resistant to corrosion. The reciprocating vane assembly used in the drill motor is brass and aluminium, the housing is an industrial high-density polyethylene, and the other componentry is stainless steel. The drill barrels are steel with an outside diameter of 75 mm and an inside diameter of 55 mm (although an improved version now has an internal diameter of 76 mm). The drill bit is a modified commercial design with tungsten carbide teeth. The drill is stabilised on a heavy stainless steel frame which weighs approximately 55 kg without the drill head (see figure 2.1). The frame is anchored tightly to the coral by 3 tensioned nylon cargo slings. Drilling proceeds in stages, and the core is brought to the surface in approximately 1 m sections.

Two parallel cores (about 15 cm apart) were drilled in the Cow and Calf Island coral. These coral cores were around 2 m in length; however, the system is designed to be able to take longer cores if required.
Figure 2.1 Coral Drilling Rig
The drill was designed and built at the RSES for taking coral cores. It is powered by two 'Davey' fire fighting pumps which force seawater through a reciprocating vane turbine in the drill motor. The use of seawater as a hydraulic fluid prevents the possibility of contamination of the coral environment if the delivery hose is ruptured. The drill is held steady by a heavy frame, which is anchored to the coral bommie by three nylon straps attached to hooks.
2.1.2 Cutting

Sectioning the Coral

Coral core sections are aligned, marked, and then split using a diamond-impregnated glass-cutting blade with fresh water as a coolant. Cores are supported on aluminium guttering (rectangular profile 3 mm thick U-bend, 25 x 50 mm), with friction preventing them from rotating during cutting. During early attempts at splitting, corals were embedded in plaster of Paris; however, this practice was discontinued because of concerns about strontium contamination.

A 7.0 mm slab is then cut from one half of the split core using the diamond blade. Slab thickness is typically uniform to within ± 0.5 mm. Slabs are cleaned in 18 MΩ water in an ultrasonic bath for 30 minutes to remove cutting debris and soluble surface contamination introduced by the saw blade, and air-dried on an absorbent paper towel. Corals obtained from AIMS had already been cut into 7.0 mm slabs.

Coral slabs are x-rayed and photographed under UV light (see next section). Chronologies are then determined by counting density bands; however, subannual density bands may obscure the major seasonal cycles. Counting dissepiments is not always reliable, as coral slabs are not always cut perfectly perpendicular to growth banding. Fluorescent bands, therefore, prove to be the most useful tool for establishing a chronology in coastal corals. The inter-annual variation in fluorescence provides useful temporal and cross-dating markers (e.g. Isdale and Parker, 1995), allowing parallel cores to be matched, and dates assigned from characteristic patterns of rainfall.

Once a chronology has been assigned to a core, a suitable growth axis for analysis is identified. This axis is then cut from the slab as a 212 mm wide strip using the diamond blade. It is often necessary to cut more than one growth axis from a coral slab in order to maintain a continuous record, as growth axes can run off the edge of the core.

The 22 mm strip is next split into 50 mm subsections for mounting in the laser ablation cell (a geometry imposed by the sample cell - see section 2.2.2). In order to minimise sample loss during this sub-sectioning, the coral is cut using a 0.15 mm diamond wafering blade. The coral strip is not cut completely through, with the sectioning cut stopping about 1 mm from the face of the coral to be analysed (see figure 2.2). The coral is then snapped so that the analytical faces mesh perfectly with each other. In most cases, the first 15 - 20 mm of the coral strip is removed to avoid the tissue layer. Subsections are ultrasonicated in 18 MΩ water for 30 minutes to remove handling contamination, and air dried on an absorbent paper towel.

Bulk sampling of corals for solution analysis is carried out by milling strips from ultrasonically cleaned coral sections. Prior to collecting milled material, 0.5 - 1.0 mm of
coral is milled from each surface of the sampled region and discarded. Milling is carried out with a 2.5 mm tungsten carbide bit, and the coral powder is collected on waxed paper and transferred to acid-cleaned polyethylene vials.

**Sectioning Coral**

![Diagram of coral sectioning](image)

**Figure 2.2 Sectioning the Coral**

When sectioning corals for analysis on the laser, some environmental information may be lost due to the thickness of the cutting blade. To avoid this, the coral samples are cut only part way through, without damaging the surface to be analysed. The coral sections are then snapped so that there is no loss of continuity on the analytical surface. The cut is made using a very fine 0.15 mm diamond wafering blade.

**Marking the Coral**

To enable precise alignment of coral laser scans and images, it is convenient to have a set of spatial markers on the coral that can be identified as reference points in images and analytical runs. In most cases these are natural features of the coral section, such as an edge, a corner, or a blemish. Some coral sections, however, do not have any easily recognisable features to measure. In these cases, small notches are carefully cut into the sides of the coral using a hack-saw.

**2.1.3 Imaging**

**X-Ray**

The 7.0 mm coral slabs are X-rayed to produce density images using standard hospital X-ray equipment. Coral samples are placed on a Kodak single sided emulsion film, using a Lamex screen, and exposed for 0.08 seconds at 50 mA and 45 kV. Contact (positive) prints are made from the X-ray negatives for routine handling.
Fluorescence

Coral slabs are photographed under ultraviolet light to obtain images of the fluorescent flood-banding. The corals are illuminated with two 40 Watt NEC 'Black-light Blue' UV tubes mounted in standard 40 W fluorescent light brackets. These are raised up on blocks and arranged so as to cast an even illumination over the surface of the coral (see figure 2.3). The fluorescent tubes are placed as close to the sample as possible to gain maximum intensity of fluorescence. Photographs are taken from directly overhead, and it is often necessary to make a montage of close-up photographs for long sections of coral. It is necessary to shield upward illumination from the camera to avoid flaring.

Under UV light, the fluorescent lines are distinguished from the blue background fluorescence of the aragonite by their characteristic yellow/green colour, rather than marked differences in the intensity of fluorescing light. For the purposes of scanning and profiling, it is convenient to be dealing with grey-scale images. The distinction between yellow bands and blue background, however, is greatly reduced when colour is excluded. The yellow bands are therefore accentuated as much as possible by using a yellow/orange filter during the photography to remove blue tones, and by controlling the development and processing to enhance the contrast.

Techniques for UV coral photography were developed by Mr Stewart Hay at 'ANU Photography'. The camera is a Hasselblad E.L.M., and the photographs are shot on black-and-white AGFA APX 25 (120) film, producing 55 mm square negatives. The filter is a Hasselblad/50 1.5x Y (yellow). Exposures vary somewhat and need to be assessed from test shots; however, f5.6 for about 4 seconds is about average. The negatives are processed using Rodinal developer at 1+ 25 at 20°C for 11 minutes, producing a gamma of 0.75. The tank is agitated for the first minute followed by 4 inversions every 30 seconds. Full size prints are made using a condenser enlarger on high contrast paper. These settings are summarised in table 2.1.

Scanning

Both X-ray and fluorescent images are scanned into Adobe Photoshop using an AGFA Arcus II flatbed scanner at a resolution of 200 pixels per inch. Scanner parameters (brightness, light point, dark point, and gamma) are optimised to ensure maximum resolution of grey-scale. The images are scanned in 16 bit greyscale, and the levels and gamma of the image adjusted further in Photoshop to enhance the contrast between light and dark bands. The images are then reduced to 8 bit grey-scale. Any digital image filtering to smooth the picture or remove speckles is carried out at this stage, although generally this is not necessary.
Table 2.1  Photographic Settings

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV Source</td>
<td>2 x 40W NEC Blacklight Blue Neon Tube</td>
</tr>
<tr>
<td>Camera</td>
<td>Hasselblad E.L.M.</td>
</tr>
<tr>
<td>Filter</td>
<td>Hasselblad/50 1.5x (yellow)</td>
</tr>
<tr>
<td>Film</td>
<td>AGFA APX 25 Black and White Film</td>
</tr>
<tr>
<td>Aperture</td>
<td>f5.6</td>
</tr>
<tr>
<td>Exposure</td>
<td>approx. 4 seconds</td>
</tr>
<tr>
<td>Developer</td>
<td>Rodinal</td>
</tr>
<tr>
<td>Development</td>
<td>20°C, 11 minutes</td>
</tr>
<tr>
<td>Print Paper</td>
<td>High Contrast</td>
</tr>
</tbody>
</table>

Profiling

Scanned images are imported into the application NIH Image, a public domain scientific image processing program written by Wayne Resband at the National Institute of Health USA, available at www:http://rsb.info.nih.gov/. UV and fluorescent images are scaled, rotated, and aligned using set reference points on the coral images (see section 2.1.2). Laser traverses on the coral are difficult to see, as the beam is very wide, does not produce a sharp edge, and leaves a very shallow track (less than 50 μm) on a complex and heterogeneous surface. Once spatially calibrated, however, it is possible to orient a laser traverse on a coral section to a precision better than 0.5 mm using measurements taken during the laser analysis.

Image density profiles (greyscale values from 0 to 255 vs. distance along the section) are taken over the same tracks as the laser traverse. Profiles are made using the 'profile' tool in NIH Image, and taken from an 8 pixel wide strip. This strip is slightly wider than the width of the laser beam (which on this scale would be equivalent to about 5.5 pixels); however, the extra averaging that this generates is negligible compared with the off-line data smoothing applied to the laser trace element profiles (see section 2.4.1).

It is worth noting that the contrast adjustments made during photography and scanning will destroy any linear relationship between the greyscale intensity of the image and the actual density or intensity of fluorescence. Without a density or fluorescence scale it is not possible to use greyscale intensity to quantitatively reconstruct skeletal density or river discharge. Fluorescence and density are therefore used only for interpreting the spatial location and seasonal timing of features.
Photographing Coral Sections under U.V.

Hasselblad
E.L.M. Camera

Yellow/Orange Filter

NEC 'Blacklight Blue'
U.V. Neon tube

Coral Sample

Black Cloth

Figure 2.3 Photographing the Coral Sections Under UV Light

The coral sections are photographed under ultraviolet light to reveal the fluorescent lines associated with river floods. The surface of the coral is illuminated with two 40 W ultraviolet tubes, and photographed in black and white. The fluorescent lines are a yellow green colour in contrast to the aragonite which fluoresces blue. For this reason it is necessary to use a yellow/orange filter in order to maximise the contrast between flood-line and background fluorescence.

2.1.4 Cleaning

While the AIMS coral cores were all bleached after drilling, the Whitsundays corals drilled by the RSES were not. All coral cores are rinsed in freshwater and dried following collection.

Routine Cleaning

Before laser analysis, coral sections are subjected to an intense ultrasonic cleaning in 18 MΩ water. A high energy ultrasonic probe is passed over the surface several times, and the water is replaced when it becomes cloudy and warm to touch. This is repeated 2 or 3 times before the coral is wrapped in a low-dust tissue and dried in a clean oven overnight at 40°C. Thereafter the coral is only handled with plastic gloves to avoid handling contamination.
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Coral sections from Kurrimine, Whitsundays, and Pandora Reef were subjected to an additional acid-leach cleaning in 0.1% HNO$_3$. The coral sections were leached twice for 10 minutes in an ultrasonic bath, with the acid being replaced between leachings. The corals were ultrasonicated several times in 18 MΩ water to remove residual acid, and then dried. During acid leaching the coral sections lost between 0.9 and 1.5 % of their mass. Corals from Davies Reef, Orpheus Island, and Christmas Island were not subjected to this acid cleaning stage.

Both corals and standards are pre-ablated twice before analysis to expose fresh surfaces. For the first ablation, the laser is pulsed at 150 Hz, and scanned at around $1/10$ mms$^{-1}$. This removes between 5 and 10 μm of the sample surface. The second pre-ablation (10 Hz, $1/30$ mms$^{-1}$) clears away residual ablation debris.

Alternative Cleaning Strategies

The major cleaning stage for the corals is ultrasonication in 18 MΩ water. While this method of cleaning is effective in removing loose particulate contaminants and water-soluble adsorbed material, it is unlikely that it will remove overgrowths of authigenic minerals and surface active contaminants. Some contaminants may remain on the surface (see section 2.5.1), where they can cause analytical anomalies (see chapter 7). One possible approach to this problem is to employ more rigorous solution based cleaning strategies.

Acid leaching is a principle element in the rigorous cleaning strategies employed by Shen and Boyle (1988) and Lea and Boyle (1993), and works by dissolving surface phases and physically removing the contaminated aragonite. Later coral analyses employed the dilute-acid leaching; however, there was no opportunity to make a systematic comparison of the effectiveness of this cleaning strategy.

The rigorous cleaning strategy determined by Shen and Boyle (1988) employs both oxidative stages for removing organic material, and reductive stages for removing authigenic overgrowths of Fe and Mn oxyhydroxides. It might be possible to modify these methods for whole coral slabs.
2.2 Hardware

2.2.1 ICP-MS

Configuration

The ICP-MS is a PlasmaQuad PQ II. Details of the ICP-MS configuration for laser ablation are presented in table 2.2, and typical solution settings have been included for comparison.

Table 2.2 ICP-MS and Gas Flow Configuration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Laser</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICP-MS Model</td>
<td>PlasmaQuad PQ II (S Option Switched Off)</td>
<td>Nickel</td>
</tr>
<tr>
<td>Cone Composition</td>
<td>Aluminium (preferred)</td>
<td>Nickel</td>
</tr>
<tr>
<td>Forward Power</td>
<td>1200 W</td>
<td>1350 W</td>
</tr>
<tr>
<td>Auxiliary Ar Flow</td>
<td>500 - 1000 ccmin⁻¹</td>
<td>500 - 1000 ccmin⁻¹</td>
</tr>
<tr>
<td>Nebuliser Ar Flow</td>
<td>1000 ccmin⁻¹ (into cell)</td>
<td>900 ccmin⁻¹</td>
</tr>
<tr>
<td>He Flow = 300 ccmin⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coolant Ar Flow</td>
<td>14,000 ccmin⁻¹</td>
<td>14,000 ccmin⁻¹</td>
</tr>
<tr>
<td>Plasma Sampling Depth</td>
<td>10 - 15 cm</td>
<td>8 - 10 cm</td>
</tr>
<tr>
<td>Typical Sensitivity on U[1]</td>
<td>3 - 6 x 10⁵ cps per ppm</td>
<td>50 - 100 x10⁶ cps per ppm</td>
</tr>
<tr>
<td>Collector Mode</td>
<td>Pulse Counting</td>
<td>Dual</td>
</tr>
<tr>
<td>Acquisition Mode</td>
<td>Time Resolved</td>
<td>Normal</td>
</tr>
<tr>
<td>Time Slice</td>
<td>1.0 s</td>
<td>-</td>
</tr>
<tr>
<td>Points per Peak</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Settling Time</td>
<td>10 ms</td>
<td>-</td>
</tr>
<tr>
<td>Nebuliser</td>
<td>-</td>
<td>Meinhardtt</td>
</tr>
<tr>
<td>Counting Time</td>
<td>-</td>
<td>60 s</td>
</tr>
<tr>
<td>Uptake Time</td>
<td>-</td>
<td>90 s</td>
</tr>
<tr>
<td>Wash-out Time (typical)</td>
<td>-</td>
<td>120 s (for each of Decon-90[2], HNO₃, and water)</td>
</tr>
</tbody>
</table>

[1] The difference between laser and solution is largely due to different sample mass delivery rates. When normalised to mass, the inherent sensitivity per atom is approximately the same.

[2] Decon-90 is an alkaline surface cleaning detergent manufactured by Sealy Scientific
Sensitivity

The tuning of the ICP-MS for laser analysis differs significantly from the settings for solution analysis. Sensitivities for the laser typically range from 3000 to 6000 cps/ppm for $^{238}$U using a 70 µm diameter laser spot ablating at a rate of 5 Hz. This is significantly lower than the sensitivity obtained for solutions, and relates mostly to the lower sample delivery rate for laser ablation.

It is possible to adjust tuning, spot size and ablation rate to increase this sensitivity; however, there are several limitations to the rate at which ablated sample can be delivered to the ICP-MS. Because the laser analysis is carried out in 'Time Resolved Acquisition' mode, it is not possible to operate the detectors in 'Dual' mode; hence, when count-rates for an isotope become too high, the detector trips out rather than switching to analog. This, therefore, imposes a limitation on the intensity of the largest signal, which in this case is $^{43}$Ca. It is important to monitor calcium in order to correct for fluctuations in the ablation rate with time, and $^{43}$Ca is the lowest abundance isotope of Ca (at 0.135%) that can be practically measured on the ICP-MS. The ultimate detectability of an isotope is therefore limited by the ratio of its signal intensity to that of $^{43}$Ca.

During routine running, the $^{43}$Ca count rates are around $2 \times 10^6$ cps. Tripout occurs at count rates around $5 \times 10^6$ cps, and dead-time caused by saturation of the detectors causes significant deviations from a linear count rate vs. trace element to calcium ratio (TE/Ca) above count-rates of about $3.5 \times 10^6$ cps.

Sensitivity and mass response of the ICP-MS can be degraded by charge build up, and contamination of the internal componentry by excessive amounts of ablated material (see also sections 2.5.4 and 2.5.5). In some experiments with high rates of carbonate ablation, the change in mass response was dramatic, with sensitivities dropping by an order of magnitude during a days running. It is, therefore, advantageous to maintain the ablation at a level somewhat below the maximum dictated by the onset of nonlinearity. Solution analysis is less limited by this contamination problem due to the time delays and washing procedures between each sample.

2.2.2 Sample Cell

Coral samples are mounted on a micrometer X-Y sample stage which is calibrated in millimetres. A linear DC motor controlled by a variable power-supply can be attached to one micrometer to allow a continuous scanning in one dimension. Coral samples are scanned at $1/30$ mm/s beneath the laser.

Ablation occurs in a sealed perspex sample chamber (see figure 2.4) under a helium atmosphere (Eggins et al, 1998), and ablated material is then entrained in an argon gas
stream for transport into the ICP-MS. Argon flow is normally around 1000 ccmin⁻¹, and helium flow is 300 ccmin⁻¹. The cell is designed principally to ensure that ablation occurs in helium rather than argon, to minimise the production of an ablation blanket and elemental fractionation during ablation.

Rhythmic signal fluctuations caused by the pulsed laser are removed by passing the gas through a smoothing manifold before it enters the ICP-MS. This manifold splits the gas steam into 8 sub-streams with different residence times, and then recombines them to integrate the signal over 1.0 s.

**Schematic of Sample Cell**

Figure 2.4 Schematic of Ablation Cell
The coral sample is ablated in a sealed perspex chamber. Ablation occurs under helium, and the ablation debris is entrained in an argon stream and carried to the ICP-MS for analysis. The coral sample is mounted on a stage which can be moved by two micrometers. For linear scanning, a DC motor is attached to one micrometer, and the sample is moved beneath the laser beam. A CCD video camera enables direct viewing of the sample during ablation, and images may be captured on computer.
2.2.3 Laser System

Laser

The laser is a LambdaPhysik LPX 120i ArF excimer with a wavelength of 193 nm. The laser produces a 3 cm x 1 cm output beam which is masked through an aperture and focused onto the sample. Long (15 cm) focal length optics are used with a 20 fold demagnification to produce a final energy density on the sample of approximately 5-10 J cm\(^{-2}\). Pulse length is nominally around 17 ns, and the drill-rate is approximately 0.1 μm per pulse when drilling into NIST 612 glass. Further details of this system can be found in Eggins et al. (1998).

The normal analytical ablation rate is 10 pulses per second (Hz). This rep-rate (coupled with the beam width and scan rate) results in a good coverage of the sample surface during analytical ablation (see section 2.2.4). The amount of ablated material entering the ICP-MS needs to be carefully controlled (see section 2.2.1), and this is achieved by keeping the laser energy around 50 mJ, and using a 50% partial reflecting mirror to produce a final laser energy of 25 mJ. For a summary of these details see table 2.3.

Table 2.3 Laser Configuration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser Model</td>
<td>LambdaPhysik LPX 120i</td>
</tr>
<tr>
<td>Gas Mix</td>
<td>ArF</td>
</tr>
<tr>
<td>Wavelength</td>
<td>193 nm</td>
</tr>
<tr>
<td>Energy</td>
<td>50 mJ with 50% mirror</td>
</tr>
<tr>
<td>Output Beam</td>
<td>3 cm x 1 cm</td>
</tr>
<tr>
<td>Focal Length</td>
<td>15 cm</td>
</tr>
<tr>
<td>Focusing</td>
<td>20-fold</td>
</tr>
<tr>
<td>Final Beam Shape</td>
<td>20 μm x 600 μm</td>
</tr>
<tr>
<td>Energy Density</td>
<td>5-10 J cm(^{-2})</td>
</tr>
<tr>
<td>Pulse Length</td>
<td>17 ns</td>
</tr>
<tr>
<td>Drill Rate</td>
<td>0.1 μm s(^{-1}) per pulse (NIST glass)</td>
</tr>
<tr>
<td>Ablation Rate</td>
<td>10 Hz</td>
</tr>
<tr>
<td>Scan Speed</td>
<td>(1/30) mms(^{-1})</td>
</tr>
<tr>
<td>Maximum Sample Size</td>
<td>50 mm x 23 mm</td>
</tr>
</tbody>
</table>

Beam Shape

One of the significant advantages of the ArF excimer laser over other laser-systems is that the output beam is large with a relatively uniform energy density. The beam can therefore be masked to produce final beam shapes that are not limited to circular spots.
The laser is masked to produce a final beam that is 600 μm wide perpendicular to the direction of the laser scan, and 20 μm wide parallel to the scan direction. This beam shape represents a compromise between maintaining resolution in the direction of analysis, averaging over a range of structural and compositional features (see figure 2.5), while keeping the amount of material entering the plasma to a practical minimum.

![Laser Spot on Coral](image)

Figure 2.5 Laser Spot Size
The laser beam is masked to produce a spot that is a narrow strip, 600 μm x 20 μm. This beam averages over a number of pores and structures in the coral while maintaining the spatial resolution in the direction of the scan (scan direction is vertical in this figure). Data is collected each second by the ICP-MS, and in this time the laser has moved 33 μm.

### 2.2.4 Effective Resolution
The time-slice for the analytical technique is 1.0 s. This means that the quadrupole lens scans once through the mass spectrum each second, integrating counts for a particular element for approximately 1/n of a second (where n is the number of elements being analysed), and recording one data point for each element. Because the quadrupole ICP-MS has only one detector, different elements are not measured simultaneously, and there can be up to 0.5 s delay between measuring one element and another. This can create
some anomalies when normalising to $^{43}\text{Ca}$, especially if the count rates are changing rapidly (such as when the laser tracks over a pore or hole in the coral). It is therefore important to keep the time-slice as short as possible; however, very short time-slices create large data files, and because the integration time is shorter, each point has a lower counting-statistics precision. The 1.0 s time-slice is a compromise between integrating for long enough to obtain a reasonably precise estimate of intensity, while keeping the time-slice short enough so that trace elements are measured approximately simultaneously.

The laser beam is typically 20 $\mu$m wide and scanned at $\frac{1}{30}$ mms$^{-1}$ (33.3 $\mu$ms$^{-1}$). In one second the laser travels 33.3 $\mu$m, but because of the width of the beam, it samples material from 53 $\mu$m of coral (20 $\mu$m + 33 $\mu$m). It takes the laser spot 0.6 s to cross any particular point in space, and therefore each point on the surface of the sample receives 6 individual laser pulses, each of which drills approximately 0.1 $\mu$m into the sample. In one second, the laser has ablated around 1200 $\mu$m$^3$ of coral, which weighs approximately 3.5 ng (assuming a density for coralline aragonite of 2.94). The laser ablates material from the surface and from the bottom of pores with almost equal efficiency due to its long focal length.

The laser spot is a long slit (600 $\mu$m), and as the slit moves away from being parallel to the banding of the coral, the apparent beam thickness increases as a sine function from 20 $\mu$m to 600 $\mu$m. If coral banding is $\lambda_{\text{tilted}}$ 15° from the laser slit, the effective beam width with respect to the banding is increased to 175 $\mu$m. While resolution is therefore somewhat dependent on the orientation of the beam relative to the coral banding, this effect is not significant once the off-line data filtering has been applied to the data (see section 2.4.1).

2.3 Analytical Method

This section summarises the analytical method. Explanations and details are presented in the following sections.

2.3.1 Isotopes Monitored

Two suites of elements are monitored during analysis. The most abundant isotope for each element is measured, with the exception of calcium, magnesium and strontium which are abundant in the aragonite. The 'major' trace elements are those for which the fully quantitative analytical method has been developed. The isotopes monitored are $^{11}\text{B}$, $^{25}\text{Mg}$, $^{43}\text{Ca}$, $^{84}\text{Sr}$, $^{138}\text{Ba}$ and $^{238}\text{U}$. The second suite of elements includes all the major
trace elements, and the following 'minor' trace elements: $^{55}$Mn, $^{58}$Ni, $^{63}$Cu, $^{66}$Zn, $^{89}$Y, $^{139}$La, $^{140}$Ce, and $^{208}$Pb.

2.3.2 Summary of Method

The full analytical method is summarised in table 2.4, along with approximate times for each stage. The analysis begins with the cleaning ablations to the standard and the coral (section 2.1.4). The second of the pre-ablations is introduced into the ICP-MS to condition the machine to carbonate material. Background counts are collected for 60 seconds before ablation. The acquisition begins with 5 minutes of coral ablation to further pre-condition the ICP-MS to carbonate. The laser is then scanned over the standard glass for 60 seconds before beginning the 25 minute coral analysis. At the end of the traverse, the first 5 mm of the coral is re-analysed to assess if any significant drift in instrumental sensitivity has occurred since the beginning of the coral analysis. The standard is then analysed again, ensuring that the laser scans over the same track on the glass as previously. NIST 612 is occasionally run after the main standard to allow an alternative calibration and semiquantitative estimates of minor trace elements, and this is followed by another 60 seconds of background. On the completion of the analysis, the spatial reference markers (see section 2.1.2) are measured to allow the laser traverse to be matched to fluorescent and density images.

During the laser analysis it is important to maintain a flow of carbonate material through the ICP-MS to ensure that the steady state created during the conditioning steps is maintained. The laser is kept on when switching between locations on the coral sample, and is switched off briefly at the start and end of each traverse creating a sudden short drop in count-rates that can be used to identify the start of an analytical run for data processing.

Laser profiles of corals display a high degree of variability, both within and between runs (see chapter 7). It is therefore important to analyse two or more parallel tracks on each coral sample to allow geochemical horizons in the coral to be distinguished from structurally induced variations.

2.4 Data Processing

All data processing is carried out off-line using spreadsheet programs. While it would now be possible to automate some of this process, the development of the analytical technique required flexibility in the data handling, and spreadsheets were most versatile option.
Chapter 2

Table 2.4 Summary of Analytical Method

<table>
<thead>
<tr>
<th>Step</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-ablation of Standard [1]</td>
<td>1:20</td>
</tr>
<tr>
<td>1st Pre-ablation of Coral [2]</td>
<td>8:33</td>
</tr>
<tr>
<td>2nd Pre-ablation of Coral [3]</td>
<td>25:00</td>
</tr>
<tr>
<td>Background 1</td>
<td>1:00</td>
</tr>
<tr>
<td>Pre-conditioning Step [4]</td>
<td>5:00</td>
</tr>
<tr>
<td>Standard 1</td>
<td>1:00</td>
</tr>
<tr>
<td>Coral Analysis</td>
<td>25:00</td>
</tr>
<tr>
<td>Driftmonitor [5]</td>
<td>2:30</td>
</tr>
<tr>
<td>Standard 2 [6]</td>
<td>1:00</td>
</tr>
<tr>
<td>Alternative Standard (Optional) [7]</td>
<td>1:00</td>
</tr>
<tr>
<td>Background 2</td>
<td>1:00</td>
</tr>
<tr>
<td>Spatial Measurement</td>
<td>-</td>
</tr>
</tbody>
</table>

[1] Actually two ablations: 20 Hz at $1/10$ mms$^{-1}$, and 10 Hz at $1/30$ mms$^{-1}$.
[2] 150 Hz at $1/10$ mms$^{-1}$.
[3] 10 Hz at $1/30$ mms$^{-1}$. Sample cell is connected so that carbonate flows into ICP-MS.
[5] Re-analysing the first 5 mm of coral.
[6] Over the same track as 'Standard 1'.
[7] For example NIST 612 glass.

The raw data is downloaded from the ICP-MS in a simple tabular format with integrated counts per second for each isotope tabulated against time. It is convenient to add a column listing time-slice number. The major steps in data processing are summarised in table 2.5.

2.4.1 Basic Processing

Background/Blank Subtraction

Backgrounds/blanks are measured at the same mass positions but with the laser switched off. They therefore represent a combination of instrumental noise plus blank contamination. Backgrounds and blanks may change during the course of an analysis as ICP-MS tuning changes and contaminants build up in the system. For the major elements analysed, the signal to background ratio is large, and backgrounds can be assumed to remain constant. For lower abundance elements, however, it is necessary to correct for these changes. Each 60 second background is averaged to produce a single value. In some cases the backgrounds are very noisy and may contain large spikes that can significantly influence the background averages. These spikes are therefore removed from the data before averaging using a running 5 point median filter.
Background drift is assumed to be linear, and in most cases this is a good approximation. On occasions, however, the background drift displays nonlinear behaviour. These cases are rare, and affect only a small proportion of elements. A linear drift is interpolated between the first and second background value, and corrected according to the following equation:

\[ A_{b(i)} = A(i) - \left[ A(b1) + \left( t(i) - t(b1) \right) \cdot \frac{A(b2) - A(b1)}{t(b2) - t(b1)} \right] \]  

(2.1)

where:
- \( A_{b(i)} \) = Background subtracted value at time i
- \( A(i) \) = Un-subtracted value at time i
- \( A(b1) \) = Value of background 1
- \( A(b2) \) = Value of background 2
- \( t(i) \) = Time
- \( t(b1) \) = Time at middle of background 1
- \( t(b2) \) = Time at middle of background 2

**Normalising to Calcium**

Porosity and structural variability in the coral results in an uneven ablation signal with time (see chapter 7, section 7.2). These variations can largely be removed by normalising the signal to an element of constant concentration in aragonite, such as calcium. The normalised signal is calculated as follows:

\[ A_{n(i)} = A_{b(i)} + A_{b(i)}Ca \]  

(2.2)

where:
- \( A_{n(i)} \) = Ca normalised ratio at time i
- \( A_{b(i)} \) = Background subtracted value at time i
\[ A_{b(i)Ca} = \text{Background subtracted value for calcium at time } i \]

**Standardisation**

The analyses of the standard are handled in a similar manner to the backgrounds. The calcium normalised ratios are filtered through a 5 point running median filter to remove large spikes, then averaged to produce a single value. Drift in mass response is assumed to be linear, and is interpolated between the first and second standard. Each calcium normalised data point is therefore divided by this interpolated standard value, and multiplied by the known TE/Ca ratio of the standard.

\[
A_{s(i)} = S \cdot A_{n(i)} + \left[ A_{n(s1)} + \left( t_{(i)} - t_{(s1)} \right) \right] \cdot \frac{A_{n(s2)} - A_{n(s1)}}{t_{(s2)} - t_{(s1)}}
\]

where:

- \( A_{s(i)} \): Standardised ratio at time \( i \)
- \( S \): Known ratio of TE/Ca in standard
- \( A_{n(i)} \): Ca normalised ratio at time \( i \)
- \( A_{n(s1)} \): Ca normalised ratio of standard 1
- \( A_{n(s2)} \): Ca normalised ratio of standard 2
- \( t_{(s1)} \): Time at middle of standard 1
- \( t_{(s2)} \): Time at middle of standard 2

**Filtering/Smoothing**

Corals are compositionally and structurally heterogeneous at the resolution inherent to the laser-ablation method (see chapter 7). The trace element signals therefore show a great deal of variability that appear to be random noise. While most of these fine-scale variations represent compositional features, they are too fine to be of use for environmental interpretation and are therefore removed by applying smoothing and filtering functions to the data. The cut-off between what is treated as 'noise' and what is treated as meaningful signal is arbitrary.

The first filtering step is the application of an 11 point running median to the data. This is a somewhat unconventional method of frequency filtering, chosen for its effectiveness, simplicity and ease of application on a spreadsheet. This is followed by an 11 point running average to smooth the resulting signal. The effect this filtering regime has on the data signal is demonstrated in figure 2.6. While the number of data points remains unchanged, features less than half the size of the filter window (i.e. 6 points) are removed, hence the effective resolution of the data series is reduced to around 250 \( \mu \text{m} \).
Figure 2.6 Demonstration of Off-line Digital Filtering

Because the analytical profiles contain high levels of fine-scale variation, the data is filtered through an 11 point running median filter, which effectively removes 'noise' on distance scales less than about 250 μm. This figure demonstrates the effect of filtering on a B/Ca profile spanning approximately 4 years. Here the unfiltered signal is in grey, while the filtered signal is in black. Figure b is an enlargement of the data region indicated in figure a.

Alternative filtering and smoothing methods were examined. Some trace element signals contain occasional extreme outlier points that probably represent dust or other discrete particulate contaminants entering the plasma. Simple smoothing functions, such as running mean or triangular filters, are sensitive to these outlier points. Running 2-sigma and Fourier filtering were both investigated, but neither was found to be a significant improvement over the presently used system, and their added complexity was a barrier to routine data processing.
2.4.2 Secondary Processing

Drift Correction (Optional)

If the standard used to calibrate the coral is not perfectly matrix-matched, it is possible to get matrix dependent drift, where (for example) the mass response for a silicate glass changes differently to the mass response of a carbonate during the course of an analysis (see chapter 3, section 3.2.2). This may lead to a residual slope in a trace element profile that is not corrected by interpolation between the two measurements of the standard.

Because the 'driftmonitor' is a replicate analysis of a section of the sample, it can be used as the basis of a secondary correction. The implementation of this correction is similar to the standardisation: a linear change in mass response is interpolated between the duplicated sections. The most objective way of making this secondary correction is to keep the average of the two standard analyses the same. Mathematically this is equivalent to 'rotating' the whole standardised data profile around a point half-way between the first and second standard. The first step is to calculate the average of the first 5 mm of coral and the driftmonitor (As(d1) and As(d2) respectively). The following equation is then applied to the data:

$$A_{d(i)} = \left( \frac{2 \cdot S \cdot A_{s(i)}}{A_{s(s1)} + A_{s(s2)}} \right) \cdot \left[ A_{s(d1)} + \left( t(i) - t(d1) \right) \cdot \left( \frac{A_{s(d2)} - A_{s(d1)}}{t(d2) - t(d1)} \right) \right]$$  (2.4)

where:

- $A_{d(i)}$ = Drift corrected (standardised) ratio at time $i$
- $S$ = Known ratio of TE/Ca in standard
- $A_{s(i)}$ = Standardised ratio at time $i$
- $A_{s(d1)}$ = Standardised ratio at middle of driftmonitor 1
- $A_{s(d2)}$ = Standardised ratio at middle of driftmonitor 2
- $A_{s(s1)}$ = Standardised ratio at middle of standard 1
- $A_{s(s2)}$ = Standardised ratio at middle of standard 2
- $t(i)$ = Time
- $t(d1)$ = Time at middle of driftmonitor 1
- $t(d2)$ = Time at middle of driftmonitor 2

Alternative Standard (Optional)

Alternative standards, such as NIST 612, are generally run to allow semiquantitative estimates of trace elements which are not well characterised in the normal standard, or to cross check the calibration of the normal standard. Processing is carried out as described above, with the two standards, or the driftmonitor being used to make the drift correction. All of the data from the analytical run is then multiplied by a constant
correction factor, calculated to bring the mean ratio of the alternative standard data in line with its known ratio.

\[ A_{s'(i)} = \frac{S'}{A_{s(i)}} \]  

(2.5)

where:
- \( A_{s'(i)} \) = Alternatively standardised ratio at time i
- \( S' \) = Known ratio of TE/Ca in alternative standard
- \( A_{s(i)} \) = Standardised ratio at time i

**Adjusting for Variation in Growth Rate**

It is common to run two or more analytical traverses in parallel so that some degree of structural and compositional variability can be averaged out. Coral growth, however, is not uniform in time or across a section of coral, and it is necessary to correct for variations in growth rate in order to compare trace element profiles to each other and instrumental records.

The simplest form of this adjustment is to identify features (tie points) common to the two records being aligned, and to linearly interpolate growth rates in one record so that these features synchronise. In the case where a high resolution instrumental record (e.g. SST) is available, it is possible to select a number of sub-seasonal features in the coral and instrumental records to use for aligning (e.g. Alibert and McCulloch, 1997). Laser profiles, however, have variations that may obscure sub-seasonal features, and often it is only possible to identify seasonal maxima and minima. For long coral records, this is adequate, and where no instrumental record exists, these long time series are generally fitted to the peaks and troughs of a regular sine wave (approximating the seasonal cycle of SST Alibert and McCulloch, 1997). The time series analysis software written by Paillard et al. (1996) has proved useful for this synchronisation.

**Re-sampling**

Before averaging two parallel trace element profiles it is necessary to have their X scales identical. Growth rate variations may lead to anomalous X scales, and slightly different motor speed settings may produce different resolutions between parallel scans, however.

The data produced by a laser-ICP-MS analysis has a very high resolution (approximately 1500 data points for a 50 mm coral scan); however, after filtering steps much of this resolution is redundant. It is therefore useful to reduce the resolution to around 200 - 300 data points. Reducing the size of the data set, and re-sampling the profile at an even and consistent scale is carried out by cubic spline interpolation, a feature common to most time-series analysis software packages.
2.5 Developmental Hurdles

The analytical method presented in the preceding sections was developed around a series of analytical problems that are inherent in a technique such as this. What follows is a brief summary of these problems, and the way in which the analytical method addresses these. The effectiveness of this method in addressing these problems is examined in chapter 4.

2.5.1 Sample Contamination

Figure 2.7 presents a profile of manganese run on the Orpheus Island coral. The 'step' in the data occurs at the point where an earlier ablation was terminated part way through the analysis. It is unlikely that this 'step' is the result of ablating a furrow into the coral, as the depth of coral ablated by one track is very small, and this step was not observed for all elements. It is concluded that the first ablation removed material that was enriched in Mn, exposing fresh aragonite that had significantly lower Mn concentrations. This would imply the presence of significant surface contamination. Other elements displaying this contamination are Fe, Y, Cd, Sn, REEs, and Pb.

Replicate analyses over the same track were characterised by progressive attenuation of the Mn/Ca until after the 5th analysis the signal remained more or less constant. The laser slit was 50 μm wide, scanned at 1/30 mms⁻¹ and pulsed at 5 Hz. Under these conditions of the analyses, 5 analytical traverses would have ablated roughly 3.75 μm from the surface of the coral. The thickness of the contaminant layer is therefore deduced to be around 3-4 μm, hence the minimum cleaning ablation must remove at least 4 μm from the coral surface in order to ensure that surface contamination has been completely removed. The pre-ablation regime presented in section 2.1.4 removes 5-10 μm from the coral surface.

2.5.2 Fractionation with Drilling

Early versions of the laser method employed a static laser spot, drilling down into the surface of the sample material. This approach was taken for both standard and coral, with coral trace element profiles being generated from a series of evenly spaced spot ablations. Static spot methods allowed long counting times, which should have produced high precisions. Trace elements fractionate with depth, however (Eggins et al., 1998), leading to unstable signals (figure 2.8). TE/Ca ratios take several seconds to settle into a constant behaviour after the laser has been switched on (Sylvestre and Ghaderi, 1997 and figure 2.8), as intensities reach maximum and sample delivery
through the smoothing manifold stabilises. As the hole gets deeper, the TE/Ca ratio drifts as elements begin to condense on the side of the hole (Eggins et al., 1998).

**Surface Contamination**

![Mn/Ca profile](image)

*Figure 2.7 Demonstration of Surface Contamination*

This figure presents a Mn/Ca profile taken over a section of coral that has been partially pre-ablated. This coral was cleaned thoroughly with an ultrasonic probe in 18 MΩ water prior to analysis. Where the coral has not been previously ablated, the Mn/Ca levels are anomalously high, resulting in the distinctive 'step' in the profile. Clearly the pre-ablation has removed coral material that is enriched in manganese, indicating that the surface of the coral was still contaminated with a coating of manganese despite the water wash.

Fractionation during the analysis of the standard was a significant source of imprecision and inaccuracy in the calibration of coral analyses. The variable, and possibly matrix specific, fractionation of the TE/Ca signal with depth precluded simple averaging of the standard signal. Sylvester and Ghaderi (1997) attempted to fit linear fractionation models to the glass analyses in order to extrapolate back to a true trace element ratio at time zero. This method is not always satisfactory for all elements, as the trace element behaviour does not always settle down into a clearly linear fractionation trend (figure 2.8). Extrapolating a slope introduces a large uncertainty into an analysis, and because the linear region of the fractionation curve is small, precision cannot be improved by counting for longer.

The method adopted here (see section 2.2.3) is to scan the laser continuously over the surface of the standard, so that the laser never drills deeply into the sample. As can be seen in figure 2.8, the signal from a scan across the surface of a standard is very stable,
in contrast with that obtained from drilling down into the glass. Because the signal is stable, the precision can be increased by counting for longer. The only disadvantage of scanning is the potential for heterogeneities in the glass to add uncertainty to an analysis (see chapter 3, section 3.2.1).

**Static vs Scanned Analysis of Standard**

![Figure 2.8 Static and Scanned Analysis of the NIST 612 Standard](image)

This figure presents analyses of the NIST 612 glass for boron, both holding the laser static in one spot (a) and scanning the laser across the surface (b). During a static analysis of a standard, the laser drills down into the surface of the material, and elemental fractionation occurs as material condenses out onto the sides of the hole. The analytical signal for a static analysis is therefore not stable with time (a), and attempts to extrapolate the B/Ca ratio back to time 0 clearly produce erroneous results. In contrast the B/Ca signal for the scanned analysis is very stable (b), allowing longer integration times which in turn produce a higher precision on a measurement.

### 2.5.3 Topology

The excimer laser used for the laser ablation can be masked and focused down into a spot smaller than 20 μm in diameter if necessary. Corals, however, are extremely heterogeneous in structure and composition on fine scales (see chapter 7), and very high resolutions are therefore redundant when extracting seasonal and sub-seasonal
environmental signals from corals (indeed fine-scale variations may sometimes be large enough to obscure seasonal signals - see chapter 7).

It would be an advantage therefore to maximise the area of material ablated by the laser, in order to smooth as much of the structural variation as possible. The maximum theoretical size of the focused laser beam is around 1500 x 500 µm, and early experiments with the laser tested beam spots of around 1000 x 200 µm. It quickly became apparent, however, that this beam size was unsatisfactory. For a start, the large beam blasted a lot of material into the ICP-MS, which led to rapid degradation of the signal through fouling of the cones. The intensity of signal also tended to be too great to monitor $^{43}$Ca, and the detectors tripped out frequently.

A compromise was to use a beam of smaller area. Instead of using a circular profile beam, the laser was masked to produce a very thin, long slit (figure 2.4). This beam averages over a range of skeletal structures, while staying relatively small in total area. Because the energy density of the spot is not uniform towards the outer edges, the beam was limited to 600 µm across. The other (smaller) dimension is not especially significant, and was chosen to be as small as could practically be adjusted on the beam mask. In most cases it is only set approximately, and could vary between about 20 and 50 µm. This has a negligible effect on the final resolution of the technique which is controlled more by the scan speed, time-slice, and off-line data filtering. Figure 2.9 demonstrates the effectiveness of the smoothing generated by the 600 µm x 20 µm beam.

### 2.5.4 Short-term Drift

Upon commencing sample ablation, or when changing between samples of significantly different matrix composition, highly non-linear changes in mass response (fractionations) are observed over time-scales of several minutes (see figure 2.10). Sudden changes to the composition of the material entering the ICP-MS affect the charge build-up on internal components, and ion-beam composition, changing the focusing of the ICP-MS (see chapter 3). This short-term drift is observed to be almost entirely reversible, and once ablation ceases, the mass-response of the ICP-MS begins to return to previous levels.

Normalising count-rates to calcium can remove some sensitivity drift. Changes are not linear across the mass spectrum, however, and residual (nonlinear) drifts in elemental ratios are observed. Ratios can drift either up or down depending on tuning, and this response can not be satisfactorily extrapolated from one mass to another. Because only initial and final sensitivity is measured, it is not possible to construct a nonlinear
sensitivity model from which to correct this change in mass response, without breaking up the analysis and making multiple measurements of the standard.

### Large vs Small Laser Spot Analyses

![Graph showing B/Ca (µmol/mol) vs Distance from top of Coral for 70 µm Diameter Spot and 600 x 20 µm Spot](image)

**Figure 2.9 Effects of Laser Spot Size on Trace Element Profile**

Two analyses have been made over the same section of coral. The first (in black) used the 600 µm x 20 µm slit, while the second (in grey) used a 70 µm diameter circular spot. The analyses with the wide slit produced a smoother signal than the small spot. Note that some fine-scale features of the data appear to match between both profiles; however, their amplitude is significantly larger using the small spot, indicating that the large slit is effective in smoothing out some of the compositional heterogeneity in the coral.

Ratios, however, converge asymptotically on stable values (see figure 2.10). It appears that the ICP-MS reaches a steady-state mass response, representing saturation or equilibrium of the analyte material within the ICP system. The analytical method takes advantage of this phenomenon by employing two pre-conditioning steps (introduction of the second pre-ablation into the ICP-MS, and the 5 minute coral ablation) to ensure that the ICP-MS is in a steady-state with respect to carbonate material entering the system during analysis of the sample (section 2.3). These steps are effective in eliminating the non-linear short-term drift observed for most elements. Because it is important to maintain a steady state, any disruption to the flow of carbonate material through the ICP-MS system, such as analysing a silicate based standard, can result in nonlinear changes in mass response.
Nonlinear Drift

![Graph showing nonlinear and linear regions of drift](image)

Figure 2.10 Example of Nonlinear (Short-term) Drift
This figure demonstrates the dramatic, highly nonlinear short-term drift that can occur when fresh material is introduced into an unconditioned ICP-MS. The grey line is the unfiltered Sr/Ca ratio for a coral, and the dark line is included as an indication of the direction of drift. Note that the drift occurs on timescales of several minutes, and signals settle down into approximately linear behaviour after about 6 - 7 minutes.

2.5.5 Long-term Drift

Drift may occur over long timescales (minutes to hours - see figure 2.11), in response to changes in room temperature, tuning, laser performance, or general degradation and contamination of internal ICP-MS componentry. This drift can sometimes be dramatic, especially when large amounts of carbonate material are entering the ICP-MS over long periods of time. Unlike the short-term drift, long-term drift is usually irreversible.

Long-term drift can be minimised by limiting the amount of ablated material entering the ICP-MS, through keeping the rep-rates down, the laser energy low, and spot sizes small. On the timescale of a single coral analysis, long-term drift is very close to linear. By interpolating a linear mass response between standards run at the beginning and end of a coral analysis, and applying secondary corrections using the driftmonitor, long-term drift can be effectively eliminated (section 2.4).
2.6 Summary

A method for quantitative analysis of trace elements in corals by laser ablation has been developed. Small sections (50 mm x 22 mm) of coral are cleaned in water and dilute acid in an ultrasonic bath, dried, and mounted in a sealed perspex ablation chamber. The surface of the coral is cleaned by a series of two preablations to remove surface contamination. The method uses a short-wavelength ultraviolet laser to ablate small amounts of coral under a helium atmosphere. Ablated material is entrained in argon and enters a quadrupole ICP-MS. The laser spot is a long, narrow slit designed to average ablation over a range of structural features, while maintaining a high spatial resolution in the direction of the scan, and keeping the amount of ablated material entering the ICP-MS relatively low to minimise contamination of internal componentry.

Before analysis, the ICP-MS is conditioned to carbonate material to prevent short-term nonlinear drift associated with matrix loading. Backgrounds are collected before and after a coral analysis to correct for linear background drift. Analysis of a coral is
bracketed by two analyses of a standard to correct for long-term linear drift, and to calibrate the trace element profiles. Independent monitoring of drift is carried out by reanalysing the first section of the coral at the end of the analysis as a drift monitor. In general at least two parallel analyses are made along a coral section to allow further averaging of structural and compositional heterogeneity in the sample.

Data reduction is carried out off-line on spreadsheets. Basic processing includes background subtraction, normalising to calcium, drift correction, calibration, and filtering/smoothing the data. Drifts in background and mass-response are assumed to be linear and are corrected by interpolation between the beginning and end of the analysis. The profiles are filtered through an 11 point running median, and smoothed by an 11 point running average to remove fine-scale structural variability. Profiles are synchronised by aligning features common to both records and interpolating linear growth between. A similar method is used to transform extension into time by matching profiles with instrumental records or a hypothetical sinusoidal forcing function. Profiles are then resampled to a uniform, constant time increment and, if desired, a lower resolution by interpolation using a cubic spline function.
CHAPTER 3: STANDARD DEVELOPMENT

3.1 Introduction

Profiles of trace element variation can be generated simply by running the laser across the surface of the coral. Turning these raw-count signals into fully quantitative concentration measurements requires finding a standard that can be used to calibrate the signal, and correct for instrumental drift. Ideally, a single standard should allow fully quantitative analysis of B, Mg, Sr, Ba, and U with an accuracy and reproducibility of ±5%, and a precision of less than 3%. This chapter discusses the theoretical aspects of selecting a standard, and presents the synthesis and characterisation of a range of different standard materials.

3.2 Theoretical Considerations

Accuracy, Reproducibility and Precision

The ICP-MS measures the concentration of an isotope in a sample by monitoring the intensity of an ion beam arriving at the detector, and integrating ion counts over a short time interval (typically \( \frac{1}{6} - \frac{1}{14} \) of a second, depending on the number of elements analysed). A stable analytical signal behaves like a population of discrete ion count observations centred on, and normally distributed around, the average signal intensity. An analytical measurement is an estimate of the signal intensity made by averaging a finite number of observations (typically 60 data points for a standard).

Precision quantifies the certainty with which a measurement represents the true average signal intensity. Precision is defined by the standard error of the measurement (\( \sigma \times \sqrt{n} \): usually expressed as a % of the population average). For a single data point taken at random from a population, the precision is simply given by the standard deviation of the population. For a larger sample, the precision improves with the square root of the number of observations.

This statistical treatment is only valid if the analytical signal is stable. If the signal is changing with time (such as might occur if the laser traverses any sort of compositional
Chapter 3

gradient), then a systematic bias is introduced into the observations, and the data points are no longer independent or normally distributed. Standard errors can still be used to approximate precision, with the caveat that they will be overestimating the uncertainty to some degree.

Reproducibility quantifies how similar replicate measurements would be expected to be to each other. Reproducibility is defined as the percentage standard deviation derived from a population of replicate measurements. Separate reproducibilities may be quoted for different populations (e.g. between-run, or day-to-day reproducibility).

Accuracy quantifies how 'correct' a measurement is. Accuracy is assessed by measurement of independently calibrated samples, and is reported as a percentage deviation between the laser ICP-MS measurement and the independently measured concentration.

3.2.1 Homogeneity

Effect of Standard Heterogeneity on Precision

The precision of a measurement can be improved by increasing the number of observations (counting for longer), providing the signal is stable (so that data points are independent, and normally distributed around the mean). If the material over which the laser is scanned is heterogeneous, however, the signal will not be stable, as the laser traverses different compositional zones. This will lead to lower precisions, and invalidate the assumptions upon which counting statistics are based.

Effect of Standard Heterogeneity on Reproducibility

If a standard material is heterogeneous, then analyses made on different regions of the standard may be in poor agreement with each other. The magnitude of this irreproducibility depends on how much the composition of the standard material varies, and how this heterogeneity is distributed relative to the area of the standard surface that is averaged during each analysis.

Figure 3.1 presents the signals that would be produced by scanning the laser across 3 hypothetical heterogeneous standards. Each standard has an average composition of 1.1, and a range of compositions from 1.0 to 1.2 (a small component of gaussian noise has also been added to the profiles). Because a measurement is an average of 60 seconds of signal, some of the compositional variations in the standard will be averaged out. The range of measurements taken from different regions of the standard will therefore be less than the maximum compositional range. The dark lines in figure 3.1 are 60 point
running averages of the three hypothetical signals, and indicate the range of measurements that could be taken from the standards.

**Models of Heterogeneity in Standard**

![Figure 3.1 Models of Heterogeneity in Standard](image)

Three heterogeneous standards have been simulated on a computer. Each standard has an average composition of 1.1 and a range from 1.0 to 1.2, however the distribution of the heterogeneity in each is different. In the first standard (a), the heterogeneity is distributed evenly over scales that are similar to the distance traversed during the measurement. In the second standard (b), the heterogeneity is more unevenly distributed over scales that are larger than the distance traversed during a measurement. In the third standard (c) the heterogeneity is present as an even compositional gradient from one side of the standard to the other. The fine grey lines represent hypothetical trace element profiles taken across 10 mm of the standard surface. The black lines are 60 point running averages of these profiles, and represent all of the possible values for single 60 second (2.0 mm) measurement across the sample surface.

The first standard (figure 3.1a) has its compositional variation distributed on a scale that is smaller than an individual analysis. This leads to poor precisions; however, much of the compositional variation is averaged out, and replicate measurements would be quite
reproducible (table 3.1). The second standard (figure 3.1b) has heterogeneities that are distributed on scales larger than an individual analysis. The reproducibility of replicate measurements would therefore be poorer than for the first standard (table 3.1). The third standard has a smooth compositional gradient. In this case, individual analysis may be relatively precise, although poorly reproducible (table 3.1).

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Model 1 (Even Distribution)</td>
<td>5.4%</td>
<td>4.0%</td>
</tr>
<tr>
<td>Model 2 (Uneven Distribution)</td>
<td>2.0%</td>
<td>14.6%</td>
</tr>
<tr>
<td>Model 3 (Smooth Gradient)</td>
<td>1.0%</td>
<td>15.5%</td>
</tr>
</tbody>
</table>

[1] Average standard deviation of a 60 point analysis expressed as a percentage of the bulk composition.
[2] Range of possible compositions[3] that may be obtained from replicate 60 second measurements of the standard, expressed as a percentage of the bulk composition.
[3] Calculated from the 60 point running average of each profile.

Reproducibility can be improved by restricting replicate measurements to a small area of the standard (ideally the same analytical track), so that broad compositional gradients in the standard are avoided. This is simplest during daily analyses of a standard, especially if the laser track is clearly visible on the surface to guide realignment of the laser. Accurate re-alignment is more difficult if the standard has been moved (re-mounted), or if its surface has been re-polished. Thus, heterogeneity will tend to affect the day to day reproducibility more than measurements made within the same day.

**Effect of Standard Heterogeneity on Accuracy**

Standards are commonly calibrated by taking a subsample of the standard material and analysing it by other methods (e.g. solution methods). If heterogeneity is finely and evenly distributed throughout the standard material, then the subsample is likely to be a good representative of the bulk composition of the standard. If heterogeneity is not evenly distributed (i.e. a compositional gradient exists), then the subsample may not be representative of the bulk composition, resulting in inaccurate calibration of the standard. Calibration of a heterogeneous standard may be improved by taking a number of subsamples from different regions of the material.
3.2.2 Matrix Matching

Ablation, transport, and ionisation processes inherently fractionate trace elements. In order to quantify the concentration of an element in an unknown, it is necessary to compare it with an analysis of a calibrated standard. It is assumed that elements fractionate similarly in the standard and the unknown. If the standard and sample differ in their physical, textural, or chemical matrix, then elements may fractionate differently, leading to inaccurate standardisation. This is defined as a 'matrix effect'.

Chemical matrix effects

A chemical matrix effect is defined as a matrix effect caused by the differing bulk compositions of the standard and the unknown. The chemical matrix effect is a well recognised problem in solution-based ICP-MS methodology, where increasing the concentration of a major matrix-element often causes a mass-dependent decrease in the sensitivity of trace level analytes (Niu and Houk, 1996; Sisi and Hieftje, 1996). Chemical matrix effects generally derive from two processes within the ICP-MS: vaporisation and ionisation, and ion extraction.

Vaporisation and Ionisation

In the ICP-MS, the sample is vaporised and ionised in the ICP torch (figure 3.2). This is a complex heterogeneous process, dependent on a range of factors in the plasma, including; diffusion and transport of sample, ionisation equilibria, thermal and electrical conductivity in the plasma, electron temperature and density, charge transfer reactions with plasma ions, etc. (as reviewed in Sisi and Hieftje, 1996). Many of these factors are influenced by the composition of the material entering the plasma, and the effects that this has on the analyte signals are often mass dependent.

Sisi and Hieftje (1996) conclude that three processes principally affect the distribution and ionisation of material in the ICP. Firstly, the presence of volatile matrix elements in the plasma results in earlier volatilisation of analyte material, hence greater diffusion of analyte and greater proportions in off-axis plasma zones (that aren't sampled by the sample cone). Light elements are dispersed more because of greater mobility. Secondly, changes in the distribution and temperature of electrons in the plasma caused by different elemental abundances results in a shift in the analyte ionisation equilibrium. Finally, changes to the matrix concentration resulted in changes in the collisional excitation efficiency of different elements.

Ion Extraction

Matrix dependent fractionation may also occur in the ion beam downstream of the skimmer cone (Niu and Houk, 1996). As the ion beam passes into the high vacuum
region of the lens chamber of the ICP-MS, electrons are readily lost to the inner wall of the skimmer cone because of their high mobility (low mass, hence low momentum). Electrons may also be stripped out of the beam at the skimmer cone because of the negative potential on the first ion lens. The loss of electrons results in a net positive charge in the ion beam, creating repulsive forces in the ions, leading to dispersion of the beam. The degree of deflection in the ion beam is influenced by subtle changes in the composition of the beam, and the ion fractionation behaviour therefore changes with changing matrix concentration and composition.

As low mass ions have a greater mobility, they are dispersed more in the charged ion beam, and consequently the transmission efficiency is attenuated at low mass. This results in a non uniform sensitivity vs. mass curve, peaking at mid to high masses, and dropping off at lower masses. Tuning the ICP-MS shifts the peak of this curve, and is usually a compromise between sensitivity at low vs. high masses. It is possible to adjust lens and plasma conditions to minimise the sensitivity of the ICP-MS to matrix effects;
however, this is often at the expense of increased oxide production and decreased sensitivity (Niu and Houk, 1996).

The tuning of the ICP-MS lens system is optimised for a narrow range of ion energies. These energies may drift with time, probably as material deposits onto the surface of the sample cone, changing its electrical properties and altering gas flow dynamics. Material may also deposit on other components further downstream, causing the charge on the skimmer cone, the ion lenses and the photon stop to change with time. This will affect the focusing of the ion beam into the quadrupole. Because the sensitivity of the ICP-MS to different sample matrices is dependent on tuning, matrix effects are not necessarily constant, but can drift significantly through time. Sensitivity to matrix effects may also vary significantly from one ICP-MS tuning to the next.

Interferences
Interferences on a mass peak can result from polyatomic ions, doubly charged ions, or similar mass isotopes of other elements. The most common polyatomic ions are those produced through the reaction of metal atoms from the sample, and gaseous species in the plasma (especially oxides from entrained air, and argides from the plasma argon). Although strictly not matrix effects, the presence and levels of these interferences can depend on the chemical composition of the sample matrix, and may be variable with time.

The dry plasma used for laser analyses produces fewer interferences than the wet plasma used for solutions. For solution analyses, interferences are corrected for by applying interference equations based on measured production rates of the various interfering species. Theoretically a similar approach could be used for laser analyses. Production rates, however, may not be constant between samples: ablated material arrives at the plasma as a dust of solid particles, whose chemical matrix is significantly influenced by the chemistry of the sample. Different matrices may create a different suite of isobaric interferences.

The production of doubly charged species and polyatomics is dependent on a number of processes in the plasma, and is affected by plasma composition, plasma energy, torch configuration, cone composition and geometry etc. Because some of these factors are variable with tuning, production rates may not be constant with time.

Physical matrix effects
A physical matrix effect is defined as a matrix effect associated with differences in the bulk physical and textural properties of the standard and the sample. In solution ICP-MS, samples are dissolved prior to analysis and introduced into the plasma as a stream of dilute aquated ions (Morrison et al., 1995). Providing the carrier solution is kept
constant, there is little need to consider the complexities of different physical matrices in the samples and the standard. Because ablated material arrives at the plasma as a dust of solid particulates, however, the physical and textural properties of this material can be an important factor in controlling the chemical fractionation. Physical matrix effects may arise during ablation, during transport from the ablation site, and during vaporisation and ionisation.

Ablation

The initial interaction of the laser with the sample is a direct adsorption of the laser energy that causes extreme localised heating and spontaneous vaporisation ('phase explosion'). This photothermal ablation liberates a cloud of vaporised material that is further heated by the laser, forming a super-hot plasma at the sample surface. After the laser pulse has ended, the hot ablation plasma continues to heat the sample surface, resulting in additional thermal volatilisation of material (plasma ablation). The intense localised heating may create a physical and thermal shock in the material that results in fracturing of the sample and explosive ejection of solid angular fragments (for more information see reviews in Eggins et al., 1998; Feng, 1994; Morrison et al., 1995).

In general, ablation using the UV excimer laser does not result in significant elemental fractionation, due to the high intensity and strong absorbence of the samples at the 193 nm wavelength. Plasma ablation may result in a thermal volatilisation of material from the sample surface, and some fractionation of elements by their volatility and lattice characteristics is possible.

Shock shattering of the surface does not result in chemical fractionation of the exploded fragments, however the degree of fracturing will be dependent on the physical characteristics of the surface. Cracking will preferentially occur along cleavage planes and between grain boundaries, and will be affected by the thermal conductivity and thermal expansion properties of the material. Thus the amount, size and shape of solid particulates produced by the laser will vary with the matrix of the sample. Any differences in the size distribution of a powder may influence the volatilisation and ionisation of the sample in the ICP.

Transport

Ablation occurs at atmospheric pressure. As a consequence, the hot ablation plume encounters a thick gaseous envelope that rapidly halts further plume expansion producing a shockwave that reflects some of the particulate and gaseous material back at the sample surface, forming an ablation blanket (Eggins et al., 1998). Larger particulates have a greater momentum, propagating further, while fine particulates are deposited close to the ablation site. Thus, ablation results in a fractionation based on particulate size.
The sample cell used with this laser system is designed to allow ablation to occur in helium, with the sample becoming entrained in argon only after ablation has occurred. Because helium has a much lower density than argon, ablated material propagates further before the expansion is halted by the ambient gas, so mass transmission (hence sensitivity) is significantly better than ablation in argon.

The ablation debris that forms the ejecta blanket is enriched in volatile elements (Eggins et al., 1998). Most of the material produced during ablation is gaseous, and volatile elements in this plasma plume clearly condense onto the sample surface. Refractory elements condense very quickly as the ablation plasma cools, and are either deposited inside the ablation hole, or form solid droplets that are projected away from the ablation site. The differential condensation of refractory and volatile elements results in a chemical fractionation of the ablated sample.

Further chemical fractionation can occur during transport of the ablated material to the ICP-MS, through depletion of refractory phases as gaseous species condense on the walls of the gas tubing, or form droplets that settle out of the gas stream. Particle size fractionation will also occur as the larger particulates are trapped in the sample cell, smoothing manifold and tubing.

**Vaporisation and Ionisation**

In solution ICP-MS, the principal processes occurring in the plasma are de-watering, and ionisation. Provided the nebuliser produces a reasonably constant size distribution of solution droplets, the physical matrix of one sample is very much like another, and the only matrix effects that occur during volatilisation and ionisation are chemical (see above). Ablated material, however, arrives at the plasma as a fine cloud of gaseous and particulate material (mostly small angular fragments and spherical droplets of condensed material Morrison et al., 1995), heterogeneous in both size and composition. Significantly, the physical matrix of these particulates will be influenced by the chemical, physical, and textural characteristics of the sample being ablated.

A particle must be broken up and dissociated into its component atoms before ionisation. Dissociation will depend on both the size of the particle, and strength of the bonding. Particles of different size and composition will have different residence times in the plasma. Since the plasma is heterogeneous, this will affect the position and environment in which ionisation finally occurs. These subtleties can significantly influence the ion populations. Ablated samples that differ in their physicochemical matrix and/or the size distribution of their particulates can behave quite differently in an ICP, leading to matrix sensitive fractionations.

During solution analysis, the continuous introduction of a relatively uniform solution matrix maintains the ICP in a steady state, and buffers it against changes in the sample
matrix. When running with a dry plasma, sudden variations in either the matrix or the particle size distribution entering the ICP can cause changes in ICP-MS response. Depending on the timescale of the variation in sample, this can lead to signal instability, or short term drift (see section 2.5.4).

**Influence of Matrix Effect on Precision**

Ablation of a fragile material that is prone to explosive fracturing can lead to angular particulate debris that is distributed unevenly over a wide range of size fractions. This heterogeneity in size manifests as an instability in the plasma ionisation processes, producing a noisier signal and lower precisions than would be obtained from a more cleanly ablating material (Perkins et al., 1991).

**Influence of Matrix Effect on Reproducibility**

Standards and samples can fractionate differently during analysis if their physical and chemical matrices differ. If this fractionation behaviour does not change with time, then reproducibility will not be affected.

Ablation related matrix effects are sensitive to laser focusing, beam shape, geometry of the laser pit, the ablation atmosphere, and the energy density in the laser spot. Condensation fractionation is a function of laser conditions, sample cell geometry, gas composition and flow, while transport processes are affected by gas flow and tubing geometry. These parameters are all well controlled, and will not vary significantly through time. Ablation, condensation, and transport related matrix effects will not, therefore, contribute significantly to irreproducibility.

Dissociation and ionisation of sample is affected by the position and geometry of the torch, coil position, power settings, gas flow rates and gas composition, etc. The depth of sampling of the plasma is generally kept constant during day to day running of the ICP-MS, however it may be altered during routine maintenance (such as changing over from solution to laser), and any time a component of the torch is replaced. Both nebuliser gas flow, and the XY position of the torch box are routinely adjusted during daily tuning of the ICP-MS. This variation in the torch geometry can influence the magnitude of matrix dependent element fractionation during ionisation. These effects can contribute significantly to irreproducibility.

Space-charge dispersion of the ion beam downstream of the skimmer cone is affected by total ion current, beam velocity, skimmer cone composition and geometry, and lens settings. Of these, the most variable parameters are the voltage settings on the ion lenses, which are routinely adjusted during tuning and often change in the course of a days running. The intensity of the ion beam is also variable, and can be affected by the intensity and efficiency of ablation, the geometry of the sample cone and the tuning of
the ICP-MS. Progressive fouling of the internal components of the ICP-MS can cause drifts in tuning, as the electrical properties (and hence charge) on the cones, photon stop and ion lenses changes. These daily, and day-to-day fluctuations in beam focusing can generate significant variability in matrix-dependent fractionation, contributing to irreproducibility in the measurement of a sample.

Influence of Matrix Effect on Accuracy

Accurate standardisation relies on elements fractionating similarly during analysis of the sample and the standard. If elements fractionate differently, the standardisation will be inaccurate. Since matrix dependent fractionation processes can change with time, any difference between sample and standard fractionation cannot reliably be corrected by applying a single correction ('fudge') factor.

The two largest contributors to matrix related inaccuracy are probably the ablation of different physical matrices, and chemical matrix effects occurring downstream of the skimmer cone. Development of an accurate quantitative analytical method will therefore need to address both chemical and physical matrix matching of standards to samples.

3.2.3 Similar levels of unknowns

The analytical method developed (section 2.3) uses a single standard to establish a calibration curve, assuming that the count-rate vs. concentration curve for all elements is a straight line passing through zero (Stix et al., 1995). Isobaric interferences can result in a non-zero intercept, however, while matrix effects, and saturation of detectors can produce a nonlinear sensitivity vs. concentration response (e.g. see figure 3.3). The inaccuracy associated with a single point standardisation can be minimised by ensuring that the concentration in the unknown is similar to the concentration in the standard (Stix et al., 1995).

Matching concentrations in the sample and the standard is important for optimising detection limits. Detection limits are limited by the sample delivery rate to the ICP-MS, which in turn is limited by the most intense element signal monitored (which cannot exceed the trip-out point of the detector). If a major trace element in a standard is too low it may be undetectable or imprecise, while if a major element is too high it will limit the maximum ablation intensity, compromising detection limits for other elements.
Nonlinearity in Count Rate Response

Figure 3.3 Nonlinear Concentration vs. Count Rate Response in ICP-MS
The ICP-MS does not generate a perfectly linear count-rate vs. concentration response. This figure presents the result of an experiment where the same section of coral was repeatedly analysed under increasingly intense ablation conditions. The B/Ca ratio is graphed against ablation intensity (in this case counts of $^{43}$Ca). If count rates were a linear function of the concentration in the sample, the result should be a horizontal straight line. Instead, the ratio of boron to calcium decreases steadily as ablation intensity increases. Normal operating intensity is around 2 - 3 million counts per second on $^{43}$Ca. The detectors tripped out at around 5 million counts per second.

3.2.4 Other Desirable Traits in a Standard

Easily Cleaned
Because the same standard is used during each analysis, it is handled much more frequently than coral samples. Even during careful routine handling it is possible to contaminate the surface. Multiple analysis of a standard can also leave a contaminant residue of ablation debris. While preablation will remove contaminants, it is still desirable to occasionally subject the standard to a rigorous cleaning such as re-polishing and ultrasonication.
Visible Ablation Track

Being able to see the ablation track is of great advantage in accurately relocating a previously analysed section of standard. While the spatial co-ordinates of all analytical tracks are recorded, the micrometer system on the sample stage does drift slightly with time, and misalignments of a few tens of microns can occur during a day. These can have a significant effect on analyses, especially if a standard is heterogeneous, or if analysis drifts onto regions that have not been preblasted.

Under routine analytical conditions a single traverse of the laser over a standard leaves a very shallow pit, 600 μm wide, 2000 μm long, but only 0.4 μm deep. It is easy to see this ablation pit on a uniform reflective surface such as a polished glass; however, it is almost invisible on a matt heterogeneous surface such as a coral or pressed powder.

3.2.5 A Note on Calibration of Standards

Accurate standardisation of an unknown requires that the composition of the standard is accurately known. The composition of a manufactured standard cannot always be assessed from the composition of its components, especially if the synthesis may have introduced contaminants or altered the composition. Both manufactured and natural standards must therefore be calibrated externally to known reference standards using independent analytical techniques. This often requires that subsamples of the standard be taken. This can be a fairly damaging process, at best inconvenient, and at worst potentially destroying a delicate standard material, or exposing it to contamination.

If a standard is at all heterogeneous, then there can be some uncertainty about how well the material subsampled for calibration represents the material analysed during routine standardisation. Sometimes, the calibration processes itself is inaccurate either because the standard material fractionates during analysis (e.g. boron is lost from glasses during dissolution by HF and heat) or because the external calibration suffers from matrix effects.

An alternative calibration philosophy is to find the 'apparent composition' of a standard: that is the composition that the standard needs to have in order to give the 'correct results'. An unknown sample is carefully analysed by independent techniques, and then used to calibrate the standard by repeated cross-analysis by laser. The 'apparent composition' of the newly calibrated standard does not necessarily relate to its real composition. Instead, it represents the hypothetical composition that would give an accurate standardisation of the calibrating sample.

There are two major advantages of this standardisation approach. Firstly, the apparent composition will automatically adjust for any constant bias between the sample and the
standard introduced by the laser ablation (e.g. time-independent matrix effects), making analyses more accurate. Time dependent fractionation will still result in irreproducibility, however, so matrix and concentration matching is still important. Secondly, subsamples are taken from the unknown rather than the standard. It is much easier to shave a section off a coral, dissolve it, and analyse it by isotope dilution, than it is to calibrate a synthetic glass, or to remove a section from a fragile fused powder that is embedded in epoxy resin.

The major disadvantage of this calibration strategy is that the 'apparent composition' is specific for a particular analytical technique and the narrow suite of samples that share the same chemical and physical properties as the externally calibrated sample (i.e. specific for one particular set of analytical fractionations).

Although this alternative method of standardisation has not been used here, in light of the accuracy problems discussed in the next chapter, it may be necessary to adopt this approach in the future.

3.2.6 Summary

An ideal standard is homogeneous, and matched with the sample in physical matrix, chemical matrix, and elemental concentrations. Ideally, it should have a robust polished surface, and be easily calibrated.

3.3 NIST 612/610

The American National Institute of Standards (NIST) manufacture a number of solid reference standards. NIST 610 and 612 are basalt composition glasses (72% SiO₂, 12% CaO, 14% Na₂O, and 2% Al₂O₃) which have been doped with trace elements to a nominal concentration of 500 ppm and 50 ppm respectively. Considerable care and effort have gone into ensuring that the glass is as homogeneous as possible, and manufacturers claim that the glass standards are homogenous to better than 5%.

3.3.1 Advantages

The NIST glasses are extremely homogeneous (figure 3.4). Small patches of heterogeneity are present in the glass; however, these can easily be detected and avoided during routine analysis. Compositional variations will not therefore affect precision, reproducibility, or accuracy, although the low concentrations of ¹¹B, ²⁵Mg, and ⁸⁴Sr generate large counting statistic errors (table 3.2).
Figure 3.4 Profiles of Scans Across NIST 612 Glass
This figure presents trace element profiles across the surface of the NIST 612 glass. As in figure 3.1 the grey lines represent the raw TE/Ca profile, and the black lines are 60 point running averages of these profiles; representing all of the possible values for a single 60 second (2.0 mm) measurement. Tabulations of the heterogeneity in this standard can be found in table 3.2. Note the very noisy signals for Sr and Mg. The low levels of these elements in the NIST 612 glass leads to unstable signals and imprecise analyses.

Because these standards are in routine use in laboratories around the world, the composition of the glass has been thoroughly measured by many independent methods and tabulations are available in the literature (e.g. Pearce et al., 1995). The addition of trace elements to the glass ensures that most analyte elements are present in levels well above the detection limits of the laser ablation technique (see next chapter for tabulations of detection limits). Finally, being a glass, laser traverses are easily seen, and a clean surface can readily be produced by polishing.
The features described make the NIST glasses attractive candidates for a coral standard, and were used by Feng (1994) to successfully calibrate laser ablation analyses of carbonates (calcite and dolomite grain mounts).

### Table 3.2 Heterogeneity in Standards

#### Compositional Range\[1\]

<table>
<thead>
<tr>
<th>Standard</th>
<th>B/Ca</th>
<th>Mg/Ca</th>
<th>Sr/Ca</th>
<th>Ba/Ca</th>
<th>U/Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIST 612</td>
<td>28%</td>
<td>26%</td>
<td>41.0%</td>
<td>9.0%</td>
<td>9.0%</td>
</tr>
<tr>
<td>Wollastonite 1</td>
<td>16%</td>
<td>12.2%</td>
<td>4.4%</td>
<td>17%</td>
<td>12%</td>
</tr>
<tr>
<td>Wollastonite 2</td>
<td>8.6%</td>
<td>5.9%</td>
<td>3.3%</td>
<td>5.7%</td>
<td>6.2%</td>
</tr>
<tr>
<td>Pressed Powder</td>
<td>23%</td>
<td>14%</td>
<td>9.5%</td>
<td>16%</td>
<td>11%</td>
</tr>
<tr>
<td>Fused Powder</td>
<td>21%</td>
<td>19%</td>
<td>6.4%</td>
<td>10%</td>
<td>26%</td>
</tr>
<tr>
<td>Standard Coral</td>
<td>24%</td>
<td>49%</td>
<td>79%</td>
<td>64%</td>
<td>30%</td>
</tr>
<tr>
<td>Aragonite</td>
<td>130%</td>
<td>220%</td>
<td>48%</td>
<td>120%</td>
<td>240%</td>
</tr>
</tbody>
</table>

#### 60 Point Average Range\[2\]

<table>
<thead>
<tr>
<th>Standard</th>
<th>B/Ca</th>
<th>Mg/Ca</th>
<th>Sr/Ca</th>
<th>Ba/Ca</th>
<th>U/Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIST 612</td>
<td>7%</td>
<td>4.0%</td>
<td>9.0%</td>
<td>1.6%</td>
<td>2.8%</td>
</tr>
<tr>
<td>Wollastonite 1</td>
<td>10%</td>
<td>10.7%</td>
<td>2.4%</td>
<td>16%</td>
<td>10.4%</td>
</tr>
<tr>
<td>Wollastonite 2</td>
<td>7.4%</td>
<td>5.8%</td>
<td>1.6%</td>
<td>2.9%</td>
<td>3.2%</td>
</tr>
<tr>
<td>Pressed Powder</td>
<td>23%</td>
<td>11%</td>
<td>4.9%</td>
<td>22%</td>
<td>7.4%</td>
</tr>
<tr>
<td>Fused Powder</td>
<td>5.7%</td>
<td>12%</td>
<td>0.77%</td>
<td>2.6%</td>
<td>8%</td>
</tr>
<tr>
<td>Standard Coral</td>
<td>20%</td>
<td>48%</td>
<td>6.1%</td>
<td>72%</td>
<td>23%</td>
</tr>
<tr>
<td>Aragonite</td>
<td>76%</td>
<td>540%</td>
<td>43%</td>
<td>160%</td>
<td>350%</td>
</tr>
</tbody>
</table>

\[1\] 90 percentile range expressed as a % of the average composition  
\[2\] 60 point average range expressed as a % of the average composition

### 3.3.2 Disadvantages

There are three significant problems with using NIST glasses. Firstly the NIST glasses are an amorphous solid solution of Ca, Na, Al silicates, and are a poor chemical matrix match with the corals which are micro-crystalline calcium carbonate. Preliminary attempts to standardise corals to NIST 612 indicated poor reproducibility. No systematic study was carried out to determine the magnitude of any inaccuracy that might be generated by this matrix difference; however, Feng (1994) reported offsets between solution and laser analyses of around 10%, and this may be a reflection of the matrix mismatch between sample and standard.
Standards

The second major problem is that the trace element composition of the NIST glasses is different from that of coral skeletons (see table 3.3), in some cases by several orders of magnitude. Magnesium and strontium are very abundant in corals, forcing the analysis of minor isotopes ($^{25}\text{Mg}$, and $^{84}\text{Sr}$). Both elements are at trace levels in the NIST 612 glass (30 - 80 ppm), and the minor isotopes are therefore very close to the detection limit of the technique, resulting in very imprecise and inaccurate measurement. The concentrations of Mg and Sr are higher in NIST 610 (approx. 500 ppm); however, the concentrations of $^{138}\text{Ba}$ and $^{238}\text{U}$ in this glass are too high and risk tripping out the detector. Because of the large differences in concentration between the NIST glasses and corals, nonlinearity in the sensitivity vs. concentration curve may also result in very poor standardisation.

The third problem is that the NIST glasses have significantly lower Sr concentrations than corals. After each coral analysis, Sr takes a significant time to 'wash out' of the system, and its memory may interfere with the low Sr signal of the NIST standard.

Because of these problems, the NIST glasses were not used for standardising coral analyses for B, Mg, Sr, Ba, and U. There is no standard that is suitable for calibrating the lower level elements (e.g. Mn, Y, La, Ce), and so the NIST 612 is often run at the end of a coral analysis to allow a semiquantitative estimate of the minor trace elements.

3.4 Pressed Powder

Although a chemically matrix-matched standard is desirable, no commercial, homogeneous, solid-state carbonate standard is presently available (Feng, 1994). Pressed powders are simple to prepare and are commonly used as both geological standards, and as a method of homogenising samples for laser analysis (e.g. Jarvis and Williams, 1993; Morrison et al., 1995; Pearce et al., 1992; Perkins et al., 1991). A pressed powder standard was therefore prepared from crushed coral.

3.4.1 Synthesis

Preparation of Crushed Coral Powder

A large chunk was broken from a coral head (collected from Davies Reef - see chapter 8 for details of the location). The tissue layer was removed with a diamond glass-cutting blade, then the coral chunk was ultrasonicated for 1 hour in 18 MΩ water and dried. Once dried the coral was coarsely crushed on a hydraulic press, producing fragments no larger than 2.5 cm$^3$. These fragments were ultrasonicated twice in 18 MΩ water, with
the water being replaced each time, and then left soaking overnight in 18 MΩ water, before being dried on absorbent paper towels.

### Table 3.3 Compositions of Standards

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Coral</td>
<td>48.6</td>
<td>960</td>
<td>7463.5</td>
<td>3.51</td>
<td>2.35</td>
<td>383230</td>
</tr>
<tr>
<td>± 0.5</td>
<td>± 10</td>
<td>± 0.7</td>
<td>± 0.04</td>
<td>± 0.02</td>
<td>± 20</td>
<td></td>
</tr>
<tr>
<td>NIST 612</td>
<td>34</td>
<td>40</td>
<td>76.4</td>
<td>38.5</td>
<td>36.9</td>
<td>84640</td>
</tr>
<tr>
<td>± 1</td>
<td>± 20</td>
<td>± 0.6</td>
<td>± 0.6</td>
<td>± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old</td>
<td>47.0</td>
<td>870</td>
<td>6200</td>
<td>6.8</td>
<td>2.07</td>
<td>320000</td>
</tr>
<tr>
<td>Wollastonite</td>
<td>± 0.2</td>
<td>± 30</td>
<td>± 100</td>
<td>± 0.4</td>
<td>± 0.06</td>
<td>± 6000</td>
</tr>
<tr>
<td>New</td>
<td>50.5</td>
<td>890</td>
<td>6210</td>
<td>4.4</td>
<td>2.19</td>
<td>320000</td>
</tr>
<tr>
<td>Wollastonite</td>
<td>± 0.3</td>
<td>± 30</td>
<td>± 100</td>
<td>± 0.3</td>
<td>± 0.06</td>
<td>± 6000</td>
</tr>
<tr>
<td>Coral Powder</td>
<td>59.1</td>
<td>1030</td>
<td>7468</td>
<td>5.0</td>
<td>2.46</td>
<td>383500</td>
</tr>
<tr>
<td>± 0.6</td>
<td>± 10</td>
<td>± 2</td>
<td>± 0.1</td>
<td>± 0.01</td>
<td>± 200</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(µmol/mol)</td>
<td>(mmol/mol)</td>
<td>(mmol/mol)</td>
<td>(µmol/mol)</td>
<td>(nmol/mol)</td>
<td></td>
</tr>
<tr>
<td>Coral</td>
<td>471</td>
<td>4.14</td>
<td>8.9087</td>
<td>2.67</td>
<td>1030</td>
</tr>
<tr>
<td>± 5</td>
<td>± 0.05</td>
<td>± 0.009</td>
<td>± 0.03</td>
<td>± 9</td>
<td></td>
</tr>
<tr>
<td>NIST 612</td>
<td>1490</td>
<td>0.7</td>
<td>0.412</td>
<td>133</td>
<td>73400</td>
</tr>
<tr>
<td>± 6</td>
<td>± 0.3</td>
<td>± 0.003</td>
<td>± 2</td>
<td>± 700</td>
<td></td>
</tr>
<tr>
<td>Old</td>
<td>545</td>
<td>4.49</td>
<td>8.92</td>
<td>6.2</td>
<td>1090</td>
</tr>
<tr>
<td>Wollastonite</td>
<td>± 3</td>
<td>± 0.04</td>
<td>± 0.02</td>
<td>± 2</td>
<td></td>
</tr>
<tr>
<td>New</td>
<td>585</td>
<td>4.59</td>
<td>8.94</td>
<td>4.0</td>
<td>1150</td>
</tr>
<tr>
<td>Wollastonite</td>
<td>± 3</td>
<td>± 0.04</td>
<td>± 0.02</td>
<td>± 2</td>
<td></td>
</tr>
<tr>
<td>Coral Powder</td>
<td>571</td>
<td>4.43</td>
<td>8.908</td>
<td>3.810</td>
<td>1080</td>
</tr>
<tr>
<td>± 6</td>
<td>± 0.05</td>
<td>± 0.002</td>
<td>± 0.008</td>
<td>± 5</td>
<td></td>
</tr>
</tbody>
</table>

[1] Boron in wollastonite glass was calibrated by 21 paired analyses against NIST 612.
[2] Strontium and Calcium in all materials was analysed by isotope dilution TIMS
All other measurements were made by isotope dilution ICP-MS.

Errors are 1σ standard deviations of 3 replicates.

The fragments were subjected to a rigorous cleaning regime modified from Shen and Boyle (1988). This involved heating the coral for 3 hours in 30% H2O2 with two 30 minute ultrasonic agitations. Coral fragments were left soaking in the peroxide for two days, rinsed twice in 18 MΩ water, ultrasonicated for an hour and then dried. The large fragments were kept.
The second step involved heating the coral for several hours in a 1:6 solution of hydrazine and then subjecting it to two 30 minute ultrasonifications. Coral fragments were left soaking for a day, then rinsed twice in 18 MΩ water, ultrasonicated in 18 MΩ water for an hour and then dried. The large fragments were again retained.

Finally, the coral fragments were leached in a 5% solution of citric acid for 5 minutes, rinsed twice in 18 MΩ water, ultrasonicated in 18 MΩ water for an hour and then dried. After drying, a few fragments were transferred to a rigorously cleaned agate pestle and mortar (mounted on an automated shaker table), crushed and discarded. The remaining fragments were then finely crushed, and the powder transferred to a polycarbonate container which was shaken and rolled extensively to homogenise it.

**Preparation of the Pressed Powder Pellet**

Two 5.0 g subsamples of the coral powder were transferred to 1 inch diameter aluminium cups. 10 drops of 2.5% poly vinyl acetate (PVA) binder was added to one cup, while the other was left binder free. Both cups were then pressed in a hydraulic press at 300 bar for 10 seconds.

Both pressed powders had relatively fragile surfaces and loose powder could be found on both. The pellet with no PVA binder proved to be too fragile for practical use and was discarded.

### 3.4.2 Calibration

Samples of the component powder were analysed by isotope dilution. The PVA binder was not analysed, and it is assumed that this did not significantly affect the trace element composition of the pressed pellet. This was considered suitable for a preliminary calibration, although B may have been contaminated by tetraborate flux used for previous glass fusions. Details of the dissolution chemistry and isotope dilution analysis of the powder are presented in the following chapter (section 4.5.1). The elemental composition of the component powder is presented in table 3.3.

### 3.4.3 Advantages

With the exception of a small amount of PVA binder, the chemical matrix of the pressed powder pellet is closely matched to an unknown coral. Chemical matrix effects will therefore be minimal. The levels of analyte trace elements in the pressed powder will also be close to those in any unknown coral, and nonlinearity in the calibration curve will not cause problems.
Once the coral powder has been prepared, it is simple to make up a pressed powder standard, and accurate calibration of the component powder is easy. It is possible to spike a powder with small amounts of trace element solutions to produce a standard with desired concentrations of analyte elements (e.g. Pearce et al., 1992); however, this was not attempted. Pressed powder standards have been successfully used for analysis of carbonate shell material (Pearce et al., 1992; Schettler and Pearce, 1996).

### 3.4.4 Disadvantages

A reconnaissance study was carried out by scanning the laser across the pressed powder surface. Figure 3.5 presents the Ca normalised trace element profiles from the pressed powder. There is clearly some heterogeneity in the standard. While this is comparatively mild (table 3.2), it appears to occur over distance scales that are much larger than the grain size of the powder. This may represent uneven mixing of the component coral powder, or an uneven distribution of the binder in the pressed pellet, leading to regions of the pellet that ablate differently. If the latter is true, then it is somewhat disturbing to see this heterogeneous signal persist despite normalising to calcium (which should correct out fluctuations in the total mass delivery rate). This may indicate that different ablation characteristics can result in surprisingly large elemental fractionations.

Count rates for ablation of a pressed powder are generally higher than for a typical coral (table 3.4), and it is possible that the fragile powder is explosively mobilised from the surface during ablation. The size distribution of particles entering the ICP may therefore be significantly greater than for coral ablation, leading to instability in the signal and physical matrix effects (see section 3.2.2). The pressed powder signals 25 - 30% less stable than the wollastonite glass, which agrees with the observations of Perkins et al. (1991). Physical (textural) matching of the standard matrix to the coral may therefore be important.

Several additional problems exist with the pressed powder. The soft porous surface can easily become contaminated, and cleaning using any kind of solution is impossible. It is also very difficult to detect the ablation track left after a laser has traversed across the surface of a pressed powder.

### 3.4.5 Pressed Powder Reconsidered

Because of the problems encountered during the reconnaissance study, the pressed powder was not considered as a standard for coral analyses until it became apparent that the mismatched chemical matrix of the CaSiO₃ glass caused significant inaccuracy (see below). The poorer signal stability generated by both the heterogeneity and ablation of the fragile powder is not significant compared with other sources of uncertainty, as the
standard errors for a 60 second measurement are typically below 1.0%, and often below 0.5%.

**Pressed Powder**

![Diagram of Pressed Powder Profiles](image)

**Figure 3.5 Profiles of Scans Across a Pressed Powder Pellet**

This figure presents trace element profiles across the surface of the pressed powder standard. As in figure 3.1 the grey lines represent the raw TE/Ca profile, and the black lines are 60 point running averages of these profiles; representing all of the possible values for a single 60 second (2.0 mm) measurement across the sample surface. Tabulations of the heterogeneity in this standard can be found in table 3.2. In general, the profiles are relatively flat, indicating that the sample is reasonably homogeneous. Some heterogeneity is evident over relatively large distances, and an unevenly distributed PVA binder may be responsible for some uneven ablation behaviour.

While the heterogeneity in the pressed powder standard can lead to inaccuracy and irreproducibility (see section 3.1.2), much of this will be averaged out during a measurement as it occurs over distances less than 2.0 mm. The range of compositions that could be measured during a 60 s (2.0 mm) analysis are presented in table 3.2. These are maximums that can be reduced significantly by restricting analysis to a narrow region.
of the pressed powder. In any case, the loss in accuracy and reproducibility associated with a heterogeneous standard must be balanced against the loss in accuracy and reproducibility caused by a chemical matrix difference in a standard like the wollastonite glass.

### Table 3.4 Count Rates for Laser Analysis of Standard Materials (ratio to a typical coral)

<table>
<thead>
<tr>
<th>Standard</th>
<th>B</th>
<th>Mg</th>
<th>Ca</th>
<th>Sr</th>
<th>Ba</th>
<th>U</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIST 612</td>
<td>0.53</td>
<td>0.061</td>
<td>0.18</td>
<td>0.0081</td>
<td>3.7</td>
<td>11</td>
</tr>
<tr>
<td>Old</td>
<td>0.63</td>
<td>0.70</td>
<td>0.60</td>
<td>0.57</td>
<td>0.80</td>
<td>0.69</td>
</tr>
<tr>
<td>Wollastonite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New</td>
<td>0.67</td>
<td>0.72</td>
<td>0.60</td>
<td>0.57</td>
<td>0.52</td>
<td>0.73</td>
</tr>
<tr>
<td>Pressed Powder</td>
<td>3.0</td>
<td>1.9</td>
<td>1.3</td>
<td>1.5</td>
<td>1.01</td>
<td>1.2</td>
</tr>
<tr>
<td>Fused Powder</td>
<td>1.3</td>
<td>1.4</td>
<td>1.1</td>
<td>1.1</td>
<td>0.59</td>
<td>0.88</td>
</tr>
<tr>
<td>Coral Standard</td>
<td>1.0</td>
<td>1.3</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Aragonite</td>
<td>0.00016</td>
<td>0.001</td>
<td>0.74</td>
<td>0.17</td>
<td>2.5</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

Improvements in the synthesis of pressed powders, has generated pellets that appear to ablate more similarly to corals, and are free from the B contamination seen here (S. Fallon, pers. comm.). Pearce et al. (1992) report that carefully synthesised pressed powder pellets approach glasses in their homogeneity and signal stability. Surface contamination of the pressed powder can be avoided by careful handling and rigorous pre-ablation. Given the simplicity with which a pressed powder standard can be synthesised, a grossly contaminated standard can simply be discarded and replaced as necessary.

### 3.5 CaSiO₃ (Wollastonite) Glass

Glass has superior ablation characteristics over pressed powders. Perkins et al. (1991) synthesised and tested a borosilicate glass made from crushed shell material, and report superior precisions and accuracy over carbonate based pressed powders. Crushed coral powder and silica were therefore fused to form a CaSiO₃ (wollastonite) composition glass.
3.5.1 Synthesis

Tetraborate fusion fluxes will contaminate a glass with boron, and compromise boron analysis in corals, so a glass was synthesised using a straight mix of silica and coral powder. Specpure silica and crushed coral powder were mixed in a 1:1 (molar) ratio, with a 5% excess of silica added to aid vitrification. Ten grams of glass required around 5.43 g of silica and 8.62 g of coral powder. The components were mixed by crushing 6 times to dryness in an agate mortar and pestle, using acetone as a wetting agent. The components were transferred to a platinum crucible (with lid) and heated overnight at 150°C to drive off moisture and any remaining acetone.

The crucible and its contents were heated up to 1100°C (ramped at 10°C per minute) and held at that temperature for 24 hours to decompose the CaCO$_3$ into CaO and CO$_2$. The crucible was weighed on cooling to ensure full decomposition, and the components were then re-crushed three times to dryness in acetone, and then heated at 150°C overnight to dry.

CaSiO$_3$ glasses melt at very high temperatures (1610°C). The components were transferred to a small cup made from platinum foil, which was placed into the bottom of a platinum crucible. The crucible was heated to 1350°C in an elevator furnace, and held there for 20 minutes to ensure temperature equilibration. The temperature was then ramped at 20°C per minute up to a final temperature of 1650°C, and held for 6 minutes. The crucible was removed from the furnace, and the contents were quenched to a glass by cooling the crucible on a graphite block.

The glass shattered when attempts were made to cut it; however, several large fragments were recovered, mounted in a 1 inch epoxy disk, and polished.

Ensuring that the melt quenched to a glass was difficult as it devitrified when poured (probably because microcrystals formed and nucleated crystallisation where the liquid encountered the cooling side-wall of the crucible). A glass formed if the melt was cooled within the crucible; however, once solid it was impossible to remove. The problem was solved by making a thin sacrificial sheath of platinum foil that sat inside the crucible. This allowed the glass to be cooled inside the crucible, yet still be removed once solid. Any platinum that could not be peeled away from the glass was simply left attached as the glass was mounted in epoxy.

Two glasses were synthesised. The first ('Old wollastonite') was used as a standard for the coral analyses presented in chapter 8. A second, more homogeneous, glass ('New wollastonite') was synthesised later, and used in combination with a pressed powder for standardising the coral analyses presented in chapter 10.
3.5.2 Calibration

The two wollastonite glasses were cross-calibrated by a series of 28 paired laser analyses over the course of 3 days and 4 different ICP-MS tunings. These glasses both had very similar compositions, reducing any inaccuracy or irreproducibility due to matrix differences, and allowing the calibration of one glass to be applied to the other. Standard errors for the cross-calibration ranged from 0.09% to 0.40%.

The new wollastonite glass was calibrated by isotope dilution ICP-MS and TIMS. Details are presented in chapter 4 (section 4.5.1). The composition of this glass is presented in table 3.3. The boron calibration was considered inaccurate as the dissolution procedure required the use of HF and heat, and volatile boron fluorides are lost from solution under these conditions. The glass was therefore calibrated for B by a series of 21 paired laser ablation analyses of the glass and NIST 612, using the B concentrations presented in Pearce et al. (1995).

3.5.3 Advantages

Figure 3.6 presents a scan across the old wollastonite glass synthesised, while figure 3.7 presents the new wollastonite. The old wollastonite displays is reasonably homogeneous (with 60 point averages ranging from 2.4% to 16%); however, the new wollastonite (which was synthesised using the refined method presented in 3.4.1) has a much better homogeneity (1.6% to 7.4%), rivalling NIST 612. Concentrations in this glass are similar to those in corals (table 3.3), although slightly diluted by the silica. Count rates are around 30 - 40% lower than for corals (table 3.4), indicating that ablation appears to be roughly similar between coral and glass, and that the wollastonite does not disintegrate into fragments like the pressed powder. The glass surface is easily cleaned, and laser traverses can be easily observed on the surface so replicating analytical tracks is simple. It would therefore be expected that this standard could produce excellent reproducibility accuracy, and precision.

3.5.4 Disadvantages

Synthesising a homogeneous glass is laborious, requiring careful and methodical grinding of components; and the temperatures necessary to fuse a CaO and SiO₂ mix can only be produced by specialised equipment. During this synthesis, uneven cooling, contamination, devitrification, and loss of volatile elements can introduce broad heterogeneities into the glass, which may compromise the accuracy of the calibration, and the reproducibility of analyses (see section 3.2.1).
Figure 3.6 Profiles of Scans Across the Old Wollastonite Glass

This figure presents trace element profiles across the surface of the old wollastonite glass. As in figure 3.1 the grey lines represent the raw TE/Ca profile, and the black lines are 60 point running averages of these profiles; representing all of the possible values for a single 60 second (2.0 mm) measurement across the sample surface. Tabulations of the heterogeneity in this standard can be found in table 3.2. There clearly exists a significant degree of heterogeneity in this glass, in the form of several very broad trends in several of the trace element compositions. These are likely to represent diffusion of contaminants, loss of volatile elements, and/or some degree of devitrification during the glass synthesis.

Calibration of the glass by solution methods requires that samples are dissolved using heat and hydrofluoric acid. Under these conditions boron is easily lost as a volatile boron fluoride. The solution calibrations for boron were all significantly below other estimates of the boron concentration in the glass. Boron was therefore calibrated by 21 paired laser analyses of the wollastonite and NIST 612. While these are both glasses, and have boron concentrations within a factor of 3 of each other, it is possible that the
different bulk compositions of the two glasses may have resulted in some matrix dependent inaccuracy.

The chemical matrix of the glass is different from corals. Experiments suggest that this poor matrix match is responsible for significant inaccuracy in the standardisation of coral analyses (see chapter 4).

New Wollastonite Glass

Figure 3.7 Profiles of Scans Across the New Wollastonite Glass
This figure presents trace element profiles across the surface of the new wollastonite glass. As in figure 3.1 the grey lines represent the raw TE/Ca profile, and the black lines are 60 point running averages of these profiles; representing all of the possible values for a single 60 second (2.0 mm) measurement across the sample surface. Tabulations of the heterogeneity in this standard can be found in table 3.2. This standard is significantly more homogeneous than the old wollastonite, mostly due to improved methods for handling the materials and synthesis of the glass. The homogeneity of this glass rivals that of the NIST 612 (see figure 3.4).
Standards

3.6 Standard Coral

Naturally the best physical and chemical matrix match for a coral is another piece of coral. Some preliminary experiments were carried out to investigate the potential for using a small section of coral as a standard for calibrating unknown corals.

3.6.1 Discussion

A small section of coral was trimmed to approximately 1.5 x 0.7 cm with a diamond blade. This coral was then handled in a similar manner other coral material (see section 2.1.4), and analysed next to the Davies Reef coral. The count rates were very close to those observed in the Davies coral, with the exception of magnesium which was approximately 30% higher in the coral chip (see table 3.4).

Because the coral chip represents an almost perfect chemical and physical matrix match to an unknown coral, accuracy and reproducibility have the potential to be extremely good. A chip of coral, however, is inherently very heterogeneous (see figure 3.8 and table 3.2). Clearly if any degree of reproducibility is to be achieved, the same section of the coral standard must be measured each time.

Unfortunately, laser pits are hard to detect on the surface of a coral due to the structural heterogeneity, and aligning a traverse to identifiable structural features in the coral (such as an edge or a corner) can result in misalignments of between $\frac{1}{3}$ and $\frac{1}{2}$ mm. A misalignment of $\frac{1}{3}$ mm over a 2 mm scan can result in differences in the measured concentration of between 2 and 20% (cf. 0.5 - 1.0% for the new wollastonite). Some of the heterogeneity in the coral standard could be avoided by a more careful selection of material: for example, the barium composition of the coral chip is influenced by a large peak, possibly a flood band (figure 3.8). Ironically this compositional heterogeneity may actually help maintain reproducibility, by acting as a spatial marker in the data set for selecting which portion of the profile to average.

Repeated ablations slowly erode the analytical surface. It is therefore necessary to occasionally relocate the analytical track. For a heterogeneous standard like the coral chip, this can introduce a significant offset between analyses calibrated on different sections of the standard, although careful cross-calibration can minimise this problem.

For similar reasons, calibration of the coral standard may be inaccurate. The bulk average composition of the coral standard can be determined very precisely and accurately using conventional solution methods, however a short scan can differ from the bulk average by $\pm$ 30 - 40% (figure 3.8).
Figure 3.8 Profiles of Scans Across the Standard Coral
This figure presents trace element profiles across the surface of a coral that was investigated as a possible standard. As in figure 3.1 the grey lines represent the raw TE/Ca profile, and the black lines are 60 point running averages of these profiles; representing all of the possible values for a single 60 second (2.0 mm) measurement across the sample surface. Tabulations of the heterogeneity in this standard can be found in table 3.2. The coral is very heterogeneous, with large, broad compositional gradients present in all of the trace elements except for Sr. The large peak in the Ba/Ca profile corresponds to a fluorescent line in the coral, and is likely to be a flood event.

3.7 Aragonite Crystal
A single homogeneous crystal of aragonite offers some of the advantages of a glass standard (solid, easily cleaned, visible ablation tracks), in addition to being chemically matrix-matched to the coral. Such a crystal could be grown from solution; however, controlled solution growth of large crystals is extremely complex, and beyond the scope...
of this research. Some naturally occurring aragonite crystals can be very homogeneous, and one such crystal was therefore examined as a potential standard.

A small section of an aragonite crystal was mounted in epoxy, sliced, polished and cleaned. The aragonite was pre-ablated in the same manner as a glass, and analysed by laser. Trace element profiles for this analysis are presented in figure 3.9.

**Aragonite Crystal**

![Aragonite Crystal Diagram]

**Figure 3.9 Profiles of Scans Across an Aragonite Crystal**

This figure presents trace element profiles across the surface of a chip from a naturally occurring homogenous aragonite crystal. As in figure 3.1 the grey lines represent the raw TE/Ca profile, and the black lines are 60 point running averages of these profiles; representing all of the possible values for a single 60 second (2.0 mm) measurement across the sample surface. Tabulations of the heterogeneity in this standard can be found in table 3.2. Although some regions of the crystal appear to be homogeneous for some elements, it is clear that regions of major heterogeneity exist in this crystal, making it unsuitable as a standard.
3.7.1 Discussion

Although an aragonite crystal has a similar chemical matrix to a coral, the highly polished surface of the crystal is clearly not texturally matched to the topologically complex clusters of aragonite micro-fibres that make up a coral. This may lead to differences in the ablation behaviour between the standard and sample.

The aragonite crystal has some regions that are relatively homogeneous for barium and strontium; however, it is clearly very heterogeneous for the other elements (figure 3.9). One advantage of the polished aragonite chip is that the laser tracks on the surface make realigning analytical traverses easy; however, the compositional gradients in some regions of the crystal are so large that even small errors in misalignment of the laser traverse can lead to large differences in measurement from one run to the next.

The biggest problem with the aragonite chip is the very low concentrations of boron, magnesium and uranium, which approach the detection limits of the technique. Accuracy, reproducibility and precisions using this particular crystal would therefore be extremely poor. If a more homogeneous aragonite crystal could be obtained with more appropriate trace element concentrations it may prove to be a very good standard for coral analyses.

3.8 Fused Powder

As an alternative to pressing coral powder to form a solid pellet, or melting it with silica to form a glass, coral powder can be fused at high temperature and pressure to form a solid crystal of calcite. Such a standard would be compositionally matched to a coral, and provide a better textural matrix match than a pressed powder. Hart and Cohen (1996) used this approach to create a carbonate standard for coral analysis; however, they reported the results to be unsatisfactory. Nevertheless, an attempt was made to synthesise a fused powder standard.

3.8.1 Preparation

A 70 mg sample of coral powder was added to a small platinum tube, which was then sealed (welded) at both ends. The tube was encased in a variety of different conductive and lubricating sheaths (see figure 3.10), and pressed at 10 Kbar and 1500°C on a hydraulic press. After quenching, the platinum tube was removed, mounted in epoxy, cut, and polished. The resulting fused carbonate had an opaque crystalline appearance, and there was some indication of the formation of crystal boundaries. Half way down the 8 mm section, a dark line indicated a major structural heterogeneity in the fused
Standards

powder, although what might have caused this is uncertain. A laser traverse was run down most of the 8 mm length of standard, and the profiles from this are presented in figure 3.11.

**Synthesising Fused Powder**

A solid calcite was synthesised by sealing a small sample of coral powder into a tube of platinum to prevent mass loss, and subjecting it to very high pressures and temperatures. The platinum capsule had to be surrounded by a number of conducting, insulating, and lubricating sheaths, which are illustrated here.

---

*Figure 3.10 Synthesis of the Fused Powder Standard*

A solid calcite was synthesised by sealing a small sample of coral powder into a tube of platinum to prevent mass loss, and subjecting it to very high pressures and temperatures. The platinum capsule had to be surrounded by a number of conducting, insulating, and lubricating sheaths, which are illustrated here.
Figure 3.11 Profiles of Scans Across the Fused Powder Standard
This figure presents trace element profiles across the surface of the first of the fused powder. As in figure 3.1 the grey lines represent the raw TE/Ca profile, and the black lines are 60 point running averages of these profiles; representing all of the possible values for a single 60 second (2.0 mm) measurement across the sample surface. Tabulations of the heterogeneity in this standard can be found in table 3.2. While some heterogeneity is evident in the form of short spikes in the signals, these occur on small distance scales relative to a normal analysis, and will be averaged out. This standard therefore behaves relatively homogeneously, as indicated by the very flat 60 point running averages.

3.8.2 Advantages

A fused powder theoretically has the advantages of both a pressed powder and a glass (i.e. chemical matrix match, some degree of physical matrix matching, similar composition to coral, reasonable homogeneity, and a polished surface on which a laser traverse is readily identified). Because the fusion occurs in a sealed environment, there is
no loss of volatile elements, and the bulk composition should therefore remain very similar to the pressed powder.

3.8.3 Disadvantages

The count rates in the fused powder are roughly similar to coral, except for barium which are quite low (table 3.4). Relative count rates decrease from low to high masses in the fused powder relative to the coral, possibly indicating a constant fractionation trend. This is unexpected as the sealed Pt tube should have prevented any net loss of elements during synthesis. Some elements may have been fractionated into a different phase, the fused powder may be zoned, or the different physical matrices of the fused powder and the coral could be causing this fractionation.

The fused powder clearly has some heterogeneity (figure 3.11), with compositions varying by up to 26% for uranium (table 3.2). Levels of heterogeneity appear roughly similar to the pressed powder, except for uranium which is significantly more variable. The heterogeneity is distributed on a relatively small spatial scale and much of it will be averaged out by a 60 second scan across the surface of the standard. For B, Sr, and Ba, the 60 point running average of the signal is significantly less variable than the pressed powder, and overall this averaged signal is comparable to the wollastonite glass in its stability.

There are several possible sources of heterogeneity in the fused powder. Superficially the heterogeneity appears similar to that found in a pressed powder, and the heterogeneity of the component powder may be preserved during fusion. Crystallisation may also add to the heterogeneity, with gross partitioning of some elements dependent on the phase being crystallised. Calcite has very different lattice affinities for some elements compared with aragonite (e.g. a greater affinity for magnesium), and there may be major changes in the partitioning of elements across crystal boundaries.

Calibration of the fused powder by solution techniques would be difficult, requiring the partial destruction of the standard, synthesis of a second fused powder specifically for calibration, or calibration by a non-destructive micro-beam technique (such as ion probe, electron probe, XRF, etc.). The small size of the standard and its relative fragility make partial destruction an undesirable option. Because the fusion of the powder occurred in a closed system, the bulk composition of the standard is not likely to have changed from the original powder. To a first approximation therefore, the composition of the fused powder can be approximated by the composition of the crushed coral powder. This would not take into account any contamination that might have occurred during the synthesis. A second option would be to calibrate the standard by laser against an externally calibrated sample to obtain an 'apparent composition' (see section 3.2.5).
Chapter 3

3.9 Summary

Table 3.5 summarises the different standards investigated and their properties.

<table>
<thead>
<tr>
<th>Analyte Concentrations</th>
<th>Chemical Matrix Match</th>
<th>Physical Matrix Match</th>
<th>Homogeneity Match</th>
<th>Polished Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIST 612</td>
<td>xx</td>
<td>xx</td>
<td>✓</td>
<td>✓/✓</td>
</tr>
<tr>
<td>Wollastonite</td>
<td>✓/✓</td>
<td>x</td>
<td>✓/✓</td>
<td>✓/✓</td>
</tr>
<tr>
<td>Coral Standard</td>
<td>✓/✓</td>
<td>✓/✓</td>
<td>xx</td>
<td>xx</td>
</tr>
<tr>
<td>Pressed Powder</td>
<td>✓/✓</td>
<td>✓/✓</td>
<td>x</td>
<td>xx</td>
</tr>
<tr>
<td>Fused Powder</td>
<td>✓/✓</td>
<td>✓/✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Aragonite</td>
<td>xx</td>
<td>✓</td>
<td>xx</td>
<td>✓/✓</td>
</tr>
</tbody>
</table>

NIST 612

Because of its high, well calibrated concentrations, the NIST 612 glass is useful for semi-quantitative calibration of the minor trace elements in corals. While it is very homogeneous, the different trace element concentrations and different chemical matrix relative to corals makes it unsuitable for standardising the 5 major trace elements (B, Mg, Sr, Ba, and U).

Wollastonite

The wollastonite glass represents a very good compromise of the desirable standard properties, and has been used as the standard of choice for much of the analyses presented in subsequent chapters. Its strengths are its homogeneity and matching of trace elements to the analyte elements, however it is by definition a poor chemical matrix match for corals, and chemical matrix effects may be contributing to an unacceptable level of inaccuracy and irreproducibility (see chapter 4).

Standard Coral

The preliminary investigation into using a coral as a standard has identified a high likelihood of poor reproducibility based on the very large inherent heterogeneity. It might be possible to develop specific analytical methodologies to circumvent this heterogeneity problem, and if this can be done, then a standard coral chip has the potential to allow very accurate and reproducible standardisations due to its excellent physical and chemical matrix match to an unknown coral.
Standards

Pressed Powder

The pressed coral-powder standards examined early in this research were rejected because of poor ablation behaviour and moderate heterogeneity, despite any advantages afforded by their chemically matched matrices, and well matched analyte element concentrations. Recent improvements in the synthesis techniques, however, has seen an improvement in both homogeneity and ablation characteristics (S. Fallon, pers. comm.). While homogeneity cannot yet rival the glass standards available, there is potential for developing these synthesis methods still further.

Fused Powder

Fused powders are at least as homogeneous as pressed powders, and possess the added advantage of a less fragile surface that can be easily polished. Synthesis techniques can be refined further, and it may ultimately be possible to produce a standard that rivals the wollastonite in homogeneity, while retaining a chemical matrix match to the coral samples.

Aragonite Crystal

Experiments with a section of naturally occurring aragonite produced disappointing results, with very poor homogeneity, and very low concentrations for a number of the analyte trace elements. The possibility of growing synthetic aragonite crystals from controlled solutions should be investigated, as a homogeneous coral-composition crystal would be an ideal standard.
CHAPTER 4: LASER METHOD EVALUATION

4.1 Introduction

This chapter evaluates the analytical method presented in chapter 2 (section 2.3). The parameters upon which the evaluation is based are precision, reproducibility, accuracy and detection limits. Working definitions of the first three terms are presented in chapter 3 section 3.2, while detection limits are defined in section 4.2. The evaluation focuses on the use of the two wollastonite glasses (new WOL and old WOL), which were the principal standards used during this research. At the RSES there has been a shift in preference towards carefully synthesised pressed powder standards which are more closely matrix-matched with corals (Fallon et al., 1998; Fallon et al., 1999). Some additional evaluation statistics are presented for the use of pressed coral-powder standards; however, as they have only been in routine use for a short time there is little data available on which to base a thorough evaluation.

4.2 Detection Limits

4.2.1 Calculation and Variability

Instrumental sensitivity was determined for the laser ICP-MS by analyses of NIST 612 glass, using the concentrations presented in Pearce et al. (1995). The sensitivity of the ICP-MS was typical on the day that these analyses were carried out, with signal intensity averaging around 2 million cps for $^{43}$Ca in the coral. Detection limits are defined as the concentration equivalent of 3 x the background standard deviation. These are presented in table 4.1.

Increases in sensitivity will usually be accompanied by a corresponding decrease in detection limits. If background count rates do not increase, then detection limits will decrease in proportion to the gain in sensitivity. Usually, however, backgrounds increase in proportion to sensitivity. In this case, the backgrounds become more stable as count rates get higher and counting statistics improve. Detection limits consequently decrease with the square root of the sensitivity increase. Occasionally the instability in
the background is caused by fluctuations in an interference species. This usually applies to element in the mid-low mass range which have gas-background isobaric interferences. Under these circumstances, changes in sensitivity may not improve detection limits at all, but variations in the gas flow and plasma conditions can significantly effect the production of these interferences (and hence detection limits).

<table>
<thead>
<tr>
<th>Table 4.1 Detection Limits</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Element</th>
<th>Detection Limit</th>
<th>Concentration Range in Corals</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B (µmol/mol)</td>
<td>6.5</td>
<td>381 - 780</td>
<td>(Gaillardet and Allegre, 1995; Vengosh et al., 1991)</td>
</tr>
<tr>
<td>Mg (mmol/mol)</td>
<td>0.010</td>
<td>1.23 - 9.7</td>
<td>(Buddemeier et al., 1981; Hart and Cohen, 1996)</td>
</tr>
<tr>
<td>Ca (mol/mol)</td>
<td>0.00042</td>
<td>1</td>
<td>(Shen and Boyle, 1988; Shen et al., 1991)</td>
</tr>
<tr>
<td>Mn (nmol/mol)</td>
<td>400</td>
<td>15 - 1030</td>
<td></td>
</tr>
<tr>
<td>Ni (nmol/mol)</td>
<td>1100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu (nmol/mol)</td>
<td>1200</td>
<td>15 - 75</td>
<td>(Linn et al., 1990)</td>
</tr>
<tr>
<td>Zn (nmol/mol)</td>
<td>610</td>
<td>25 - 150</td>
<td>(Shen and Boyle, 1988)</td>
</tr>
<tr>
<td>Sr (mmol/mol)</td>
<td>0.0033</td>
<td>8.5 - 9.5</td>
<td>(e.g. McCulloch et al., 1994)</td>
</tr>
<tr>
<td>Y (nmol/mol)</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ba (µmol/mol)</td>
<td>0.0035</td>
<td>1.5 - 44</td>
<td>(Allison, 1996a; Buddemeier et al., 1981)</td>
</tr>
<tr>
<td>La (nmol/mol)</td>
<td>2.7</td>
<td>0.76 - 2.72</td>
<td>(Sholkovitz and Shen, 1995)</td>
</tr>
<tr>
<td>Ce (nmol/mol)</td>
<td>2.3</td>
<td>1.84 - 6.41</td>
<td>(Sholkovitz and Shen, 1995)</td>
</tr>
<tr>
<td>Pb (nmol/mol)</td>
<td>17</td>
<td>0.9 - 95</td>
<td>(Delaney et al., 1989; Dodge et al., 1984)</td>
</tr>
<tr>
<td>U (µmol/mol)</td>
<td>0.00041</td>
<td>0.95 - 1.51</td>
<td>(Shen and Dunbar, 1995)</td>
</tr>
</tbody>
</table>

Sensitivity for the high mass elements does not generally change more than a factor of 2 from one tuning to the next; hence, the detection limits quoted for Y, Ba, La, Ce, Pb, and U are likely to be within ± 40% of the values quoted in table 4.1. Detection limits for the lower masses may change significantly more than this as tuning affects their background signals; however, this variation is unlikely to be more than a factor of three up or down.

As noted in (section 3.2.3), the high count-rate for $^{43}$Ca places a ceiling on the total signal intensity, and it is not possible to improve detection limits by increasing ablation rate. Some improvement may be gained by tuning for a higher sensitivity in one region of the mass spectrum; however, this is usually at the expense of other regions of the mass spectrum.
Signal Smoothing

Smoothing a signal makes it more precise (although this is at the expense of resolution). The detection limit defined above applies to a single observation (data point) taken at random from a population (stable signal). If the signal is smoothed with an 11-point running average, then each data point is now an average of 11 observations. The standard error of this average is $\sigma/\sqrt{n}$, and the detection limits of a smoothed signal are therefore a factor of $\sqrt{11} (= 3.3)$ lower than for the unsmoothed signal. Strictly, the filter is an 11 point median (section 2.4.1), although for statistical purposes it behaves much like an average.

4.2.2 Major Trace Elements

Table 4.1 lists detection limits, tabulated against coralline trace element concentrations reported in the literature. The major trace elements in corals (B, Mg, Ca, Sr, Ba, and U) are clearly well above detection limits, with concentration-to-detection-limit ratios ranging from about 90 for boron up to 6700 for barium. Such clearly resolved signals can be accurately and precisely quantified.

4.2.3 Minor Trace Elements

Nickel Copper and Zinc

Copper and zinc have very poor detection limits. The very low concentrations generally found in corals, coupled with the high and unstable backgrounds make these elements undetectable by LA-ICP-MS. Although no data for coralline nickel can be found, it is likely that this element is also undetectable.

The skeletal concentrations taken from the literature (table 4.1) were generally from mid-ocean corals. Copper and zinc may be significantly enriched in the skeletons of coastal corals, due to the high concentration of particulates in these waters. It is hard to imagine the concentrations being enriched by factors of more than 8 and 25, however, which is what would be required for these elements to become detectable by this method. Occasionally Cu, Zn, and Ni, signals are resolved above background; however, these are believed to be surface contamination, detrital contamination or background interferences. No further consideration will be made of these elements in this research.

Lead

According to the detection limits presented in table 4.1, lead is detectable using this method, and may even be tentatively quantifiable. Lead signals are often seen above background; however, these are inevitably extremely variable, with very amplitude noise
that is characteristic of small contaminant particles. Lead is ubiquitous in the environment and samples are therefore extremely prone to contamination. Specific rigorous cleaning techniques had to be devised by other researchers in order to obtain meaningful lead concentrations in corals by bulk solution methods (Delaney et al., 1989; Shen, 1986; Shen and Boyle, 1987; Shen and Boyle, 1988). No such cleaning was undertaken for any of the laser analyses, and while preablation may well expose clean aragonite, it may not remove all contamination. Lead signals are therefore treated with caution.

**Manganese, Lanthanum, Cerium, and Yttrium**

The average levels of coralline manganese, lanthanum and cerium reported in the literature are all very close to the reported detection limits for this technique (table 4.1). No data is available for yttrium; however, it is expected that this element is present in corals at similar levels to the rare earth elements, and will therefore be close to detection limits too. Any attempts to quantify these elements will therefore be very inaccurate and imprecise, although it may be possible to gain semiquantitative information about their concentrations.

The range of Mn La and Ce concentrations reported in table 4.1 are all derived from analyses of offshore corals. Manganese is enriched in coastal waters through diagenetic release from nearshore anoxic sediments, while the rare earth elements (and Y) are transported as colloidal material in fresh water and desorbed in estuaries (see chapter 9 for review). Concentrations of these elements in the skeletons of coastal corals may therefore be significantly higher than open ocean corals, making detection and quantification easier. River flood plumes are rich in La, Ce and Y (chapter 9), and these elements are readily detectable in flood bands in the coral skeleton.

Because these elements are close to the detection limit, their signals are inherently very noisy, and no attempt has therefore been made to quantify them. Nevertheless, the shape of the TE profiles in smoothed signals can usually be clearly seen. The real scientific value of these minor trace element profiles lies not in their magnitude, but their shape and the timing of particular features (e.g. flood spikes), both of which can be very important for interpreting environmental processes (see chapter 10).

### 4.3 Precision

A working definition of the precision of a technique is given in section 3.2. In essence precision is a measure of the stability of a trace element signal, or how much noise a
signal contains. In this discussion, precisions are reported as a percentage standard deviation of the average TE/Ca signal.

Precision was calculated for a typical wollastonite glass analysis by taking the percentage standard deviation of the calcium normalised trace element signals. These values are presented in the 4th column of table 4.2. Precision was calculated for a typical coral analysis by averaging the output from an 11 point running standard deviation. A small windowed standard deviation was used to avoid including the seasonal compositional cycle, which would have resulted in an overestimate of the imprecision in the signal. These values are presented column 2 of table 4.2.

### Table 4.2  Precisions

#### Glass Precision

<table>
<thead>
<tr>
<th>Element</th>
<th>Precision</th>
<th>Temperature Equivalent</th>
<th>Counting Statistics</th>
<th>Sample Heterogeneity</th>
<th>Machine Noise</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/Ca</td>
<td>3.8%</td>
<td>0.9 °C</td>
<td>2.7%</td>
<td>2.4%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Mg/Ca</td>
<td>1.4%</td>
<td>0.4 °C</td>
<td>1.0%</td>
<td>1.0%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Sr/Ca</td>
<td>1.3%</td>
<td>1.7 °C</td>
<td>1.0%</td>
<td>0.8%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Ba/Ca</td>
<td>2.9%</td>
<td>-</td>
<td>1.6%</td>
<td>1.6%</td>
<td>1.9%</td>
</tr>
<tr>
<td>U/Ca</td>
<td>2.8%</td>
<td>0.7 °C</td>
<td>1.4%</td>
<td>2.3%</td>
<td>0.7%</td>
</tr>
</tbody>
</table>

#### Coral Precision

<table>
<thead>
<tr>
<th>Element</th>
<th>Precision</th>
<th>Temperature Equivalent</th>
<th>Counting Statistics</th>
<th>Sample Heterogeneity</th>
<th>Machine Noise</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/Ca</td>
<td>3.9%</td>
<td>0.9 °C</td>
<td>1.8%</td>
<td>3.5%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Mg/Ca</td>
<td>5.1%</td>
<td>1.3 °C</td>
<td>0.5%</td>
<td>5.1%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Sr/Ca</td>
<td>1.6%</td>
<td>2.0 °C</td>
<td>0.6%</td>
<td>1.5%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Ba/Ca</td>
<td>14.4%</td>
<td>-</td>
<td>1.2%</td>
<td>14.2%</td>
<td>1.9%</td>
</tr>
<tr>
<td>U/Ca</td>
<td>5.3%</td>
<td>1.3 °C</td>
<td>1.6%</td>
<td>5.0%</td>
<td>0.7%</td>
</tr>
</tbody>
</table>

#### 4.3.1 Components of Precision

There are three major factors that can contribute to imprecision in an analytical signal: counting statistics, machine noise, and sample heterogeneity. Independent components of normally distributed variance add in quadrature to give a final precision. To a first
approximation, variance from several non-normal distributions (e.g. binomial, Poisson, and even distributions) will also sum in quadrature, providing they act independently of each other. The total precision of an analysis can therefore be written as:

\[
\sigma_p = \sqrt{\sigma_{cs}^2 + \sigma_{sample}^2 + \sigma_{machine}^2}
\]  

(4.1)

where:

\( \sigma_p \) = precision for an analysis
\( \sigma_{cs} \) = counting statistics errors
\( \sigma_{sample} \) = variance caused by the sample, including heterogeneity, and ablation behaviour
\( \sigma_{machine} \) = machine noise, including plasma instability

**Counting Statistics**

Counting statistics have been modelled by a Poisson distribution, which has the property that the standard deviation of a set of data points averaging \( n \) counts per point is given by \( \sqrt{n} \). Percentage standard deviations for each element and calcium have been combined to produce the values in column 4 of table 4.2.

Two suites of elements are commonly monitored: the major trace elements suite consists of B, Mg, Ca, Sr, Ba, and U, while the extended trace element suite includes these and Mn, Ni, Cu, Zn, Y, La, Ce, and Pb. The ICP-MS records one data point per element each second, and the integration time for each element is therefore \( \frac{1}{6} \) of a second for the major suite, or \( \frac{1}{14} \) of a second for the extended suite. The precisions in table 4.2 were calculated for the major element suite, whereas using the extended suite these would be expected to be larger by a factor of 1.53 (\( =\sqrt{14+6} \)).

**Machine Noise**

Fluctuations in gas flow, sample delivery, and variability within the plasma can all contribute to imprecision. The ICP plasma is an inherently noisy ion source, and large particle size distributions from ablation of a sample can result in unstable vaporisation and ionisation processes, leading to noisy signals (Perkins et al., 1991).

The machine noise component is calculated by subtracting (in quadrature) the components of counting statistics and sample heterogeneity (see below) from the observed precision for the wollastonite glass.

\[
\sigma_{machine} = \sqrt{\sigma_{Pglass}^2 - \sigma_{cs}^2 - \sigma_{sample}^2}
\]  

(4.2)

These values are presented in column 6 of table 4.2.

Fragile samples can fracture during ablation, resulting in a large particle size distribution in the plume of ablated material. If the micro-crystalline aragonite of a coral skeletons is
more fragile than the wollastonite glass, resulting in a broader distribution of fragment sizes, the machine noise for a coral could be larger than for a glass. The UV laser ablates carbonates cleanly, however, and this effect is likely to be small. Machine noise is therefore assumed to be the same for coral and glass.

The machine noise and counting statistics together make up the 'inherent' precision of the LA-ICP-MS technique, which is the precision expected when ablating a perfectly homogeneous sample.

Sample Heterogeneity

The final contributor to a noisy signal may be the sample itself. If a sample contains physical or compositional heterogeneity, then scanning a laser over its surface will result in a fluctuating signal. This is not strictly 'noise', as compositional variations may be reproduced in replicate analysis over the same material (e.g. chapter 7, figure 7.1). It will nonetheless contribute to estimates of precision. The wollastonite glass contains some broad heterogeneities (chapter 3, figure 3.6), which add a minor component of variance to the precision.

Heterogeneity in the glass was measured by de-trending the wollastonite signal before calculating the standard deviation. Heterogeneity in the coral signal was calculated by subtracting the counting statistics and machine noise components from the observed coral precision.

\[
\sigma_{\text{sample}} = \sqrt{\sigma^2_{\text{Coral}} - \sigma^2_{\text{cs}} - \sigma^2_{\text{machine}}} \tag{4.3}
\]

Heterogeneities are presented in column 5 of table 4.2.

4.3.2 Major Trace Elements

The precisions measured for the wollastonite glass are relatively low, and in general represent less than 1 °C when converted into a temperature equivalent (using the temperature dependencies presented in chapter 8). The exception is Sr, which has a very sensitive temperature dependence.

Counting statistics are low for the major trace elements because of their relatively high concentrations, and account for around half of the total glass precision. Machine noise contributes relatively little to the precision, which is encouraging, as it means that the ICP-MS isn't significantly compromising the signal stability. The inherent precision (defined above) of the method should therefore allow quantitative temperature reconstruction to within 1 °C.

The precisions calculated for a coral are significantly lower than for the glass, due almost entirely to the fine-scale compositional heterogeneity in the sample. Temperatures
reconstructed from a coral would not be precise to better than 1 °C. This heterogeneity is discussed further in chapter 7. All coral data is smoothed through an 11 point filter (see section 2.4.1). Because this filter approximates an 11 point average, the precision of the smoothed signal is better estimated from the standard error of the data within the smoothing window. Precisions for the smoothed signal will therefore be lower than the values presented in table 4.2 by a factor of \( \sqrt{11} \), bringing temperature uncertainties down to around the 0.3 - 0.4 °C mark.

4.3.3 Minor Trace Elements

Because no attempt has been made to quantify minor trace elements, the precisions and their components have not been tabulated. Counting statistics are the principal contributors to the imprecision (ranging from 10 - 50%), because of the very low concentrations of the minor trace elements. For the rare earth elements, each data point is represented by around 5 to 8 individual ion arrivals, resulting in counting statistic uncertainties as high as 46%. During river floods, however, counts can be elevated by a factor of 3 to 10, and the counting statistic precision improves to around 10% for the peaks in the signal. Signal filtering helps to further improve the precision.

4.4 Reproducibility

Reproducibility has been examined in two data sets. The reproducibility of a matrix-matched sample and standard is demonstrated by 28 analyses of the new wollastonite glass standardised to the old wollastonite glass (section 4.4.2). The reproducibility of a non-matrix-matched sample and standard is demonstrated by 17 analyses of a coral standardised to the old wollastonite glass (section 4.4.3).

Reproducibility is a measure of how closely replicate analyses of a sample cluster, and is defined in section 3.2. The analytical method was designed to maximise reproducibility in a sample analysis by ratioing all counts to an internal standard element (\(^{43}\text{Ca}\)), and by standardising to a reference material of known composition. Reproducibility is therefore reported as the standard deviation of replicated ratios of \( \text{TE}/\text{Ca} \) in the sample over the \( \text{TE}/\text{Ca} \) in the standard material, expressed as a percentage of the average.

4.4.1 Components of Reproducibility

The major factors contributing to irreproducibility are precision, heterogeneity (in the sample and the standard), and matrix effects. The reproducibility can therefore be modelled as:
Laser Method Evaluation

\[ \sigma_R = \sqrt{\sigma_{P(R)}^2 + \sigma_{h(sample)}^2 + \sigma_{h(standard)}^2 + \sigma_m^2} \]  

(4.4)

where:

- \( \sigma_{P(R)} \) = precisions for both sample and standard measurements (added in quadrature)
- \( \sigma_{h(sample, standard)} \) = variance associated with heterogeneity in the sample and standard
- \( \sigma_m \) = variance caused by matrix effects

Two reproducibilities are considered: daily reproducibility (defined as the reproducibility observed for replicates made within a day), and day-to-day reproducibility (defined as the reproducibility observed for replicates made over several days). Day-to-day reproducibility will be poorer than daily reproducibility, because matrix effects will be enhanced by re-tuning of the ICP-MS, and realigning laser tracks is less accurate. Note, that all the factors that contribute to daily reproducibility also contribute to day-to-day reproducibility, so:

\[ \sigma_{R(dtd)} = \sqrt{\sigma_{R(daily)}^2 + \sigma_{additional}^2} \]  

(4.5)

where:

- \( \sigma_{R(dtd)} \) = day to day reproducibility
- \( \sigma_{R(daily)} \) = daily reproducibility
- \( \sigma_{additional} \) = components of variance that are specific to day-to-day reproducibility

4.4.2 Wollastonite Glass Reproducibility

The 28 analyses of the wollastonite glasses were part of the cross-calibration of the new and old wollastonites. Samples were pre-ablated as described in chapter 2, section 2.3. A 600 x 20 \( \mu \)m laser spot was scanned at \( 1/30 \) mms\(^{-1} \) across the glasses, and each 60 second analysis was background subtracted, normalised to calcium, and averaged to produce a single data point. No drift correction was carried out; however, as each analysis was short, this was not considered necessary. The glasses were analysed in pairs, and the new wollastonite measurements were normalised to the corresponding old wollastonite analyses. These values are graphed in figure 4.1.

Analyses were carried out over a period of 3 days with 4 separate ICP-MS tunings, and the data has been grouped into 4 sets of analyses, corresponding to each re-tuning (see table 4.3). Analytical conditions (spot size, ablation rate, laser energy, etc.) were all identical from one day to the next. Measurements were constrained to very narrow regions of both glasses: the samples were not re-polished so that the laser track remained visible, allowing accurate realignment of analyses.

'Day-to-day' reproducibility is given as a percentage standard deviation for the whole data set. 'Daily' reproducibility was calculated by removing the variability associated
with re-tuning the ICP-MS. Each block of data (1-9, 10-14, 15-20, 21-28) was normalised to its average before the percentage standard deviation was taken. These observed precisions are presented in column 2 of table 4.4.

**Glass Replicates**

![Glass Replicates Diagram](image)

**Figure 4.1 Replicate Analyses of the Wollastonite Glass**

In order to examine the reproducibility of a matrix-matched sample, 28 analyses of the new wollastonite glass (NW) were made, standardising to the old wollastonite glass (OW). These analyses were carried out over a period of 3 days and 4 ICP-MS tunings. Data for each element are displayed at the same scale. The Y axes are the uncalibrated ratios of TE/Ca for the new wollastonite over TE/Ca for the old wollastonite. The X axis lists the analysis reference numbers, which are tabulated in table 4.3. Crosses mark outlier data points that were excluded from the reproducibility calculations.
Table 4.3  List of All Analyses Used in Reproducibility Calculations

### Wollastonite Analyses

<table>
<thead>
<tr>
<th>Ref. #</th>
<th>Date</th>
<th>Material</th>
<th>Replicates</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 9</td>
<td>20/6/97</td>
<td>New WOL</td>
<td>9</td>
<td>Normal[1]</td>
</tr>
<tr>
<td>10 - 14</td>
<td>20/6/97</td>
<td>New WOL</td>
<td>5</td>
<td>ICP re-tuned[2]</td>
</tr>
<tr>
<td>15 - 20</td>
<td>21/6/97</td>
<td>New WOL</td>
<td>6</td>
<td>Normal</td>
</tr>
<tr>
<td>21 - 28</td>
<td>22/6/97</td>
<td>New WOL</td>
<td>8</td>
<td>Normal</td>
</tr>
</tbody>
</table>

### Coral Analyses

<table>
<thead>
<tr>
<th>Ref. #</th>
<th>Date</th>
<th>Material</th>
<th>Replicates</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 2</td>
<td>24/9/96</td>
<td>Davies 2 Coral</td>
<td>2</td>
<td>70 µm spot</td>
</tr>
<tr>
<td>3 - 4</td>
<td>24/9/96</td>
<td>Davies 2 Coral</td>
<td>2</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(same track)</td>
</tr>
<tr>
<td>5 - 6</td>
<td>27/9/96</td>
<td>Davies 2 Coral</td>
<td>2</td>
<td>70 µm spot</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(same track)</td>
</tr>
<tr>
<td>7 - 9</td>
<td>10/10/96</td>
<td>Davies 2 Coral</td>
<td>3</td>
<td>Normal</td>
</tr>
<tr>
<td>10 - 11</td>
<td>14/11/96</td>
<td>Davies 2 Coral</td>
<td>2</td>
<td>Normal</td>
</tr>
<tr>
<td>12</td>
<td>14/11/96</td>
<td>Davies 2 Coral</td>
<td>1</td>
<td>70 µm spot</td>
</tr>
<tr>
<td>13</td>
<td>14/12/96</td>
<td>Davies 2 Coral</td>
<td>1</td>
<td>On Axis</td>
</tr>
<tr>
<td>14</td>
<td>14/12/96</td>
<td>Davies 2 Coral</td>
<td>1</td>
<td>Off Axis</td>
</tr>
<tr>
<td>15</td>
<td>14/12/96</td>
<td>Davies 2 Coral</td>
<td>1</td>
<td>On Axis (new)</td>
</tr>
<tr>
<td>16</td>
<td>14/12/96</td>
<td>Davies 2 Coral</td>
<td>1</td>
<td>Off Axis (new)</td>
</tr>
<tr>
<td>17</td>
<td>unknown</td>
<td>Davies 2 Coral</td>
<td>1</td>
<td>New WOL[3]</td>
</tr>
</tbody>
</table>

[1] Normal analytical conditions are described in chapter 2, section 2.3.

[2] These analyses were carried out after the ICP-MS had been re-tuned. Under normal circumstances the ICP-MS is only tuned once at the start of the day.

[3] Analyses performed by Mr. Stewart Fallon using the new wollastonite glass as a standard.

**Precision**

The maximum obtainable reproducibility is limited by the precision with which the sample and standard can be analysed. The precision for a 60 second measurement is given by the standard error: \(\sigma_p/\sqrt{60}\); where \(\sigma_p\) is the inherent precision for a single data point (the counting statistics and machine noise - see section 4.3.1). The precision of both the sample and the standard contribute to the reproducibility, and therefore add in quadrature giving:

\[
\sigma_{P(R)} = \frac{\sigma_p}{\sqrt{30}} \tag{4.6}
\]

These values are presented in column 3 of table 4.4.
Heterogeneity

Slight misalignments in the laser between replicate analyses can cause some irreproducibility because the glasses are heterogeneous. For the purposes of modelling, it is assumed that both daily and day-to-day realignments are within $\frac{1}{3}$ mm of each other. This probably represents a slight overestimate of the realignment uncertainty.

Table 4.4 Reproducibility of Replicate Wollastonite Glass Analyses

**Daily Reproducibility for Glass**

| Observed Components | Precision | Hetero. Hetero. Matrix Additional |
|---------------------|-----------|----------------------------------|-------------------------------|
| Reproducibility     | Precision | Hetero. (old WOL) | Hetero. (new WOL) | Matrix Effects | Additional |
| B/Ca                | 0.5%      | 0.4%                   | 0.3%                 | 0.1%           | ≈ 0%       |
| Mg/Ca               | 0.3%      | 0.2%                   | 0.2%                 | 0.1%           | 0.2%       |
| Sr/Ca               | 0.3%      | 0.2%                   | 0.04%                | 0.04%          | 0.2%       |
| Ba/Ca               | 0.8%      | 0.7%                   | 0.3%                 | 0.1%           | 0.2%       |
| U/Ca                | 1.8%      | 0.5%                   | 0.2%                 | 0.1%           | 1.7%       |

**Day-to-Day Reproducibility for Glass**

| Observed Components | Precision | Hetero. Hetero. Matrix Additional |
|---------------------|-----------|----------------------------------|-------------------------------|
| Reproducibility     | Precision | Hetero. (old WOL) | Hetero. (new WOL) | Matrix Effects | Additional |
| B/Ca                | 0.8%      | 0.4%                   | 0.3%                 | 0.1%           | 0.6%       |
| Mg/Ca               | 0.4%      | 0.2%                   | 0.2%                 | 0.06%          | 0.2%       |
| Sr/Ca               | 0.5%      | 0.2%                   | 0.04%                | 0.04%          | 0.4%       |
| Ba/Ca               | 1.3%      | 0.7%                   | 0.3%                 | 0.07%          | 1.1%       |
| U/Ca                | 1.9%      | 0.5%                   | 0.2%                 | 0.09%          | 1.8%       |

The average reproducibility expected for replicates over this distance can be calculated from the compositional profiles presented in chapter 3 (figures 3.6 and 3.7). A 60-point smoothed trace element profile spans all of the possible 60 second measurements. A 10-point standard deviation taken around any point on this profile quantifies the variability that could be expected for measurements taken within $\frac{1}{3}$ mm of the selected point (10 points = $\frac{1}{3}$ mm). The reproducibility for replicate analyses of a glass was therefore estimated by taking a 10 point running standard deviation of the 60-point smoothed profile, and averaging the resulting data. Reproducibilities have been calculated for both new and old wollastonites, and are presented in columns 4 and 5 of table 4.4.
Matrix Effects

Subtracting the variance caused by precision and heterogeneity from the observed reproducibility leaves the residual component $\sigma_m$.

$$\sigma_m = \sqrt{\sigma_R^2 - \sigma_{P(R)}^2 - \sigma_{h(sample)}^2 - \sigma_{h(standard)}^2} \quad (4.7)$$

This variance contains all of the unaccounted for sources of irreproducibility, of which matrix effects are likely to be the largest contributor. These values have been calculated for daily and day-to-day replicates, and are presented in column 6 of table 4.4.

Additional Day-to-day Irreproducibility

The day-to-day reproducibility is poorer than the daily reproducibility, because factors such as ICP-MS re-tuning, and less accurate realignment of laser traverses introduce additional variance into replicate analyses. This extra irreproducibility can be calculated by rearranging equation 4.5:

$$\sigma_{\text{additional}} = \sqrt{\sigma_{R(dtd)}^2 - \sigma_{R(daily)}^2} \quad (4.8)$$

This component has been calculated for the glass analyses and is presented in column 7 of table 4.4.

Discussion

Both day-to-day and daily replicates of the wollastonite glass are very similar (table 4.4 and figure 4.1), illustrating the reproducibility possible for the LA-ICP-MS technique under ideal circumstances. Within a day, most of the reproducibility can be accounted for by the inherent precision of the ICP-MS, and heterogeneity in the two glasses. The exception is uranium, for which most of the variance is found in the residual component. This is curious, as the close matrix match between standard and sample should eliminate any matrix effects. There does not appear to be any identifiable drift that might have caused this variance (figure 4.1); however, the irreproducibility is small, and may represent an underestimate in one of the other components.

Some additional variance does appear in the day-to-day replicates, which may be caused by subtle matrix effects associated with minor differences between the trace element concentrations of the two glasses. Again, this component is small, and may be caused by overestimating the accuracy of the laser realignment, or underestimating one of the other components.
4.4.3 Coral Reproducibility

Estimates of coral reproducibility have been made from a compilation of 17 analyses of the Davies 2 coral (for details of this sample, refer to Alibert and McCulloch (1997) and chapter 8), standardising to the old wollastonite glass. In contrast to the glass replicates discussed in section 4.4.2, the coral replicates were not well constrained in a number of parameters: laser spot sizes differed from one run to the next (either 600 x 20 μm or 70 μm diameter), and the analytical tracks on the wollastonite and the coral were not replicated from one day to the next (see table 4.3 for details). Most of the replicate analyses made of the Davies coral lie very close to, or directly superimposed on each other. The principal exceptions are analyses #14 and #16 which were made in the valley between growth axes (figure 4.2).

Processing of coral data was as described in section 2.4, and all standardised coral analyses were then averaged to produce a single data point. These are graphed in figure 4.3. Day-to-day reproducibility was calculated by taking a standard deviation of the whole data set. Daily reproducibility was calculated by taking any two or more 'matched' replicates (replicate analyses of the same coral material, on the same day, under the same instrumental conditions, with the same laser spot sizes), normalising them to their average, and combining all the normalised data to calculate a standard deviation. Analyses that did not have a matched replicate were not included (i.e. analyses 12 - 17). These values are presented in column 2 of table 4.5.

Precision

As described in section 4.4.2, the precision for a 60 second measurement of the standard is given by $\sigma_p/\sqrt{60}$. While the precision for a coral signal was poor (section 4.3.2), this was almost entirely due to fine-scale compositional variations that are reproducible in replicate analyses over the same track (see chapter 7, figure 7.1). The precision that affects the reproducibility of replicate coral analyses is the random component ('inherent' precision ($\sigma_p$), which is a combination of counting statistics and machine noise - see section 4.3.1).

A bulk coral composition is determined from an average of around 1500 data points. The standard error of this measurement is therefore given by $\sigma_p/\sqrt{1500}$. Coral and glass precisions add in quadrature:

$$\sigma_{p(R)} = \sqrt{\left(\frac{\sigma_p}{\sqrt{60}}\right)^2 + \left(\frac{\sigma_p}{\sqrt{1500}}\right)^2} = \sigma_p \cdot \sqrt{\frac{26}{1500}}$$ \hspace{1cm} (4.9)

These values are presented in column 3 of table 4.5.
**Figure 4.2 Replicate Laser Analyses on the Davies 2 Coral**

This figure marks the positions of the laser tracks and the bulk milled sample on an X-Ray of the Davies 2 coral. Note that the laser analyses are shifted to one side of the major growth axis. All laser tracks cluster within a region of ~2.0 mm, except analyses #14 and #16, which were made along the valley to one side of the growth axis. For reference numbers and analytical conditions for each analysis refer to table 4.3.

**Heterogeneity**

**Day-to-day**

As discussed in section 4.4.2, misalignments in the laser track can lead to irreproducibility in replicate analyses of a heterogeneous material. Both coral and glass replicates were not as well constrained from one day to the next as those presented in 4.4.2 (e.g. see figure 4.2), and replicates were assumed to be within 2.0 mm of each other. The calculations are similar to those in section 4.4.2: the glass component was estimated by taking a 60 point running standard deviation (60 points = 2.0 mm) of the 60-point smoothed profile (60 seconds = duration of glass measurement), and averaging the resulting data. These values are presented in column 4 of table 4.5.
Figure 4.3 Replicate Analyses of the Davies 2 Coral
17 replicate analyses of the Davies 2 coral are presented in this figure. All analyses except for # 17 were standardised to the old wollastonite glass. These analyses were carried out over a period of 6 days and represent a range of analytical conditions (see table 4.3). Data for each element are displayed at the same scale. The Y axes are the uncalibrated ratios of TE/Ca for the coral over TE/Ca for the old wollastonite. The X axis lists the analysis reference numbers, which are tabulated in table 4.3. Due to anomalous behaviour in the ICP-MS, no data is available for Sr or U in analysis # 3. Ba data for the two off-axis analyses (# 14 and # 16) have been excluded from the reproducibility calculation.
### Table 4.5 Reproducibility on Replicate Analyses of Davies 2 Coral

#### Daily Reproducibility for Coral

<table>
<thead>
<tr>
<th>Element</th>
<th>Reproducibility</th>
<th>Precision (old WOL)</th>
<th>Hetero. (coral)</th>
<th>Hetero. (coral)</th>
<th>Matrix Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/Ca</td>
<td>1.8%</td>
<td>0.3%</td>
<td>0.3%</td>
<td>0.4%</td>
<td>1.7%</td>
</tr>
<tr>
<td>Mg/Ca</td>
<td>3.7%</td>
<td>0.1%</td>
<td>0.2%</td>
<td>0.4%</td>
<td>3.7%</td>
</tr>
<tr>
<td>Sr/Ca</td>
<td>0.4%</td>
<td>0.1%</td>
<td>0.04%</td>
<td>0.06%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Ba/Ca</td>
<td>9.5%</td>
<td>0.5%</td>
<td>0.3%</td>
<td>0.5%</td>
<td>9.5%</td>
</tr>
<tr>
<td>U/Ca</td>
<td>3.6%</td>
<td>0.3%</td>
<td>0.2%</td>
<td>0.4%</td>
<td>3.6%</td>
</tr>
</tbody>
</table>

#### Day-to-Day Reproducibility for Coral

<table>
<thead>
<tr>
<th>Element</th>
<th>Reproducibility</th>
<th>Precision (old WOL)</th>
<th>Hetero. (coral)</th>
<th>Hetero. (coral)</th>
<th>Matrix Effects</th>
<th>Additional</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/Ca</td>
<td>5.5%</td>
<td>0.3%</td>
<td>1.5%</td>
<td>0.4%</td>
<td>5.3%</td>
<td>5.2%</td>
</tr>
<tr>
<td>Mg/Ca</td>
<td>9.2%</td>
<td>0.1%</td>
<td>0.8%</td>
<td>0.4%</td>
<td>9.2%</td>
<td>8.4%</td>
</tr>
<tr>
<td>Sr/Ca</td>
<td>4.3%</td>
<td>0.1%</td>
<td>0.2%</td>
<td>0.06%</td>
<td>4.3%</td>
<td>4.3%</td>
</tr>
<tr>
<td>Ba/Ca</td>
<td>34.7%</td>
<td>0.5%</td>
<td>1.4%</td>
<td>0.5%</td>
<td>34.6%</td>
<td>33.3%</td>
</tr>
<tr>
<td>U/Ca</td>
<td>11.9%</td>
<td>0.3%</td>
<td>0.6%</td>
<td>0.4%</td>
<td>11.9%</td>
<td>11.3%</td>
</tr>
</tbody>
</table>

The coral data was treated similarly, except that the profiles were smoothed with a 550 point running average (550 points = 18.3 mm or approximately 1.5 seasonal cycles). Strictly this should have been a 1500 point running average; however, this was not possible due to limitations imposed by the length of the data set. The estimates of reproducibility caused by coral heterogeneity are therefore likely to be a slight overestimate. These values are presented in column 5 of table 4.5.

**Daily**

Daily replicates the glass were constrained to within $\frac{1}{3}$ mm. The calculation is as described in section 4.4.2. Daily replicates of the coral are assumed to be the same as calculated above. These values are presented in columns 4 and 5 of table 4.5.

**Matrix Effects**

As described in section 4.4.2, the residual component of irreproducibility is given by:

$$\sigma_m = \sqrt{\sigma_R^2 - \sigma_p^2(R) - \sigma_{h(sample)}^2 - \sigma_{h(standard)}^2} \quad (4.7)$$
For the coral, $\sigma_m$ includes matrix effects, and any additional variance associated unconstrained analytical variables (such as laser spot size). Matrix effects, however, are still likely to be the dominant component. Daily and day-to-day values are presented in column 6 of table 4.5.

**Additional Day-to-day Irreproducibility**

The component of variance in day-to-day reproducibility that is additional to the daily reproducibility is calculated from equation 4.8 (as described in section 4.4.2).

$$\sigma_{\text{additional}} = \sqrt{\sigma^2_{R(\text{DTD})} - \sigma^2_{R(\text{daily})}}$$

(4.8)

These values are presented in column 7 of table 4.5.

**Discussion**

The 'daily' reproducibility of the coral analyses (table 4.5) is somewhat larger than the glass reproducibility (table 4.4), and precision and heterogeneity cannot account for this variance. Normalising matched replicates to their average to produce the 'daily' reproducibility should have controlled factors such as spot size, and ICP-MS re-tuning, while the heterogeneity component accounts for the mis-alignment of laser analyses. The major component of the residual variance ($\sigma_m$) must therefore be matrix-dependent machine drift during a day. While this drift only seems to cause moderate irreproducibility in B, Mg, Sr and U, it seems to significantly affect Ba.

'Day-to-day' reproducibility is significantly poorer than 'daily' reproducibility (table 4.5). Again, precision and heterogeneity contribute negligibly to this variance. Laser spot size is uncontrolled in day-to-day replicates; however, matrix effects associated with re-tuning the ICP-MS are likely to be the dominant cause of the irreproducibility. The magnitude of this irreproducibility is sufficient to introduce 1.5 - 3.0 °C uncertainties into temperature reconstructions (calculated using the temperature dependencies presented in chapter 8). The irreproducibility in Ba is quite alarming (table 4.5), and suggests that barium might be inherently more sensitive to matrix effects than other elements, although there are no reports of this in the literature, and no obvious reason why it should be so.

Coral analyses 7, 8, and 9 represent the 3 most controlled coral replicates. These were analysed consecutively, under the same conditions, over the exact same tracks on the coral and glass. These analyses are very close to each other (figure 4.3 and table 4.6), and although $n = 3$ is a small sample, these results suggest that tightly constrained replicate coral analyses may be quite reproducible. It is possible, however, that these analyses happened to be run on a day when the ICP-MS tuning was unusually stable.
The effectiveness of using a matrix-matched standard is illustrated with 4 analyses of a coral from Shirigai Bay in Japan that performed by Mr Stewart Fallon (details of the coral and the technique can be found in Fallon et al., 1998; and Fallon et al., 1999). These analyses were standardised against a pressed powder. Although the replicates were analysed on different days, and the laser tracks were not well constrained on the standard (it is difficult to see laser traverses on the matt surface of the pressed powder), the reproducibility is still within 2 - 7% (table 4.6).

**Table 4.6** Coral Reproducibility

<table>
<thead>
<tr>
<th>Element</th>
<th>Analyses 7 - 9</th>
<th>Shirigai Bay Corals (Pressed Powder)[1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/Ca</td>
<td>1.1%</td>
<td>5%</td>
</tr>
<tr>
<td>Mg/Ca</td>
<td>1.5%</td>
<td>7%</td>
</tr>
<tr>
<td>Sr/Ca</td>
<td>0.1%</td>
<td>2%</td>
</tr>
<tr>
<td>Ba/Ca</td>
<td>1.7%</td>
<td>2%</td>
</tr>
<tr>
<td>U/Ca</td>
<td>2.4%</td>
<td>5%</td>
</tr>
</tbody>
</table>

[1] 4 analyses performed by Mr. Stewart Fallon

4.5 Accuracy

The accuracy of the LA-ICP-MS method was assessed by comparing the average of the replicate laser analyses of the Davies 2 coral (section 4.4.3) to a bulk sample that had been analysed by isotope dilution. In order to quantify the laser analyses it was necessary to independently calibrate the wollastonite standard. Both the glass and the coral were therefore calibrated by isotope dilution together, under the same conditions and using the same solutions and spikes. Thus, any inaccuracies in the isotope dilution method should be the same for all samples, and the glass and coral analyses will be *self consistent*. Theoretically, any inaccuracy in a laser measurement of a coral that has been standardised to the wollastonite glass must derive from the laser method.

The 17 coral replicates have been averaged, and this value is presented in column 2 of table 4.7. The isotope dilution measurement is presented in column 5.
Table 4.7  Accuracy

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>B/Ca (μmol/mol)</td>
<td>455</td>
<td>1.3%</td>
<td>17</td>
<td>471</td>
<td>1.1%</td>
<td>381[5]</td>
<td>780[6]</td>
</tr>
<tr>
<td>Sr/Ca (mmol/mol)</td>
<td>8.78</td>
<td>1.1%</td>
<td>16</td>
<td>8.9087</td>
<td>0.01%</td>
<td>8.890[8]</td>
<td>8.894[8]</td>
</tr>
<tr>
<td>Ba/Ca (μmol/mol)</td>
<td>3.2</td>
<td>9.0%</td>
<td>15</td>
<td>2.67</td>
<td>1.0%</td>
<td>3.9[9]</td>
<td>6.5[10]</td>
</tr>
<tr>
<td>U/Ca (nmol/mol)</td>
<td>979</td>
<td>3.0%</td>
<td>16</td>
<td>1030</td>
<td>0.90%</td>
<td>880[11]</td>
<td>1510[9]</td>
</tr>
</tbody>
</table>

[1] Average of the replicate coral laser analyses presented in section 4.4.3.
[3] Some outlier analyses were excluded from the average: see figure 4.3.
[10] Shen and Sanford (1990)

4.5.1 Experimental

Laser Analyses

Details of this data set are presented in section 4.4.3. The laser analyses of the Davies coral were made close to the edge remaining from the high resolution milling carried out by Alibert and McCulloch (1997). It was not possible to analyse precisely along the centre of the growth axis, as this had been removed by the earlier sampling. Analytical traverses were therefore offset from the growth axis by about 6.5 mm (see figure 4.2). This growth axis is very broad, however, and a small offset is not believed to cause any significant geochemical anomaly (of the type described by Alibert and McCulloch, 1997; de Villiers et al., 1995; de Villiers et al., 1994).
**Bulk Sampling**

The bulk sample of the Davies 2 coral was taken from the ledge remaining after earlier milling (Alibert and McCulloch, 1997): approximately 5.5 mm from the section analysed by the laser (see figure 4.2). The coral was ultrasonicated in 18 MΩ water and dried overnight at 40°C in a clean oven. Sampling was carried out on a computer controlled milling machine using a 2.5 mm tungsten carbide milling bit. Half a millimetre was shaved off the outside surfaces of the coral region to remove any surface contamination introduced by sample handling, and a section of coral measuring approximately 27 mm x 4 mm was sampled. Care was taken to avoid sampling the visible discolouration of the tissue layer.

**Dissolution**

**Carbonates**

Four replicate 0.05g samples of the coral powder were weighed out into teflon vials and dissolved directly in quartz distilled 2% nitric acid. After ultrasonication for a half an hour, these were transferred to 100 ml polyethylene screw-cap bottles that had been cleaned in 'Decon-90', and 10% nitric acid (soaked overnight in each case) and rinsed in 18 MΩ water. Coral powder solutions were then made up to 100 ml with quartz distilled 2% nitric acid.

**Glass**

Five 0.05 g subsamples of the new wollastonite glass were taken from different regions of the original disc. These were transferred to acid washed 1.5 ml centrifuge tubes, and cleaned of handling contamination by 5 minute ultrasonications in 1 ml of clean ethanol and 1 ml of 2% nitric acid; rinsing in between with 1 ml of 18 MΩ water and finishing with two 5 minute ultrasonications in 18 MΩ water. The glass was thereafter handled with plastic gloves only.

The dried glass samples were re-weighed, and had lost approximately 4% of their mass. The samples were transferred to teflon screw-cap vials (with one empty vial being handled in the same manner as a procedural blank). 1.0 ml of HF, and 6 drops of concentrated HNO₃ were added to each vial. Vials were alternately ultrasonicated for 30 minutes, and refluxed for 2 hours, twice before being dried down. The dried solid was slurried with 1.0 ml of 18 MΩ water, 1.0 ml of HF, and 2.0 ml of conc. HNO₃. The solution was ultrasonicated for 5 minutes, and left refluxing on the hotplate overnight. Next morning, samples were ultrasonicated for 30 minutes, and dried down. 7.5 ml of 6N HNO₃ was added, and the solution was ultrasonicated twice for 30 minutes, before being left to reflux overnight. Samples were dried down next morning, taken up in 2% HNO₃ and refluxed overnight. Finally the solutions were ultrasonicated for 30 minutes,
transferred to clean polyethylene bottles, and made up to 100 g with quartz distilled 2% HNO₃.

Analysis

Carbonate, glass, and procedural blank solutions were left for a day to equilibrate, then 0.05 g splits were taken for Sr and Ca analysis by TIMS (and the solutions re-weighed). A standard solution of B, Mg, Ba, and U was prepared gravimetrically from Plasmachem solution standards (see table 4.8 for composition). All solutions were then spiked with 5.0 g of enriched ²⁵Mg spike, 0.50 g of enriched ¹⁰B spike, and 0.20 g of mixed ¹³⁷Ba + ²³³U spike. 2.0 g splits of all solutions were taken and diluted 20-fold for solution ICP-MS analysis of magnesium. The undiluted parent solutions were analysed for B, Ba, and U by solution ICP-MS. Natural abundance (unspiked) solutions were analysed in addition to the standards, samples, and procedural blanks.

Table 4.8 Compositions of Isotope Dilution Solutions

<table>
<thead>
<tr>
<th>Concentration in Standard</th>
<th>ICP-MS Analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td>Concentration (nmol/g)</td>
</tr>
<tr>
<td>B</td>
<td>5.358</td>
</tr>
<tr>
<td>Mg</td>
<td>1.921</td>
</tr>
<tr>
<td>Ba</td>
<td>0.1031</td>
</tr>
<tr>
<td>U</td>
<td>0.007987</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration in Spike</th>
<th>TIMS Analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotope</td>
<td>Concentration (nmol/g)</td>
</tr>
<tr>
<td>¹⁰B</td>
<td>1358</td>
</tr>
<tr>
<td>²⁵Mg</td>
<td>221</td>
</tr>
<tr>
<td>¹³⁸Ba</td>
<td>0.192</td>
</tr>
<tr>
<td>²³³U</td>
<td>0.0266</td>
</tr>
<tr>
<td>⁴³Ca</td>
<td>14.79</td>
</tr>
</tbody>
</table>

The 0.05 g sample split was spiked with 0.15g of mixed ⁸⁴Sr ⁴³Ca spike, and analysed for Sr and Ca using a method similar to Alibert and McCulloch (1997) (the only modification to that method was the use of a ⁴³Ca spike instead of a ⁴²Ca ⁴³Ca spike).
The following isotope ratios were monitored on the ICP-MS: $^{11}\text{B}/^{10}\text{B}$, $^{25}\text{Mg}/^{24}\text{Mg}$, $^{26}\text{Mg}/^{24}\text{Mg}$, $^{135}\text{Ba}/^{138}\text{Ba}$, $^{137}\text{Ba}/^{138}\text{Ba}$, $^{233}\text{U}/^{238}\text{U}$, and $^{235}\text{U}/^{238}\text{U}$, while $^{84}\text{Sr}/^{86}\text{Sr}$, $^{88}\text{Sr}/^{86}\text{Sr}$, $^{42}\text{Ca}/^{44}\text{Ca}$, and $^{43}\text{Ca}/^{44}\text{Ca}$ were measured by TIMS. Instrumental bias factors were estimated using a power law relationship for Mg, Ba, and U. Boron has only two isotopes, and the bias factor for this element was therefore estimated from analysis of the normal abundance solution.

The isotopic abundances for the B and Ba spikes were taken from the manufacturers specifications. The other spikes were checked by TIMS against standard reference materials. Spike compositions were calculated from the spiked standard solution using the isotope dilution equation:

$$\frac{\left(\frac{^{86}\text{Sr}}{^{86}\text{Sr}_{\text{spk}}}\right)_{\text{sa}}}{\left(\frac{^{86}\text{Sr}}{^{86}\text{Sr}_{\text{spk}}}\right)_{\text{spk}}} = \frac{\left(\frac{^{84}\text{Sr}}{^{86}\text{Sr}}\right)_{\text{Mix}} - \left(\frac{^{84}\text{Sr}}{^{86}\text{Sr}}\right)_{\text{Spk}}}{\left(\frac{^{84}\text{Sr}}{^{86}\text{Sr}}\right)_{\text{Sa}} - \left(\frac{^{84}\text{Sr}}{^{86}\text{Sr}}\right)_{\text{Mix}}}$$  \hspace{1cm} (4.10)

where:

$^{86}\text{Sr}_{\text{Sa,Spk}}$ = Moles of $^{86}\text{Sr}$ in the sample and spike

$^{84}\text{Sr}$\text{Mix,Spk,Sa} = $^{84}\text{Sr}/^{86}\text{Sr}$ ratio in the mixture, spike and sample

Abundances and concentrations are presented in table 4.8. Once the spike composition was established, the concentrations of the samples were calculated using the isotope dilution equation.

Boron is volatile, and can be lost as a boron fluoride when heated with HF. Solution based calibration of this element is therefore inaccurate, and B in the old wollastonite glass was therefore measured by 21 paired laser analyses against the NIST 612 glass (see chapter 3, section 3.5.2).

The old wollastonite glass was calibrated by 28 paired laser analyses against the new wollastonite glass. The data from these replicates is presented in section 4.4.2, and uncertainties in the calibration amount to less than 0.4 % (the standard error of the reproducibility presented in table 4.4).

### 4.5.2 Discussion

The laser measurement in column 2 of table 4.7 is compared both to trace element concentrations reported in the literature (columns 7 and 8), and to the isotope dilution measurement (column 5). The laser measurements of the Davies coral all appear reasonable given the range of concentrations quoted in the literature for corals. The concentration of strontium falls somewhat below the value calculated from the high precision isotope dilution analyses of this coral sample presented in Alibert & McCulloch (1997). The laser analyses are slightly offset from the main growth axis (figure 4.2),
and the Sr/Ca ratio can be shifted by several % when traversing a growth margin instead of a growth axis (Alibert and McCulloch, 1997; Cohen and Hart, 1997; de Villiers et al., 1994). This shift is typically towards higher Sr concentrations in marginal aragonite, whereas the value estimated from the laser falls below the isotope dilution average of Alibert and McCulloch (1997).

There are definite differences between the laser and the isotope dilution measurements of the coral, with offsets from 1.5% for Sr, up to 19% for Mg. While these appear large, the poor reproducibility of the technique makes it uncertain whether these discrepancies are statistically significant.

**Statistical Comparison**

It is possible to construct a simple statistical test to determine the significance of the difference between the laser and the solution estimates of the coral. The hypothesis being tested is that "the average of the replicate laser analyses for trace element X is significantly greater/less than the bulk composition determined by isotope dilution". The null hypothesis is naturally that "any observed difference between the laser and solution analyses is due to chance".

This implies a simple 1-tailed test for the difference between populations. The test statistic is the difference between the laser and isotope dilution measurements, divided by the standard deviation for the difference.

\[
 z = \frac{n_1 - n_2}{\sigma_{s-l}}
\]

where:

\[ n_1 = \text{average of solution analysis.} \]

\[ n_2 = \text{average of laser analysis.} \]

\[ \sigma_{s-l} = \text{standard deviation for difference between solution and laser estimates.} \]

The null hypothesis is rejected at the 5% level for a critical value of the test statistic of 1.645.

The uncertainties in the isotope dilution measurement were estimated by propagation of counting statistics and measurement uncertainty through the isotope dilution equation. These errors are around 1% of the ICP-MS analyses, and are much lower for the high precision TIMS analysis of Sr/Ca. The uncertainty in the laser measurement of the coral is given by the standard error of the reproducibility (\( = \sigma_R/\sqrt{n} \) where \( n = 15, 16, \) or 17 - see table 4.7). These uncertainties range from around 1% for Sr to 9% for Ba. The laser and solution uncertainties are independent, and the standard deviation of the difference
between solution and laser measurements is given by adding the absolute uncertainties in quadrature.

\[
\sigma_{\text{solution-laser}} = \sqrt{\left(\sigma_{\text{solution}} \cdot \bar{x}_{\text{solution}}\right)^2 + \left(\sigma_{\text{laser}} \cdot \bar{x}_{\text{laser}}\right)^2}
\]  \hspace{1cm} (4.12)

where: \(\sigma_{\text{solution}}\) and \(\sigma_{\text{laser}}\) are the values in columns 6 and 3 of table 4.7 respectively.

Values of the test statistic are presented in column 4 of table 4.9. The difference between the solution and laser estimate for Mg is clearly significant, and indicates that there is a major inaccuracy in the calibration for this element. The differences for Sr are small and are therefore not significant. For B, Ba, and U, the test statistic is close to (but slightly larger than) the critical value, suggesting that the laser and solution measurements may be significantly different.

**Table 4.9 Statistical Analysis of Accuracy**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>B/Ca ((\mu\text{mol/\text{mol}}))</td>
<td>16</td>
<td>8.0</td>
<td>2.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Mg/Ca ((\mu\text{mol/\text{mol}}))</td>
<td>0.73</td>
<td>0.091</td>
<td>8.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Sr/Ca ((\mu\text{mol/\text{mol}}))</td>
<td>0.13</td>
<td>0.095</td>
<td>1.36</td>
<td>0.09</td>
</tr>
<tr>
<td>Ba/Ca ((\mu\text{mol/\text{mol}}))</td>
<td>-0.53</td>
<td>0.29</td>
<td>-1.84</td>
<td>0.03</td>
</tr>
<tr>
<td>U/Ca ((\mu\text{mol/\text{mol}}))</td>
<td>51</td>
<td>31</td>
<td>1.67</td>
<td>0.05</td>
</tr>
</tbody>
</table>


Critical value for a 1 tailed test = 1.645 (\(P = 0.05\)).

[3] Probability that the null hypothesis scenario could produce the observed test statistic by chance.

Note: Assumes that the test statistic is normally distributed.

The accuracy of these probability estimates should be treated with caution. This statistical test is based on the assumption that the test statistic is normally distributed, and there has been no attempt made to validate this assumption. Nor is it certain that the uncertainties for isotope dilution or laser analyses are normal. It is also important to note that this critical level of \(P = 0.05\) is an arbitrary limit that is conventionally used in statistics, and has no absolute significance as a cut-off between the null hypothesis and the working hypothesis. With these caveats in mind it is concluded that the laser
technique is inaccurate for Mg, and may be inaccurate for B, Ba, and U. There is no evidence that the technique is inaccurate for Sr.

**Sources of Inaccuracy**

Factors that result in inaccuracy are by definition constant with time. If a factor changed with time, then its variance would have been accounted for in the analysis of the reproducibility. A factor can have both time variable and invariable components (e.g. matrix effects); however, the time variable component will have been removed by averaging the replicates.

**Solution Analysis**

Some of the inaccuracy may derive from the solution analysis of the samples and the standard. The accuracy of the isotope dilution measurement ultimately depends on how well known the standard solution compositions, and spike isotope ratios are. After sample dissolution, however, all samples were treated identically, and measured on the same day. All samples had very similar concentrations of unknowns, and although carbonates have a different matrix to silicates, the loss of C as CO₂ and Si as SiF during dissolution results in solutions that are very similar in their chemical matrix. Thus, even if there is inherent inaccuracy in the isotope dilution calibration of the standard, the same inaccuracies should also apply to the analysis of the Davies 2 coral, and the data will be self consistent.

The coral and glass samples were treated differently during the dissolution stage, however, and as discussed in chapter 3 (section 3.5.2), B was lost from solution when dissolving the glass. Some loss of Mg, Sr, or U may also have occurred, explaining their underestimates in the laser measurements.

**Heterogeneity in Standard**

During the cross-calibration of the old and new wollastonite, only a single track on each standard was replicated. These tracks were in regions of the glass that were commonly analysed, and should be representative of the standard analyses. Nonetheless, an offset as small as 0.5 mm on the old wollastonite glass can result in shifts of up to ±4% in B, 2% in Mg, 0.4% in Sr, 2.5% in Ba, and 1.5% in U.

The chips of the new wollastonite glass analysed by solution may not be representative of the region of the glass analysed by laser, especially if significant broad compositional gradients exist in the glass. The new wollastonite appears to be very homogeneous, however (figure 3.7), and the 5 subsampled glass chips used for solution calibration surround the region of the glass analysed by laser. Inaccuracy in the sampling is therefore expected to be minimal.
Coral Milling

The region of the coral milled for the bulk sample lies approximately 5.5 mm to one side of the laser traverses (figure 4.2). Because the growth axis being analysed is very broad it is assumed that this offset makes little difference to the bulk composition of the coral. It is possible, however, that significant lateral heterogeneity exists in the bulk coral composition. There is certainly evidence in the literature that Sr can change in composition by several % across the coral (see discussion in section 4.5.1), and further work should be carried out to assess whether this could cause some of the observed inaccuracy.

The bulk coral sample that was milled for solution analysis did not span exactly the same time range covered by the laser analyses (figure 4.2). A correction factor was calculated from the observed offsets and the seasonally varying composition of the coral; however, applying this correction did not improve accuracy.

Matrix Effects

The final factor that might be responsible for inaccuracies in the technique is the matrix difference between the calcium carbonate of the coral, and the calcium silicate of the wollastonite. A more detailed discussion of matrix effects was presented in the previous chapter (section 3.2.2). Matrix effects clearly have a significant influence on reproducibility (section 4.4), and it is reasonable to suggest that they are also responsible for a constant offset between glass and coral. Unfortunately it is not possible to estimate other components of inaccuracy, and the magnitude of any matrix effect cannot be quantified. Anecdotal evidence does suggest that coral analyses standardised to the matrix matched pressed powder are significantly more accurate (Fallon, S. pers. comm.).

Correcting for Inaccuracy

Because inaccuracy is constant with time, it may be possible to calculate a single correction factor that can be applied to each coral analysis. Alternatively, it may be worth calculating an 'apparent value' for the wollastonite glass (see discussion in chapter 3, section 3.2.5) by calibrating it to the Davies 2 coral. Unfortunately, no other corals have been independently calibrated by isotope dilution, and it is not therefore possible to assess whether this strategy is significantly more accurate.

Irrespective of any improvement in accuracy, the largest contribution to the uncertainty of an individual laser analysis is still the poor reproducibility of the technique, which cannot be corrected by applying a single factor. A matrix-matched standard could therefore significantly improve accuracy by reducing both time-dependent and time-independent matrix effects.
4.6 Summary

The detection limits for the technique are well below the concentrations of B, Mg, Ca, Sr, Ba, and U in the coral. Normal skeletal concentrations of Cu, Ni, and probably Zn are significantly below detection limits, and signals seen for some of these elements are likely to be contaminants. Lead levels in coral skeleton are slightly above the detection limits of this technique, and semiquantitative analyses might be possible if Pb contaminants can be removed. Manganese, Y, La, and Ce are all close to the detection limits of the technique; however, quantitative information about environmental processes may be obtainable from smoothed profiles.

Instrumental precision is typically between 0.5 and 3%, corresponding to temperature uncertainties of less than 1 °C (except for Sr). Fine-scale compositional variability in the coral will add significantly to imprecision, although temperature uncertainties may be reduced to around 0.3 - 0.4 °C by smoothing the trace element signals.

Reproducibility is good for a matrix-matched sample, and can be largely accounted for by heterogeneity in the standard, and counting statistics. Reproducibility is poor for coral replicates standardised to the wollastonite glass, and appears to be dominated by tuning-dependent matrix effects. Coral reproducibility seems to be significantly better when using the matrix-matched pressed powder standard.

Statistically significant differences between solution and laser estimates of the coral were observed for B, Mg, Ba and U. Fractionation during solution calibration, or heterogeneity in the samples may have contributed to this inaccuracy; however, it is likely that matrix effects during laser analyses dominate. It may be possible to calculate a single correction factor to correct for much of this inaccuracy; however, the inaccuracy associated with a single laser analysis is still dominated by the poor reproducibility of the technique.

Overall, matrix matching between the sample and the standard appears to be important, and use of a pressed powder standard may significantly improve reproducibility and accuracy.
CHAPTER 5: LITERATURE REVIEW: CORAL CALCIFICATION AND PARTITIONING OF TRACE ELEMENTS INTO CORAL SKELETON

5.1 Introduction

The mechanisms that control trace element incorporation into coral aragonite are poorly understood. Many researchers assume that trace elements are passively precipitated from seawater under the rigorous and constant laws of inorganic chemistry. Others treat corals as 'black boxes', and empirically correlate trace elements with environmental signals, relying on corals to partition trace elements consistently through time and across species. While there is no doubt that corals partition most trace elements at close to inorganic values, more and more evidence is being compiled suggesting that physiology may play a significant role in controlling the uptake of trace elements into the coral skeleton. The evidence also suggests that these physiological processes are not constant through time or between individuals. An understanding the mechanisms of coral biomineralization is therefore a critical requisite to understanding how physiological and environmental parameters may influence the coralline chemical record.

The model of coral biomineralization developed by McConnaughey in the mid 1980s to explain oxygen and carbon isotope fractionations in coral skeletons (McConnaughey, 1986) has proved to be robust, providing a good framework for understanding the general processes that occur during coral skeletal deposition. The McConnaughey mechanism, however, is based on a relatively simple model of coral physiology, many details of which still remain unknown. It is important, therefore, to examine in more detail the complexities of calcification physiology and chemistry, and to highlight the current gaps in scientific understanding.

This chapter is presented in two parts. Sections 5.2 to 5.4 review the basic coral calcification physiology and biochemistry; highlighting the current debates and uncertainties, and presenting the major calcification model upon which further discussion is presented. Sections 5.5 to 5.8 take this calcification model and examine the implications that it has for trace element partitioning into the coral skeleton. Included in
this is a discussion of the inorganic factors controlling trace element partitioning, and ion-transport pathways through the coral polyp.

PART 1: REVIEW OF CALCIFICATION BIOCHEMISTRY AND PHYSIOLOGY

5.2 Skeletal Deposition

There are two schools of thought regarding the way in which corals form their skeleton: those who believe that coral skeletons form from, or are templated by an organic matrix, and those who believe that crystals form inorganically (physicochemically) inside membrane-enclosed pockets of fluid. An understanding of the deposition process is important for understanding and predicting the inorganic mechanistics of crystal growth, and the distribution of trace elements within the coral skeleton. If calcification occurs from a solution, then the aragonite composition and crystal morphology are subject to the thermodynamic and kinetic constraints of inorganic crystal growth, and the distribution of trace elements within the coral skeleton. If calcification occurs from an organic matrix, then crystal deposition is largely controlled by biological factors, such as physiological transport and membrane 'excretion'. Under these circumstances inorganic considerations will not necessarily apply.

5.2.1 Calcification from an Organic Matrix

Coral skeletons contain small amounts (0.01 - 0.1%) of organic material intimately dispersed throughout the aragonite (Johnston, 1980; Wainright, 1963). Most of the organic material is inaccessible to oxidation by solution, and even complete pulverisation of the skeleton does not release more than 10% of these organics (Young, 1971). Wainright (1963) demonstrated that careful demineralisation of the aragonitic skeleton of a sample of *Pocillopora* revealed an intricate and delicate matrix of organics that retained the exact shape of the intact skeleton, and histochemical analysis of this material revealed it to be distinct from coral tissue.

This organic material has polarised the calcification community, with some researchers interpreting it as a structured organic material upon which the coral skeleton is built. One of the earliest ideas regarding calcification is the concept of a template of organic material that aids calcification and guides skeletal formation. As early as the turn of the century, light microscopy revealed amorphous organic material at the surface of the skeleton (see reviews in Johnston, 1980; Wainright, 1963). Goreau (1959), identified this material as an amorphous mucopolysaccharide gel, while Wainright (1963) observed a spongework of organic fibrils of chitin at the surface of the skeleton of a *Pocillopora damicornis*. 
Organic material is believed to play a role in skeletal construction, by adsorbing $\text{Ca}^{2+}$ and $\text{HCO}_3^-$ ions, and concentrating them spatially so that $\text{CaCO}_3$ precipitation can occur. Other possible roles include the nucleation and seeding of mineral crystals, determining the crystal form that is precipitated, controlling the size, shape and orientation of the crystals, and altering the structural properties of the skeleton (Goreau, 1959; Johnston, 1980; Wainright, 1963).

### 5.2.2 Physicochemical Calcification

Proponents of physicochemical skeletal growth interpret the skeletal organic material differently. They suggest that this organic material represents small amounts of tissue that have been trapped and 'pinched off' by the formation of crystals (Barnes, 1970). Inorganic growth can result in a myriad of sub-µm pores and holes, that could easily preserve the trapped organic material from oxidation by solution, even during extensive crushing of the skeleton (Constantz, 1986).

Physicochemical growth of crystals is defined here as unrestricted inorganic crystal growth from a physiologically modified pool of calcifying fluid. Most of the evidence for physicochemical calcification derives from analysis of the aragonite crystal morphology and arrangement within the coral skeleton. Corals are constructed from spherulitic bundles of acicular aragonite fibres (Barnes, 1970; Constantz, 1986; Johnston, 1980; Lowenstam and Weiner, 1989; Wainright, 1963), with each crystal elongated along its c axis. This structure is characteristic of inorganically precipitated crystals grown rapidly from supersaturated solutions (Constantz, 1986), and is commonly seen in calcitic marine cements. Such structures are hard to explain by epitaxial growth within an organic matrix, or direct secretion from a membrane, and some researchers therefore take this to be an indication that although calcification may be biologically initiated, it then progresses under physicochemical control (Barnes, 1970; Constantz, 1986; Hayes and Goreau, 1977).

### Crystal Growth Within Membrane Sheaths

For crystals to grow physicochemically, solution containing the skeletal components (ions and seed nuclei) must be delivered to an isolated pocket of calcifying fluid. A thorough review of the observations made of calcifying tissue over the last century is presented in Johnston (1980). The critical observations, made on specimens of *Pocillopora damicornis*, include the presence of sheets and fibrils extending onto and into the skeleton from the undersurface of the calicoblastic ectoderm, the transient presence of sheaths around each individual aragonite crystal, and the appearance of small extracellular vesicles that are interpreted to be precursors to the crystal sheaths (Johnston, 1980). Johnston (1980) hypothesises that calcification occurs only within the
crystal sheaths. Vesicles fuse with the sheaths, discharging fluids containing skeletal components (organics and ions).

A related mechanism was earlier proposed by Hayes and Goreau (1977) who suggested that *intracellular* vesicles may nucleate small crystals. These vesicles eventually fuse with the cell wall, providing transport for seed nuclei, organic components, and ionic calcium to the skeletal surface.

Both sheaths and vesicles may actively control the calcifying environments through membrane bound enzymes. The sheaths grow to accommodate the expanding crystal, and occasionally a vesicle will nucleate its own crystal, thereby becoming a new sheath. Crystal shape, placement and orientation is entirely controlled by the orientation of the sheath, and of the surrounding sheaths.

**Free Crystal Growth**

In contrast to the idea that single crystals are nucleated and grow within individual membrane sheaths, a number of researchers propose that crystal growth and nucleation instead occurs freely within a larger (membrane bound) pocket of supersaturated fluid (Barnes, 1970; Constantz, 1986; McConnaughey, 1989b; McConnaughey, 1986). The volume and ionic composition of this fluid is modified and buffered by transport from extracellular and/or intracellular sources, and by enzymes bound to the membrane (McConnaughey, 1986).

**Skeletal Organisation**

Crystals nucleate and grow outwards from structures called 'centres of calcification'. These nucleating structures appear darker than the surrounding aragonite in thin sections analysed by light microscope, and have a grainy texture in SEM analyses (Lowenstam and Weiner, 1989). The growth form of crystals in centres of calcification differs from the fibrous aragonite that makes up the bulk of the skeleton. Crystals tend to be more platy and tabular rather than elongate laths and rods (Johnston, 1980). Centres of calcification have been found to display a significantly different trace element chemistry (Allison, 1996b; Allison and Tudhope, 1992), and there is a possibility that these structures contain a different mineralogy. Constantz and Meike (1988) report finding traces of calcite in the centres of calcification of the coral *Mussa angulosa*. There is some precedent for aragonite nucleation from calcitic structures, as the initial basal plates deposited by recently settled coral larvae are calcite (Lowenstam and Weiner, 1989). It is postulated that centres of calcification contain high amounts of organic material (Allison, 1996b; Allison and Tudhope, 1992; Johnston, 1980); however, histochemical tests revealed no differences in the composition of these structures compared with other parts of the coral skeleton (Wainright, 1963).
Clusters of acicular aragonite crystals grow concentrically and radially outwards from their nucleation points at the centres of calcification, forming fan-shaped structures called sclerodermites (Barnes, 1970) or fasciculi (Constantz, 1986). Additional crystal nucleation occurs spontaneously in spaces between growing crystals (Barnes, 1970; Constantz, 1986). New crystals nucleate on the walls and ends of existing crystals, and depending on the conditions in the solution, this can be syntaxial (oriented the same way as seed crystals) or non-syntaxial (Barnes, 1970). The amount of spontaneous nucleation, and non-syntaxial growth increases with the degree of supersaturation in the calcifying fluid.

In the 'free growth' models (Barnes, 1970; Constantz, 1986), sclerodermites (fasciculi) grow inorganically, without direct intervention by tissues, competitively interacting with neighbouring crystal-fans. Individual crystals elongate until they intersect another crystal or their growth is terminated by contact with the membrane wall. Crystals with their c axes oriented obliquely to the predominant crystal growth direction will quickly be 'blocked off' by other crystals. This competition for space therefore maintains the predominant crystal fabric, despite the tendency for non-syntaxial crystals to form new divergent fans (Barnes, 1970; Constantz, 1986). These groups of fasciculi, bounded by their enclosing membrane, are the basis of the larger structures seen in corals (septae, trabeculae, epitheca and dissepiments: see Barnes, 1970).

**Biological Control of Skeletal Formation**

The general morphology of coral skeletons is taxa specific, and indeed is often an important diagnostic tool for classifying coral lineage (Constantz, 1986; Lowenstam and Weiner, 1989). Corals clearly have some control over their gross skeletal form, and this requires that some aspects of crystal growth are influenced by the coral polyp. Under the 'inorganic growth' hypothesis, nucleation of new crystals seems to be the easiest way by which the corals might affect the growing skeleton. Constantz (1986) hypothesises that calcification is initiated by seed nuclei packets which may be generated intracellularly by the coral (see also Hayes and Goreau, 1977). Under this hypothesis, the seed nuclei form the centres of calcification (see previous section), and the coral directs skeletal growth through the positioning and orientation of these calcification centres.

Aragonite is not the thermodynamically stable crystal phase at atmospheric temperature and pressure, and seawater composition. Lowenstam and Weiner (1989) note that the shape of individual aragonite crystals has a taxonomic regularity, suggesting some degree of biological control over the crystal form. It is therefore suggested by some researchers that the coral must modify organic and ionic composition of the calcifying environment to ensure the correct phase and morphology of crystal is precipitated (Barnes, 1970 and references therein; Lowenstam and Weiner, 1989). The coral may
also manipulate the nucleation of CaCO₃ crystals, and therefore the different mineralogy and crystal form observed for the centres of calcification could be a requirement for initiating aragonite formation.

On the other hand, McConnaughey (1986) noted that the precipitation kinetics and high Mg²⁺ content of natural seawater were enough to favour aragonite formation, and there was no need for the coral to adjust the organic concentration of the calcifying solution to control either crystal shape or form. Similarly, Constantz (1986) noted that the acicular crystal form in corals is well within the bounds normally observed for inorganic aragonite cements (see also appendix A).

It is clear that some of the macroscopic features of the coral skeleton are directed by the coral polyp. The shape and size of the membrane-bound pocket of calcifying fluid will ultimately have some control over the architecture of the growing skeleton. Barnes (1970) observed calcifying tissues and noted that the calicoblastic membrane swells up and lifts away from actively extending sections of skeletal elements. He proposed that the coral was opening up a fluid-filled gap to allow precipitation of CaCO₃ onto the growing skeleton. Johnston (1980) disputes this observation, stating that under the conditions in which Barnes manufactured his thin sections, tissue shrinkage may have resulted in contraction of the membrane away from the skeletal surface. Despite this assertion, McConnaughey (1986) concluded that the coral is likely to control crystal growth by contact inhibition (rather than contact initiation). Constantz (1986) extends this idea, suggesting that in some regions of the coral skeleton, the tissue actively 'planes off' crystals, and smooths the skeleton by covering it in a fine-grained mineral sheath.

Theories proposed by Barnes (1970) do not require the presence of centres of calcification for nucleation of crystal growth. Instead nucleation is spontaneous (either syntaxial or non-syntaxial) at high enough supersaturations. Neither are centres of calcification required for directing skeletal growth, and instead the geometry of the basal structure, the mechanisms of sclerodermite competition, and the shape of the enclosing membrane are sufficient to explain the range of structures observed in corals. The structures identified as centres of calcification are the natural apices of crystal fans.

5.2.3 Summary

The debate concerning the deposition of CaCO₃ in coral skeletons remains unresolved. In essence the two contrasting ideas relate to the degree to which the coral influences the deposition of individual crystals. Theories of organic control suggest that skeletal growth may occur through the calcification of an organic structural matrix that facilitates and templates CaCO₃ deposition. In contrast, some researchers propose that calcification
proceeds physicochemically, where crystals nucleate and grow without direct interference from the coral tissue. Crystal growth occurs from a thin, membrane bounded pocket of calcifying fluid that surrounds each structural element.

Although there does not appear to be a clear consensus amongst coral calcification researchers, the physicochemical theory seems to be able to account for most of the morphological and structural features in coral skeletons, without requiring more complex ideas of calcification from an organic matrix.

5.3 Metabolic Controls on Calcification

5.3.1 Introduction

Corals clearly have some degree of physiological control over their calcification rate. Calcification rates are higher over corals than over sand and lagoon, calcification in zooxanthellate corals is faster during the daytime than at night (table 5.1), and polyps growing on the same colony may calcify at different rates (Goreau, 1959; Pearse and Muscatine, 1971).

5.3.2 Metabolic Processes and Biochemistry

Information on the physiological mechanisms for coral calcification is mostly derived from experiments examining the effects of various metabolic inhibitors on the incorporation of $^{45}$Ca into coral skeletons, and the transport of $^{45}$Ca across isolated membranes. As more research is undertaken on the subject, the more complex the physiological chemistry of calcification is revealed to be. Marshall (1996) reports evidence for at least three different calcification mechanisms in corals, as calcification in zooxanthellate corals responds to a different suite of environmental and chemical conditions in the light than in the dark, and dark calcification does not follow the same biochemistry as calcification in non-zooxanthellate corals. There is also clear evidence that the calcification mechanism differs different between different genera and species of corals, with some being more sensitive to particular inhibitors or environmental conditions than others (Goreau, 1959). For instance: *Porites divaricata*, which is a shallow growing species adapted to high light conditions is more affected by being grown in darkness, and is more sensitive to carbonic anhydrase inhibitors than *Cladacora arbuscula* which grows in deeper shadier waters (Goreau, 1959).
Table 5.1  Calcification Rates

Coral Community Calcification

\((kg \text{CaCO}_3 \text{m}^{-2} \text{yr}^{-1})\)

<table>
<thead>
<tr>
<th>Coral Reef</th>
<th>Lagoon</th>
<th>Sand/Algal Flats</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 - 6(^1)</td>
<td>0.5(^1)</td>
<td>0.3(^1)</td>
</tr>
</tbody>
</table>

Day vs. Night Calcification

<table>
<thead>
<tr>
<th>Coral Species</th>
<th>Light/Dark Ratio(^2)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acropora cervicornis</td>
<td>(4.2 \pm 1.5)(^3)</td>
<td>(45\text{Ca Uptake})</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>(5.4 \pm 0.4)(^4)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>(7.5)(^5)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Acropora palmata</td>
<td>(13.2)(^5)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Acropora formosa</td>
<td>(2.6 \pm 0.8)(^6)</td>
<td>Alkalinity Anomaly</td>
</tr>
<tr>
<td>Porites divaricata</td>
<td>(2.0 \pm 0.1)(^7)</td>
<td>(45\text{Ca Uptake})</td>
</tr>
<tr>
<td>Porites compressa</td>
<td>(1.1 \pm 0.1)(^7)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Porites porites</td>
<td>(3.2)(^5)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Porites furcata</td>
<td>(8.1)(^5)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Porites astreoides</td>
<td>(4.5)(^5)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Galaxea fascicularis</td>
<td>(1.5 \pm 0.3)(^8)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Cladacora arbuscula</td>
<td>(1.0 \pm 0.1)(^7)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Acropora prolifera</td>
<td>(1.7 \pm 1.4)(^7)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Montipora verrucosa</td>
<td>(1.2 \pm 0.2)(^7)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Pocillopora damicornis</td>
<td>(1.5 \pm 0.2)(^7)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Oculina diffusa</td>
<td>(2.0 \pm 0.2)(^7)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Colpophyllia natans</td>
<td>(4.3)(^5)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Manicina aerolata</td>
<td>(7.5)(^5)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Montastrea annularis</td>
<td>(22.9)(^5)</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Millepora complanata</td>
<td>(7.5)(^5)</td>
<td>&quot; &quot;</td>
</tr>
</tbody>
</table>

Zooxanthallate vs. Non-Zooxanthallate Calcification

\((\mu\text{mol}^{-1}\text{hr}^{-1})\)

<table>
<thead>
<tr>
<th>Tubastrea fualkneri ((\text{non-zooxanthallate}))</th>
<th>Galaxea fascicularis ((\text{zooxanthallate}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0.63 \pm 0.13)(^9)</td>
<td>(0.57 \pm 0.07)(^9)</td>
</tr>
</tbody>
</table>

\(^{[1]}\) See reviews in Smith, (1983) and Davies (1983).
\(^{[2]}\) Presented as a ratio because calcification units are not consistent between studies.
\(^{[3]}\) Calculated from 5 experiments presented in Chalker and Taylor (1975).
\(^{[4]}\) Pearse and Muscatine (1971)
\(^{[5]}\) Goreau and Goreau (1959)
\(^{[6]}\) Calculated from 4 values in Barnes (1985).
\(^{[7]}\) Goreau (1959)
\(^{[8]}\) Krishnaveni et al. (1989): photosynthesis inhibited by DCMU.
\(^{[9]}\) Calculated from 2 values in Marshall (1996).
Dependence on Metabolic Energy

Biological energy is stored and transported as a high energy triphosphate called ATP, which is formed by an organism during the metabolism of large organic molecules. Daytime calcification in corals is dependent on this metabolic energy. This has been demonstrated by a number of researchers who have shown that coral calcification is significantly inhibited by adding respiratory poisons (uncouplers of oxidative phosphorylation) to the seawater in which the corals are growing (Chalker and Taylor, 1975; Krishnaveni et al., 1989). These respiratory poisons had no effect on dark calcification, suggesting that the mechanism for Ca uptake in the dark was largely independent of metabolic energy, or that dark calcification utilises a source of metabolic energy that is not derived from oxidative phosphorylation (Chalker and Taylor, 1975). It appears, therefore, that the enhanced daytime calcification requires metabolic energy, while the slower night-time calcification does not. Some conflicting results have been reported, however, with Barnes (1985) observing that respiratory inhibitors had no effect on light calcification but inhibited dark calcification.

Light and Photosynthesis: Role of Algal Symbiosis

Most coral researchers agree that the photosynthetic symbiotic algae enhance calcification rates during the day (Goreau, 1959), and some researchers find that calcification rates are linearly dependent on the intensity of light under which corals are grown (Chalker and Taylor, 1975). It is clear, however, that photosynthesis is not a requirement for calcification, as the non-photosynthesising non-zooxanthellate corals can still calcify (Constantz, 1986; Goreau, 1959). It is also apparent that the link between photosynthesis and calcification is not necessarily a simple or intimate relationship between the zooxanthallae and the polyp that is their host. The fastest calcifying regions of *Acropora cervicornis* are the tips of branches; however, these are relatively depleted in zooxanthallae, compared with the more basal regions of the coral (Pearse and Muscatine, 1971).

The photosynthetic inhibitor DCMU abolishes photosynthesis in the zooxanthallae without affecting the coral's metabolic processes. It has been found to completely inhibit light-enhanced calcification in zooxanthellate corals (Chalker, 1976; Chalker and Taylor, 1975; Krishnaveni et al., 1989; Marshall, 1996), but to have no effect on the growth of an non-zooxanthellate coral (Marshall, 1996). Zooxanthellate corals grown without zooxanthallae display a depressed calcification, with calcification rates similar to corals grown in darkness (Goreau, 1959). These observations suggest that photosynthesis within the symbiotic zooxanthallae somehow increases coral calcification rates. Photosynthetically enhanced calcification may allow corals to keep pace with erosive
forces, explaining why zooxanthellate corals build reefs, but non-zooxanthellate corals do not.

Several hypotheses have been proposed to explain how zooxanthallae enhance calcification, and these are reviewed in Johnston (1980) and Krishnaveni et al. (1989). The first possibility is that the zooxanthallae excrete substances that are required by the coral tissues for calcification. Low molecular weight organic molecules secreted by the photosynthesising zooxanthallae may be metabolised by the coral to generate the energy required to maintain the Ca$^{2+}$-ATPase pump (Chalker and Taylor, 1975; Pearse and Muscatine, 1971). Pearse and Muscatine (1971) used radioactively labelled carbon to demonstrate that photosynthesis generated metabolisable organics which were translocated to the rapidly calcifying tips of *Acropora cervicornis*. Similarities between the effects of metabolic poisons, and the effects of photosynthetic inhibition by DCMU lend weight to this idea. Alternatively, the zooxanthallae may synthesise organic molecules that are required for forming centres of nucleation or an organic matrix upon which the skeleton is templated (Wainright, 1963 and see also the debate in section 5.2).

Zooxanthallae may also play a secondary role in calcification. Goreau (1959) found that calcification without zooxanthallae was slightly slower than dark calcification, and proposed that zooxanthallae may help the coral by removing waste products generated by the coral such as PO$_4^{3-}$, SO$_4^{2-}$, and NO$_3^-$ (Goreau, 1959; see also review in Johnston, 1980). Phosphates have been found to act as crystal poisons, inhibiting the nucleation and growth of aragonite crystals (Simkiss, 1964a and see also appendix A), and it is possible that uptake by zooxanthallae removes these phosphates from the calcifying system (Simkiss, 1964b).

Finally, the removal of CO$_2$ by photosynthesis may aid calcification by 'soaking up' protons that are generated during CaCO$_3$ deposition (equations 5.1 - 5.3). Protons generated by the calcification process are probably discharged into the coelenteron, where a drop in pH may damage tissue and deplete the water of CO$_3^{2-}$, halting calcification. By removing CO$_2$ for photosynthesis, the coupled calcification/photosynthesis reaction is pH neutral (equation 5.1+5.2+5.3). A number of corals are found to exhibit a 1:1 ratio of calcification to photosynthesis (McConnaughey and Whelan, 1997).

\[
\begin{align*}
\text{HCO}_3^- + \text{Ca}^{2+} & \rightarrow \text{CaCO}_3 + \text{H}^+ \\
\text{[calcification]} & \quad \text{(5.1)} \\
\text{H}^+ + \text{HCO}_3^- & \rightarrow \text{CO}_2 + \text{H}_2\text{O} \\
\text{[generation of CO}_2] & \quad \text{(5.2)} \\
\text{CO}_2 + \text{H}_2\text{O} & \rightarrow \text{CH}_2\text{O} + \text{O}_2 \\
\text{[photosynthesis]} & \quad \text{(5.3)} \\
2\text{HCO}_3^- + \text{Ca}^{2+} & \rightarrow \text{CH}_2\text{O} + \text{CaCO}_3 + \text{O}_2 \\
\text{(5.1+5.2+5.3)}
\end{align*}
\]

Recent controversial research suggests that calcification may functionally precede photosynthesis in zooxanthellate corals, generating protons that liberate CO$_2$ from the
seawater HCO₃⁻ for the benefit of the symbiotic zooxanthallae (McConnaughey, 1993; McConnaughey and Whelan, 1997). This idea is taken a step further by Marshall (1996) who proposes that, rather than enhancing daytime calcification (as suggested by Goreau, 1959), zooxanthallae suppress calcification in the coral at night when protons are not required for photosynthesis.

**Enzyme Mediation in Ca²⁺ Transport**

Krishnaveni et al. (1989) demonstrated that calcification was temperature dependent, with an optimum temperature of around 22°C. Such a temperature optimum is a property that is characteristic of enzyme systems, and suggests that calcification is under enzymatic control. Strontium ions can act as a competitive inhibitor of both calcification (Chalker, 1976; Ip and Lim, 1991) and active Ca²⁺ transport across the oral epithelium (Wright and Marshall, 1991); further evidence for an enzyme mediated transport of Ca²⁺ during calcification. ⁴⁵Ca incorporation into coral skeletons follows Michaelis Menton saturation kinetics, with incorporation rates slowing to an asymptote as Ca²⁺ concentrations increase (Chalker, 1976; Krishnaveni et al., 1989). Michaelis Menton kinetics are characteristic of enzyme mediated reactions, where the maximum reaction rate is dictated by the number of enzyme particles in a system, and the rate at which they can catalyse the reaction. The asymptotic behaviour occurs as the enzymes become saturated by the substrate (in this case Ca²⁺) as concentrations increase.

**ATPases and Active Ion Transport**

A number of organisms have been observed to actively transport ions for the purpose of skeletogenesis, some of which can deposit calcium carbonate skeletons in waters that are normally undersaturated with respect to CaCO₃ (McConnaughey, 1989b; McConnaughey, 1986; McConnaughey, 1993; McConnaughey and Whelan, 1997). Corals do possess active Ca²⁺ transport mechanisms as the cells that make up coral tissues generally have a lower Ca²⁺ concentration than the seawater that surrounds the polyp (Constantz, 1986; Ip and Lim, 1991; Krishnaveni et al., 1989). In order to maintain this low intracellular calcium against the osmotic pressure from the ocean, coral cells must have a way of removing excess Ca²⁺ from the cytosol. It is therefore reasonable to propose that active transport of Ca²⁺ plays a role in coral skeletogenesis.

Energy is required to actively transport an ion against a concentration gradient. One class of enzymes known to be involved in the energy dependent transport of ions are the ATPases. These enzymes exchange cations across a membrane, using energy derived from ATP. Ca²⁺ATPase is central to the calcification models of McConnaughey (1989b; 1986), where it is hypothesised to pump Ca²⁺ across the membrane isolating the calcifying fluid, exchanging one Ca²⁺ for 2 H⁺.
Several inhibitors are known to block ATPase activity. Ruthenium Red specifically targets Ca\(^{2+}\)ATPase, and has been shown by a number of researchers to block calcification in corals (Ip and Lim, 1991; Krishnaveni et al., 1989; Marshall, 1996). The fact that Ruthenium Red inhibits both light and dark calcification in the zooxanthellate *Galaxea*, as well as calcification in the non-zooxanthellate *Tubastrea* (Marshall, 1996), is a testimony to the ubiquitous nature of Ca\(^{2+}\)ATPase in the calcification of corals. Other more general ATPase inhibitors also block calcification (Chalker, 1976; Krishnaveni et al., 1989).

Although Ca\(^{2+}\) transport is directly linked with calcification, other ion transport mechanisms are also implicated. Ouabain inhibits calcification (Krishnaveni et al., 1989; Marshall, 1996). This inhibitor targets Na\(^{+}/K^{+}\)ATPase, disrupting the normal sodium gradient across the cell membrane, suggesting the presence of a Na\(^{+}/Ca^{2+}\) exchange mechanism within the coral cells (Marshall, 1996). A range of other inhibitors also affect calcification, despite targeting other areas of coral physiology that are not obviously linked to calcification. It therefore appears as if enhanced calcification is sensitive to other unidentified aspects of coral physiology, raising the possibility that the general 'health' of the coral could affect calcification rates.

### 5.3.3 Summary

Calcification is an enzyme mediated process that appears to require the use of metabolic energy during the day, but not at night. Calcification rates are not constant through time, and are highest during the daylight hours. The presence of photosynthetic symbionts appears to be responsible for this diurnal variation, either suppressing night-time calcification rates or enhancing daytime calcification (possibly through the excretion of metabolisable photosynthetic byproducts). Ca\(^{2+}\)ATPase is clearly important in the calcification process; however, it may not be the only enzyme system involved. The physiology is complex, and several metabolic pathways are implicated, with light calcification differing from dark calcification, and calcification in zooxanthellate corals differing from non-zooxanthellate corals. Different species may also display different degrees of sensitivity to metabolic and photosynthetic inhibitors, suggesting that calcification mechanisms may vary depending on niche and taxonomy. It is also possible that the general health of the coral can influence calcification rates.

### 5.4 Basic Calcification Model

This section presents a model of coral calcification derived from the evidence and observations presented in the previous sections. McConnaughey derived a similar model
in his thesis (McConnaughey, 1986) to account for skeletal carbon and oxygen isotopes in coral skeletons, and developed it further in subsequent publications (McConnaughey, 1989a; McConnaughey, 1989b; McConnaughey, 1993; McConnaughey et al., 1997; McConnaughey and Whelan, 1997).

The waters in which zooxanthellate corals typically grow are warm, slightly alkaline, and supersaturated with respect to CaCO₃. It therefore appears that there is little need for these corals to expend significant amounts of energy to generate a supersaturated calcifying fluid from which to grow a skeleton (Constantz, 1986). In an environment already supersaturated with respect to CaCO₃, this can be as simple as providing a nucleation site for calcium carbonate, increasing the reactive surface area, or removing a crystal poison. The McConnaughey model, however, proposes that corals enhance calcification by raising the ion product of [Ca²⁺] and [CO₃²⁻]. Increasing the concentration of one or both of these species will increase CaCO₃ deposition (equation 5.4).

\[
\text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3
\]  

Figure 5.1 schematically presents the basic anatomy of a coral polyp, while figure 5.2 illustrates the 'trans' calcification mechanism proposed in McConnaughey (1993). Calcification is restricted to the underside or 'aboral' regions of the coral ectoderm. Studies of coral tissue reveal a layer of specialised cells called the calicoblastic ectoderm, which are believed to control calcification (see review in Johnston, 1980). Calcification is assumed to occur physicochemically from a thin fluid sheath surrounding the growing structural element.

A thin membrane separates the calcifying fluid from the calicoblastic ectoderm. This membrane is impermeable to ions and large molecules; however, small uncharged molecules such as CO₂ can readily diffuse through this layer into the calcifying fluid. Pores, vesicles, and/or pericellular channels in the calicoblastic ectoderm allow transport of fluids either extracellularly or excreted intercellularly (Johnston, 1980; McConnaughey, 1986).

The calcifying fluid is assumed to be close to seawater composition, transported directly and without modification from the coelenteron through the membrane by vesicles and pericellular channels. This fluid transport accounts for the bulk of the Ca²⁺ flux to the skeleton, and is consistent with the indiscriminate incorporation of small particulates, dyes and other macromolecules into the coral skeleton (McConnaughey, 1986).

According to the McConnaughey model, the coral raises the supersaturation of the calcifying fluid by increasing the pH. It does this by pumping H⁺ out of the calcifying fluid, in exchange for calcium ions in a 2:1 ratio, simultaneously raising the pH and increasing the Ca²⁺ concentration. This pumping is energetically expensive as Ca²⁺ ions
are being pumped against a diffusion gradient, and the coral must expend metabolic energy through the hydrolysis of high energy adenosine triphosphate (ATP). The enzyme responsible is Ca$^{2+}$ATPase (Krishnaveni et al., 1989), which is found in the membrane surrounding the calcifying fluid (McConnaughey, 1986).

**Basic Coral Anatomy**

![Basic Coral Anatomy](image)

Figure 5.1 Basic Coral Anatomy
Schematic cross section through a coral polyp showing tissue, coelenteron and skeleton (modified from Johnston, 1980). Corals are simple organisms, consisting of a double layered epidermis (ectoderm and gastroderm). This surrounds a fluid-filled cavity called the coelenteron which acts as a simple stomach. The aboral ectoderm is modified in adult corals and a specialised layer of cells called the calicoblastic ectoderm are responsible for secreting aragonitic skeleton. The oral ectoderm and gastroderm are infected with photosynthesising algae (zooxanthallae), which have a symbiotic relationship with the host polyp.
Basic Calcification Model

Coelenteric Fluid  Ca$^{2+}$  2HCO$_3^-$  

\[ \text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow 2\text{CO}_2 + 2\text{H}_2\text{O} \]

Calicoblastic Ectoderm

\[ 2\text{H}^+ + 2\text{HCO}_3^- \rightarrow 2\text{CO}_2 + 2\text{H}_2\text{O} \]

\[ 2\text{H}^+ + \text{CO}_3^{2-} \rightarrow \text{CO}_2 + \text{H}_2\text{O} \]

Photosynthesis: Zooxanthallae in Oral Ectoderm

Figure 5.2 'Trans' Calcification Mechanism

Basic calcification mechanism in corals (modified from McConnaughey and Whelan, 1997). In this model, corals calcify by pumping protons from the calcifying fluid, in exchange for Ca$^{2+}$ ions from the coelenteron. This raises the pH of the precipitating microenvironment, generating high CO$_3^{2-}$ concentrations, and significantly increasing the saturation state of CaCO$_3$. Protons discharged into the coelenteron react with bicarbonate, liberating CO$_2$ which is used for photosynthesis by the zooxanthallae.

Raising the pH of the calcifying fluid shifts the CO$_2$/HCO$_3^-$/CO$_3^{2-}$ equilibrium towards higher carbonate concentrations (equations 5.5 & 5.6).

\[ \text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}^+ \]  \hspace{1cm} (5.5)

\[ \text{HCO}_3^- \leftrightarrow \text{CO}_3^{2-} + \text{H}^+ \]  \hspace{1cm} (5.6)

\[ \text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{CO}_3^{2-} + 2\text{H}^+ \]  \hspace{1cm} (5.5+5.6)

Because the membrane is impermeable to ionic species, the elevated pH is maintained and does not dissipate into the surrounding tissues (McConnaughey, 1989b; McConnaughey, 1986). The membrane, however, is permeable to CO$_2$. As reactions 5.2 and 5.3 shift to the right, CO$_2$ is consumed, creating a diffusion gradient of CO$_2$ into the cell. The CO$_2$ is hydroxylated (with the aid of carbonic anhydrase), generating
more CO$_3^{2-}$ and significantly raising the carbonate concentration of the fluid. Together with the pumped Ca$^{2+}$, the increased CO$_3^{2-}$ concentration raises the state of supersaturation and hence the rate at which calcium carbonate is deposited.

Carbonate concentrations are very sensitive to pH, increasing with the square of the OH$^{-}$ concentration. Thus even small changes in pH can have a significant effect on the overall saturation state of the liquid. McConnaughey (1986) proposes that physiological pumping of H$^+$ raises the pH from seawater levels (about 8.2) to between 8.5 and 9.0, resulting in an increase in saturation of between 10 and 100 times.

The physiologically pumped Ca$^{2+}$ does not contribute much to the increased saturation state of the calcifying fluid. Seawater is naturally rich in Ca$^{2+}$, and the McConnaughey model predicts that between 89% (pH = 8.5), and 47% (pH = 9.0) of the total Ca$^{2+}$ in the calcifying fluid is carried in by fluid transfer rather than being enzymatically pumped. Seawater is also rich in dissolved inorganic carbon (DIC); however, this is largely in the form of HCO$_3^{-}$ rather than CO$_3^{2-}$. Raising the pH of the fluid results in a dramatic shift in the carbonate equilibria, and the increase in supersaturation is therefore largely a consequence of increased CO$_3^{2-}$ concentrations. The CO$_2$ that diffuses into the high pH calcifying fluid contributes significantly to the total DIC pool, and McConnaughey's model suggests that between 49% (pH = 8.5) and 89% (pH = 9.0) of the carbonate in the calcifying fluid is derived from external CO$_2$.

These budgets are rough, and probably represent underestimates, as it is assumed that the calcifying fluid is replenished constantly during the calcification process. If the fluid is not replaced periodically by the coral, then it will quickly be depleted of ions, and elevated calcification rates must be maintained by pumped Ca$^{2+}$ and diffused CO$_2$. Other unidentified physiological modifications to the composition of the calcifying fluid may also occur, or respiration and photosynthesis may change the pCO$_2$ of the calcifying tissues, affecting the diffusion of CO$_2$ into the calcifying fluid (evidence for a photosynthetic influence on the skeletal CO$_2$ can be found in the form of positive shifts in the d13C McConnaughey, 1986).

### 5.4.1 Summary

In the basic calcification model, calcification occurs physicochemically from a membrane-bound pocket of calcifying fluid. The fluid in the calcifying microenvironment is derived largely from seawater, transported from the coelenteron to the skeleton via vesicles and pericellular channels. Ca$^{2+}$ATPase enzymes in the membranes surrounding the calcifying fluid pump Ca$^{2+}$ ions into, and H$^+$ ions out of the fluid, raising the calcium concentration and the pH. The high pH results in a shift in the carbonate equilibria towards CO$_3^{2-}$, and CO$_2$ from the tissues diffuses into the fluid.
through the membrane. The increased Ca\({}\text{2+}\) and CO\({}\text{3}\text{-}\) concentration raises the supersaturation state of the calcifying fluid, enhancing the calcification rate. Between 47% and 89% of the Ca\({}\text{2+}\) ions in the calcifying fluid are derived from the seawater component, with the remainder being transported by enzyme pumping. In contrast, only 11% - 51% of the carbonate ions in the calcifying fluid are derived from the seawater component, with the bulk coming from CO\(_2\) that has diffused through the membrane.

**PART 2: FACTORS AFFECTING TRACE ELEMENT PARTITIONING INTO THE CORAL SKELETON**

### 5.5 Kinetics and Thermodynamics of Trace element Partitioning

The morphology of the aragonite crystals precipitated by a coral indicate that growth has occurred rapidly from a supersaturated solution. Before considering the physiological controls over trace element concentrations in coral skeletons, it is important to understand how the basic inorganic chemistry of rapid physicochemical calcification can influence trace element partitioning.

The discussion and formulae presented in this section are derived from basic inorganic reaction kinetics using a simple model of competitive trace element coprecipitation. The model is a simple 2 cation, 1 anion system (Ca\({}\text{2+}\), Sr\({}\text{2+}\), and CO\({}\text{3}\text{-}\)), where cations precipitate independently in simple bimolecular forward reactions. Details of this model, and the derivation of the equations are presented in appendix A.

The model is not intended to be a realistic representation of calcification kinetics, as the mechanisms of crystallisation are extremely complex (see appendix A). The equations model the substitution of a minor cation (Sr\({}\text{2+}\)) for Ca\({}\text{2+}\). Similar models could be constructed for the substitution of a minor anion for CO\(_3\text{2-}\), and the reasoning remains the same. For ions that do not substitute directly into the lattice, the partitioning behaviour is likely to be mechanism specific, and much harder to predict. Some discussion on non-lattice ion partitioning is presented in chapter 6.

The basic reactions are:

\[
\begin{align*}
\text{Ca}^{2+} + \text{CO}_3^{2-} & \rightarrow \text{CaCO}_3 & \text{rate constant } k_1 & (5.7) \\
\text{CaCO}_3 & \rightarrow \text{Ca}^{2+} + \text{CO}_3^{2-} & \text{rate constant } k_2 & (5.8) \\
\text{Sr}^{2+} + \text{CO}_3^{2-} & \rightarrow \text{SrCO}_3 & \text{rate constant } k_3 & (5.9) \\
\text{SrCO}_3 & \rightarrow \text{Sr}^{2+} + \text{CO}_3^{2-} & \text{rate constant } k_4 & (5.10)
\end{align*}
\]
5.5.1 Solution Concentration

The partitioning of a trace component (e.g. Sr$^{2+}$), and its carrier phase (Ca$^{2+}$) between solution and crystal is usually given by the following relationship:

\[
\frac{[\text{Sr}]/[\text{Ca}]}{[\text{Sr}]/[\text{Ca}]}_{\text{crystal}} = D
\]

(5.11)

where:

- $D$ = the distribution coefficient
- $[X]$ = the molar concentration of an ion in the solution or crystal

The partitioning of two competing ions into the crystal is therefore always dependent on the ratio of the two ions in the precipitating solution. In essence this is because partitioning is a competition between two reactions, the rates of which are dependent on the numbers of collisions between ions and the crystal surface (which is a function of the solution concentrations).

5.5.2 Calcification Rate

Distribution coefficients, and the factors that influence them, depend on the mechanistics of the reaction. A simple chemical reaction will pass through a number of distinct behavioural stages, defined by increasing reaction rate. At each of these stages, the partitioning of trace elements will be dependent on a different suite of physical and chemical parameters. It is possible to identify two semi-independent processes: the transition from thermodynamic (equilibrium) to kinetic partitioning of trace elements, and the transition from reaction controlled kinetics to diffusion controlled kinetics.

A crystal is at thermodynamic equilibrium when it is neither growing or dissolving, and its composition is not changing with time. This occurs when the rates of all forward and reverse reaction pairs are identical. Equilibrium growth is therefore somewhat of a contradiction in terms; however, if growth is very slow (i.e. the forward and reverse reaction rates are fast relative to the net calcification rate), then the trace element partitioning in the crystal approximates that of equilibrium.

The equilibrium distribution coefficient is given by:

\[
D_{\text{thermodynamic}} = \frac{k^3 \cdot k^2}{k^4 \cdot k^1} \left( \frac{\gamma_{\text{Sr}} \cdot f_{\text{Ca}}}{\gamma_{\text{Ca}} \cdot f_{\text{Sr}}} \right)
\]

(5.12)

where:

- $k_1$-$4$ = rate constants for reactions 5.7 to 5.10 respectively
- $\gamma_X$ = solution activity coefficient for ion $X$
\( f_X = \) solid state activity coefficient for ion X in aragonite

As the concentrations of reactants in solution rise, the rate of the forward reactions (reactions 5.7 and 5.9) begins to exceed the rate of reverse reaction, and the crystal therefore begins to grow. As the degree of supersaturation continues to rise, forward reaction rates become very large, and the reverse reaction rate becomes negligible. The limit at high reaction rates (kinetic growth) therefore becomes:

\[
D_{\text{kinetic}} = \frac{k_3}{k_1} \left( \frac{\gamma_{\text{Sr}}}{\gamma_{\text{Ca}}} \right)
\]  \hspace{1cm} (5.13)

As reaction rates become very fast, the diffusion of ions through solution starts to limit the rate at which calcification can occur. The concentration of ions at the crystal surface becomes depleted, and the reaction is transport limited. At the transport limited extreme:

\[
D_{\text{transport}} = \frac{D_{f(Sr)}}{D_{f(Ca)}}
\]  \hspace{1cm} (5.14)

where \( D_f \) is the diffusion coefficient that links the flux of an ion to the concentration gradient for that ion in solution (Fick's Law: see appendix A).

Clearly as the net calcification rate increases from zero, the partitioning of a trace element into a growing crystal will change as the reaction kinetics shift from equilibrium to kinetic to transport limiting. In reality, there may be several more stages of reaction, defined by different rate limiting steps in the more complex crystal kinetics. Simple calculations presented by McConnaughey (1989b), suggest that the calcification rate in corals is some way between kinetic and thermodynamic extremes, which would correspond to the region of the rate continuum where distribution coefficients are most sensitive to changes in calcification rate.

### 5.5.3 Temperature Dependence

The distribution coefficients can be shown to have the following form (see appendix A):

\[
D_{\text{Thermodynamic}} = a_T \cdot e^{\frac{(\Delta G_{\text{Sr}} - \Delta G_{\text{Ca}})}{RT}} \cdot \left( \frac{\gamma_{\text{Sr}^{2+}} \cdot f_{\text{Ca}^{2+}(\text{aragonite})}}{\gamma_{\text{Ca}^{2+}} \cdot f_{\text{Sr}^{2+}(\text{aragonite})}} \right)
\]  \hspace{1cm} (5.15)

and

\[
D_{\text{Kinetic}} = a_K \cdot e^{\frac{(E_{a\text{Sr}} - E_{a\text{Ca}})}{RT}} \cdot \left( \frac{\gamma_{\text{Sr}^{2+}}}{\gamma_{\text{Ca}^{2+}}} \right)
\]  \hspace{1cm} (5.16)

where:

- \( a_T \) and \( a_K \) = pre-exponential constants for thermodynamic and kinetic reactions
- \( E_{a\text{Sr}}, E_{a\text{Ca}} \) = activation energies for Sr and Ca forward reactions (5.7 and 5.11)
- \( \Delta G_{\text{Sr}}, \Delta G_{\text{Ca}} \) = free energy change for Sr and Ca precipitation reactions
Both thermodynamic and kinetic distribution coefficients are clearly temperature dependent. The magnitude of the thermodynamic temperature dependence is a function of the free energies for the Sr and Ca precipitation reactions ($\Delta G_{Sr}$ and $\Delta G_{Ca}$). These energy terms are dependent on the stability of an ion in both the solution and the crystal. These terms, however, are independent of the intermediate stage in the reaction (see appendix A for details), and the reaction mechanism will not affect partitioning or temperature dependence.

In contrast, the kinetic temperature dependence is a function of the activation energies for the precipitation reactions ($E_{a_{Sr}}$ and $E_{a_{Ca}}$ - see appendix A). These values are independent of the stability of the ion in the crystal, so under kinetic control, crystal composition no longer affects the partitioning of the trace elements. On the other hand the forward reactions are dependent on the energy of the intermediate, so the reaction pathway, and hence the reaction mechanism will play a role in controlling partitioning.

Although no equations have been derived, the transport limited distribution coefficient will also be dependent on temperature, as factors such as the speciation of an ion in solution, and viscosity of the fluid change with temperature. As with reaction rates, therefore, the temperature dependence changes as the reaction passes through different kinetic stages. Predicting the temperature dependence from an equilibrium partitioning model may not therefore be accurate.

### 5.5.4 Solution Composition

The composition of the calcifying fluid may influence trace element partitioning in several ways. The thermodynamic and kinetic distribution coefficients depend on $\gamma$, and $Ea$ or $AG$, while the transport limited distribution coefficient depends on $Df$. All of these parameters are affected by the speciation of ions in solution, which can change the apparent size, reactivity and stability of the ions. The kinetic and thermodynamic distribution coefficients are also sensitive to the reaction mechanism and the physical properties of the crystal surface. Surfactants and crystal poisons may affect the mechanism of crystallisation, while certain inclusions (e.g. Mg$^{2+}$) may influence the stability of other ions in the crystal lattice. Even if corals don't change the ionic composition of the calcifying fluid much, the presence of other species, especially organic ligands and surfactants, can significantly influence the partitioning of trace elements into the skeleton.

### 5.5.5 Summary

It is clear that the partitioning of trace elements into aragonite crystals is complex even when growing according to the well defined dictates of thermodynamic and kinetic
theory. This is especially true when precipitating from a solution with the complexity of normal seawater. Too little is known about the exact reaction mechanism and rate limiting steps to make accurate predictions about how inorganic factors might affect trace element partitioning into aragonite.

Simple models, however, predict that trace element partitioning into coral skeletons should be sensitive to the ionic composition in the calcifying fluid, the calcification rate, and the temperature. Three distinct partitioning stages are identified - thermodynamic, kinetic, and transport limited. At each stage the distribution coefficient and temperature dependence is a function of a different suite of factors.

Trace element partitioning for a thermodynamically controlled reaction is dependent on the relative stabilities of ions in the solution and crystal. As calcification rates increase, the mechanism becomes kinetically controlled, and partitioning becomes more dependent on the reaction mechanism, and less on the stability of ions in the crystal. As reaction rates get very high, ion transport controls the partitioning behaviour. The reaction mechanism ceases to become important, and the relative rates of ion diffusion control the partitioning of elements into the crystal. Trace element partitioning is also predicted to be influenced by the composition of the precipitating fluid, as ligand-molecules can affect the complexation and stability of the precipitating ions, and impurities and crystal poisons can alter the reactivity of the crystal surfaces.

Detailed modelling of aragonite calcification, using realistic models and a full range of thermodynamic and kinetic data for each ion, may help to elucidate other processes that are important for trace element partitioning. Such models are, however, beyond the scope of this thesis. Nevertheless, observations of the trace element composition of coral skeletons may shed light on a number of areas of the calcification process, at the very least by eliminating some of the many possible conflicting alternatives.

5.6 Implications of Calcification Model for Trace Element Partitioning

The inorganic theory presented above and in appendix A identifies 3 factors that may influence trace element partitioning: temperature, calcification rate, and solution composition. The effects of temperature on trace element partitioning are covered explicitly in chapter 8 (and also to some degree in chapters 6 and 7), and are not considered further here. Both calcification rate and solution composition may be influenced by the coral physiology.

According to the basic calcification model, the coral enhances calcification rates by altering the composition of the calcifying fluid. The calcifying fluid is therefore not...
**Chapter 5**

exactly seawater composition. Most trace-ions are coprecipitated with CaCO$_3$ in proportion to their ratio to Ca$^{2+}$ or to CO$_3^{2-}$ in the precipitating solution. Because Ca$^{2+}$ ions are pumped into the coral's calcifying fluid, while CO$_3^{2-}$ concentrations are raised by an influx of diffused CO$_2$, minor elements in the seawater component of the calcifying fluid will be effectively 'diluted' by these additional major ions.

Trace anions that compete with CO$_3^{2-}$ for lattice sites in the aragonite would be expected to be diluted more than trace cations competing with Ca$^{2+}$. According to McConnaughey's calculations, cations would be diluted in coralline aragonite (relative to inorganic precipitation from seawater) by factors of between 1.12 and 2.13 while anions would be diluted by factors of 1.96 to 9.09 (for pH 8.5 to 9.0). For more detail on the dilution of B and U, refer to chapter 6 and appendix B. Increasing coral calcification is accomplished by increasing the amount of ion pumping, hence pH, Ca$^{2+}$ concentrations, and CO$_2$ diffusion should all increase. Thus, increases in the calcification rate should be accompanied by greater dilution of trace elements.

Dilutions will be greater than calculated here if the coral tissue has a higher pCO$_2$ than the atmosphere, or if the seawater component of the calcifying fluid is not refreshed regularly, so that greater amounts of ion pumping are required to maintain supersaturation. The dilution will be less if the calcifying pH is less than 8.5, or if the pumping of Ca$^{2+}$ is accompanied by active transport of other ions.

### 5.7 Conceptual Model of Ion Transport

The transport of minor ions is not explicitly addressed in the McConnaughey calcification model, although the implication is that minor elements are transported incidentally in the unmodified seawater component. A review of the ion transport literature, however, reveals several other possible pathways by which trace ions might be transported to the calcifying surface. Figure 5.3 presents a conceptual model summarising these pathways. This model is almost certainly an oversimplification of the coral ion transport physiology, but provides a conceptual framework on which to base discussion.

The simplest route for trace elements into a coral skeleton is by direct deposition from seawater (see figure 5.3, pathway 1). Brown et al. (1991) describe a mechanism for iron incorporation into coral skeleton in which stress events cause the tissue to retract, exposing bare skeleton to the seawater. Iron rich compounds precipitate onto the exposed skeleton, and are subsequently calcified over once the tissue returns. This mechanism may be significant for trace elements that have a tendency to form authigenic
overgrowths such as iron and manganese, or for elements that adsorb strongly to surfaces such as lead and rare earth elements.

5.7.1 Transport of ions into the Coelenteron

While some skeletal components may be transported directly from seawater or an intercellular source, it is the general consensus of the calcification community that the penultimate source of fluids and/or skeletal components is the coelenteron (e.g. Constantz, 1986). The coelenteric fluid is likely to be mostly seawater ingested by the coral (pathway 3). Calcification will deplete this fluid of Ca\textsuperscript{2+}, and Wright & Marshall (1991) suggest that coelenteric fluid would need to be recharged with seawater between 10 and 50 times an hour in order to sustain daytime calcification rates. The fluid in the coelenteron is recharged when the coral polyp's mouth is opened to ingest food; however, calcification is maximal during the day when most polyps are closed. It can therefore be argued that ionic recharge of the coelenteric fluid must be occurring by ion transport across the oral epithelia (pathways 2 and 5) (Bénazet-Tambutté et al., 1996; Wright and Marshall, 1991).

Wright and Marshall (1991) suggest that calcium is actively pumped across the oral epithelia of scleractinian corals and into the coelenteron (pathway 2). In contrast, research by Bénazet-Tambutté et al. (1996) shows that the oral epithelia from two different cnidarian genera are freely permeable to small ions from the surrounding seawater (pathway 5). These later experiments were not carried out on corals, however, and it is possible that different genera and species have different mechanisms for recharging the coelenteron.

If Ca\textsuperscript{2+} is actively pumped across the oral epithelium, and if the ion pump displays a specificity for one ion over another, then the composition of the coelenteric fluid may be under some degree of biological control. On the other hand, if the epithelia is 'leaky' and diffusion is free, then the ionic composition of the coelenteron may be buffered by the surrounding oceanic composition. Although Bénazet-Tambutté et al. (1996) found no evidence for active transport of Ca\textsuperscript{2+}, they did report that epithelial diffusion was not equal for all chemical species, and while small ions and molecules passed freely to and from the epithelium, larger molecules such as low molecular weight amino acids were not transmitted. Thus, in either case it is possible that the daytime composition of the fluid in the coelenteron may not be identical to the composition of the surrounding seawater. The system will be 'flushed out' any time the coral polyp opens its mouth and ingests fresh seawater; however, during extended intervals without seawater recharge, the composition of the coelenteric fluid may drift.
Figure 5.3 Conceptual Model of Ion Transport pathways
Conceptual model detailing the major pathways for ion transport from the ocean to the coral skeleton. This model assumes three pools of ions: the seawater, the coelenteron, and the calcifying fluid. 1) direct incorporation into the skeleton when tissue retracts during stress events. 2) active pumping of ions across oral epithelium. 3) ingestion of seawater. 4) ingestion of particulates and organic material. 5) passive (diffusive) transport across the oral epithelium. 6) fluid transport from coelenteron to skeleton. 7) active transport to skeleton. 8) diffusive transport to skeleton (e.g. CO₂).
Another possible source of trace elements to the coelenteron is from digesting food (pathway 4). Corals ingest particulate material including sediment and organic detritus. These ingested particulates may be significantly enriched in some trace elements (e.g. barium, which can be highly concentrated in some tissues, or REEs which are adsorbed to organic coatings on particulate surfaces). If these elements are released during digestion, then they could significantly affect the chemical composition of the small isolated volume of fluid in the coelenteron.

5.7.2 Transport of ions to the Calcifying Surface

Transport of ions from the coelenteron to the skeletal surface may occur through active pumping by enzymes bound to the calicoblastic membrane (pathway 7), or through fluid transport via vesicles or pericellular channels (pathway 6) (e.g. Constantz, 1986; Hayes and Goreau, 1977; Johnston, 1980; Lowenstam and Weiner, 1989; McConnaughey, 1989b; McConnaughey, 1986). Active transport of ions from the coelenteron to the calcifying site is almost certainly occurring. It is entirely possible that both processes are occurring together, with active modification of the coelenteric fluid occurring during fluid transport from to the calcifying space.

Chalker (1976) proposes that transport is active across the calicoblastic plasmalemma membrane. This is similar to McConnaughey's (1986) ideas of active Ca\(^{2+}\) pumping across the membrane that isolates the calcifying fluid, facilitated by membrane-bound ATPase. Johnston (1980), and Hayes and Goreau (1977) suggest that Ca\(^{2+}\) transport occurs across the membrane of intracellular vesicles.

5.7.3 Balance of Different Ion Transport Paths

Of the pathways presented in figure 5.3, fluid transport (pathways 2 and 7) is the most indiscriminate, and does not fractionate ions relative to the source. The trace element composition of coral aragonite is similar to inorganic aragonite precipitated from seawater (see chapter 6), indicating that relatively little discrimination is occurring during transport. It therefore appears that fluid transport from an unfractionated coelenteron to the calcifying environment is a significant mechanism for the delivery of ions to the skeleton.

None of the pathways described are exclusive of any of the others, and it is highly likely that several operate in concert. Which pathway is dominant may depend on species, time, physiology, trace element, and environment. The biggest change is likely to occur for night vs. day calcification. During the day the coral polyp is closed and fluid recharge to the coelenteron is therefore minimal. Daytime calcification is clearly energy dependent and linked to the photosynthetic activity of the zooxanthallae. Active ion
transport pathways (pathways 3, 4, and 6) may therefore dominate over fluid transport during the day. At the same time, digesting food may be modifying the composition of the coelenteric fluid. At night, when the polyp is open and photosynthetic activity is minimal, the coelenteron is flushed with ingested seawater, and fluid transport mechanisms may dominate over energetically demanding active transport.

While some corals derive almost all of their metabolic energy from photosynthesis by their algal symbionts, others have a greater dependence on ingested food (the extreme example being non-zooxanthellate corals that do not photosynthesise at all). Therefore another factor that may affect the balance of ion transport pathways is the availability of food. When food is abundant, active ion transport mechanisms may be relatively more important in the ion transport budget. The sensitivity of different corals to food availability will depend on their balance of ingested vs. photosynthesised energy.

A study by Chalker (1976) illustrates the complexity of the ion transport physiology. Chalker examined the kinetics of Ca$^{2+}$ transport in *Acropora cervicornis*, and *Acropora formosa*. He observed Michaelis-Menton enzyme kinetics for both light and dark calcification, as well as calcification that had been poisoned by the photosynthetic inhibitor DCMU. In each case the half-saturation constant ($K_m =$ the Ca$^{2+}$ concentration at which calcification rates reach half of the saturated maximum Voet and Voet, 1990) was found to be identical, despite very different calcification rates, and a clear demonstration that light and dark calcification proceed by significantly different pathways (light calcification was poisoned by a range of different metabolic inhibitors, while dark calcification was not). This result appears to be contradictory: the identical $K_m$s suggest that the same enzyme mediates both calcification mechanisms, yet daytime calcification is energy dependent, while night time calcification is not, and different metabolic pathways are clearly involved.

It is possible to construct a model in which the same enzyme mediates both light and dark calcification. Calcification during the day proceeds by active pumping of Ca$^{2+}$ to the skeleton from the coelenteron. Because the polyps are shut, the coelenteron is recharged with calcium ions by an enzyme mediated passive diffusion across the oral epithelium. The same enzyme also mediates a passive diffusion of Ca$^{2+}$ from the coelenteron to the skeleton, which provides a low level of calcification that is independent of metabolic energy. At night, the active transport of Ca$^{2+}$ to the skeleton stops, but the low rate of energy-independent calcification continues.

This is just one interpretation of the physiology and in reality ion transport is probably more complex (for example, transport of ions across the oral epithelium may be active rather than passive, several transport mechanisms may be operating, and different species may have different biochemistry).
5.7.4 Implications for Trace Element Partitioning

**Ca\(^{2+}\) Transport**

As described above, \(^{45}\text{Ca}\) incorporation into the coral skeleton displays Michaelis-Menten enzyme kinetics. The \(K_m\) for the reaction was around 4 mM \(\text{Ca}^{2+}\). This compares with typical seawater concentrations of 10 mM (Bruland, 1983), suggesting that under normal conditions, enzyme mediated calcium transport is approaching maximum, and the enzyme system is beginning to show saturation. This means that calcium transport and calcification are not going to be linearly related to the calcium concentration in the water (or coelenteron), and factors such as rainfall, evaporation, or river floods that change the dilution of seawater may not produce a proportional change in the calcium concentration at the calcifying surface. How nonlinear this effect is will depend on the balance of enzyme to fluid transport for \(\text{Ca}^{2+}\). McConnaughey's models suggest that up to 90% of all \(\text{Ca}^{2+}\) in the skeleton is derived from fluid transport, and therefore nonlinearity in the enzyme transport of \(\text{Ca}^{2+}\) may have a relatively minor impact on the overall \(\text{Ca}^{2+}\) concentration in the calcifying fluid.

Not all elements will share the \(\text{Ca}^{2+}\) enzymatic transport pathway (with most probably transported via indiscriminate fluid transport), and may not experience the same nonlinearity. Because trace elements are incorporated into the coral in proportion to their ratio to calcium, however, nonlinearity in the \(\text{Ca}^{2+}\) behaviour will translate as nonlinearity in all of the TE/Ca ratios.

**Other Ions**

The trace element behaviour predicted for the basic calcification model (section 5.4) assumes that calcium ions are pumped while all other ions are transported without fractionation by fluid transport from the seawater. In reality the transport of minor ions to the calcifying surface may be partially or wholly mediated by enzyme systems which may or may not be the same as those responsible for calcium transport. The critical questions to ask when considering trace element incorporation into the coral skeleton are therefore "is a trace ion transported by the same mechanism as calcium?" and "is a trace ion transported without fractionation from seawater?".

While the mechanisms and physiology of \(\text{Ca}^{2+}\) transport during calcification has received a lot of attention, there have been few experimental studies of the manner in which minor elements are transported to the skeleton. Of the other ions, the most attention has been focused on \(\text{Sr}^{2+}\). The chemical properties of strontium are very similar to calcium, and strontium has been observed to be a competitive inhibitor of calcium transport in the coral (Chalker and Taylor, 1975; Ip and Krishnaveni, 1991; Wright and Marshall, 1991). This competitive inhibition is usually taken as an indication
that Sr$^{2+}$ is an acceptable alternative substrate for Ca$^{2+}$ in the calcium transport enzymes, and that both ions are therefore transported by the same mechanism (see reviews in Ip and Krishnaveni, 1991; Ip and Lim, 1991). Chalker (1976), however, warned that the specificity of the Ca$^{2+}$ transport enzymes for Sr and Ca may differ, which would lead to discrimination in their transport. Recent studies by Ip and Krishnaveni (1991) also find that Sr$^{2+}$ exhibits mixed rather than pure inhibition behaviour, suggesting that the mechanistics of Sr$^{2+}$ interaction with the Ca$^{2+}$ transport enzyme is not as simple as earlier thought (see also Chalker, 1976).

The recent work by Ip and Krishnaveni (1991), and Ip and Lim (1991) indicates that the cellular transport of Sr$^{2+}$ is significantly different to Ca$^{2+}$ transport mechanism. Incorporation of $^{90}$Sr into coral skeletons is linearly dependent on Sr$^{2+}$ concentration up to 20 mM, and is not affected by KCN (a respiratory inhibitor), darkness, or the photosynthetic inhibitor DCMU. In contrast, Ca displays Michaelis Menton saturation kinetics, and is inhibited by darkness, DCMU, and KCN (Ip and Krishnaveni, 1991). In extracts of tissue from Galaxea fascicularis, Sr$^{2+}$ failed to activate Ca$^{2+}$ATPase leading to the conclusion that the enzyme could discriminate between calcium and strontium (Ip and Lim, 1991). No Sr$^{2+}$ATPase could be detected in the tissue.

These significant differences in the transport behaviour of Sr and Ca have major implications for the control that physiology or environment may exert over the relative transport of Sr and Ca. If Ca$^{2+}$ transport is energy dependent, but Sr$^{2+}$ transport is not, then anything that influences the energy budget of the coral will result in a change in the relative rates at which the two ions are transported to the calcifying fluid. Thus changes in light, nutrients, food, growth rate, symbiosis, and availability of metabolic energy may result in changes in the Sr/Ca ratio in the coral skeleton. Another implication of these different pathways is that the Ca$^{2+}$ transport is enzyme mediated and close to saturation, hence changes in the Ca$^{2+}$ concentration in the seawater will have relatively little effect on transport rate. In contrast, the Sr$^{2+}$ transport is linear with concentration. Thus any conservative change in the dilution of seawater (e.g. evaporation, rainfall, or flood plumes) will still result in a change in the Sr/Ca ratio.

Information on the physiological transport of other trace elements is scarce. It is generally accepted that Ca$^{2+}$ATPase mediated ionic transport in cells is relatively indiscriminate (Ip and Lim, 1991). This would suggest that even if active transport of ions was occurring across the oral epithelia into the coelenteron, or from the coelenteron to the calcifying fluid, the ionic ratios in both would remain relatively close to seawater. On the other hand, Ip and Lim (1991) concluded that active transport in the coral was an exception to this generalisation (see above), and the selectivity shown for strontium would suggest that this transport path could be highly selective against ions with different physicochemical characteristics to Ca$^{2+}$.
Even the experiments of Bénazet-Tambutte et al. (1996), which suggested free diffusion of ions across the oral epithelia, found that the diffusional process was selective on the basis of size, and favoured anion diffusion. If diffusion across the epithelia was limiting (or close to limiting) calcification, then the composition of the coelenteric pool would to some degree reflect the rates at which different ions diffuse across the epithelium. A simple calculation suggested that the trans-epithelial flux was similar in magnitude to the rate at which a coral calcified (Bénazet-Tambutte et al., 1996). While they tentatively concluded that epithelial flux of Ca\(^{2+}\) was not rate limiting, the magnitude of the diffusive and calcification fluxes were sufficiently similar that diffusional modification to the composition of the coelenteric fluid should not be disregarded.

It is possible that specific active transport mechanisms exist for ions other than Ca\(^{2+}\). While it was demonstrated that Sr\(^{2+}\) has no active transport mechanism (Ip and Krishnaveni, 1991; Ip and Lim, 1991), evidence of MnATPase activity was detected in extracts of coral tissue (Ip and Lim, 1991). During calcification, it is the elevated pH, and the consequent increase in CO\(_3^{2-}\) ion concentration that is mostly responsible for elevated supersaturations in the calcifying fluid. If McConnaughey's later theories are correct (1993; 1997), active transport is more geared up to pumping protons out of the calcifying fluid than pumping Ca\(^{2+}\) ions in. In this case, any other ion-pump systems (e.g. Na\(^+\)/K\(^+\)ATPases, Mn\(^{2+}\)ATPases, and Mg\(^{2+}\)ATPases) that exchange protons for cations would be advantageous to the coral, and the calcifying fluid may become charged with a lot of surplus cations that the polyp sacrifices in order to generate acidity for photosynthetic CO\(_2\) uptake.

5.7.5 Summary

A simple conceptual model of ion transport involves three reservoirs of ions: the ocean, the coelenteron and the small pocket of calcifying fluid. Ions may be fractionated during transport between these reservoirs. Ion transport to the skeleton is likely to be via several different pathways at once (summarised in figure 5.3). The contribution of each different pathway to the overall ion transport budget may be different between different taxa, and may change with time depending on the environment, nutritional status and health of the coral. The major changes in pathway are likely to occur between day and night, as the polyp is open and ingesting food and water during the night, while it is closed and photosynthesising during the day.

The most indiscriminate form of transport is via fluids, including the ingestion of seawater into the coelenteron, and the transport via vacuoles and pericellular channels from the coelenteron to the calcifying fluid. The indiscriminate incorporation of a number of ions (and other chemical species), and the similarity between coralline and
inorganic aragonite implies that these pathways are significant in the overall transport of ions from the ocean to the skeleton.

Enzyme mediated diffusion, and active pumping are the other major ion-transport mechanisms. These mechanisms are likely to be significant for Ca^{2+} transport, and may be involved in the transport of other ions as well (e.g. Sr^{2+}). It is likely that these transport paths are not indiscriminate, and fractionation of other ions relative to calcium would be predicted. Physiological pumping of Ca^{2+} would be expected to dilute cations with no active transport mechanism by between 11% - 50% in the calcifying fluid relative to seawater. This dilution would be less for any ions that have an active transport mechanism, but could be greater if the coelenteric fluid is recharged by Ca^{2+}-selective active ion transport, rather than by ingestion of more seawater. Greater dilutions would be expected for higher calcification rates.

It is possible that coralline TE/Ca ratios may be sensitive to salinity if the coral physiologically buffers the Ca^{2+} or CO_3^{2-} concentration in the calcifying fluid to maintain a particular calcium carbonate saturation state. Min et al. (1995) proposed this idea to explain the lack of observed variation in the U/Ca in corals growing in waters of different carbonate concentration. If the principal pathway for transport of a TE to the precipitating microenvironment is fluid transfer from seawater, then it might be expected that the activity of the TE in the calcifying fluid would fluctuate along with salinity. If the pH (and hence the carbonate concentration) in the calcifying fluid is static (physiologically buffered), the TE/Ca^{2+} or TE/CO_3^{2-} ratio of the precipitating fluid will decrease, and the biologically precipitated aragonite would display a TE/Ca ratio that is directly proportional to salinity.

Thus all species substituting for anions or cations in the aragonite should have their ratios to calcium decrease in direct proportion to any drop in salinity. This has never been reported for strontium, despite several studies examining Sr/Ca ratios in corals experiencing large river floods (Gagan et al., 1994; McCulloch et al., 1994). This might suggest that cellular transport mechanisms transport all cations with equal efficiency, effectively eliminating any effects of seawater concentration changes.

5.8 Skeletal Growth and Structure

It is important to understand the structure and growth of coral skeleton when considering the distribution of trace elements on fine spatial scales, and the way that environmental signals may be distorted when being recorded by the coral. The following is a summary of the major ideas regarding coral skeletal growth presented by Barnes and coworkers.
Coral Calcification

(Barnes, 1970; Barnes and Lough, 1993; Barnes et al., 1995; Taylor et al., 1993; Taylor et al., 1995).

The skeletons of *Porites* corals are formed mostly from vertical spines. The tops of these spines do not form a horizontal surface, but rather each coral polyp sits in a U-shaped depression called a calyx. Skeletal growth is controlled by the tissue, and extension is initiated by the uplift of the tissue surrounding the growing spine to create a 'pocket' of fluid into which the structural element can extend (Barnes, 1970). This tissue uplift appears to occur primarily during the early evening (Vago et al., 1997), and extension continues until the growing crystals encounter the top of the tissue pocket. The tips of the extending spines consist of fine 'fingers' of aragonite, which are eventually filled in during subsequent thickening. Although extension stops during the day, the structural element continues to calcify, resulting in thickening of the spine. Occasionally some of the 'fingers' continues to grow out laterally, eventually forming one of the cross-struts or 'synapticulae' (Barnes, 1970).

Staining experiments reveal that some degree of calcification continues throughout the thickness of the coral's tissue layer, which can occupy up to 9 mm depth (possibly representing more than 9 months growth in slow growing corals). Septal spines and synapticulae become visibly more robust as this thickening progresses (Barnes and Lough, 1993). Some estimates suggest that thickening through the tissue layer is responsible for up to 50% of the total aragonite deposited.

Every month or so the bottom of the tissue layer of a polyp 'uplifts' a short distance to keep pace with the skeletal extension, and deposits a new dissepiment. Dissepiments appear to form almost 'instantaneously', and are not thickened by tissue-layer smoothing. Because *Porites* is a perforate coral genus, with the tissue from adjacent polyps being in intimate contact, tissue uplift is co-ordinated across the entire colony (Barnes and Lough, 1993; Wells, 1969).

**Implications for Trace Element Distribution**

The trace element composition of aragonite deposited by a coral varies with time in response to a range of chemical, biological and physical factors (e.g. temperature), on a range of timescales (diurnal to yearly). Because new layers of aragonite can be deposited over old, and the growth surface of a coral is not uniform, the trace element distribution throughout the skeleton could potentially be very complex.

The initial extension of coral structural elements appears to occur during the night, while the initial thickening occurs during the day. As described in section 5.7.3 the variation in calcification mechanism and trace element partitioning can vary significantly from day to night. Thus the initial deposit may be quite heterogeneous with respect to the fine scale
distribution of trace elements. The presence of fine 'fingers' on the end of the structural element will add to this heterogeneity.

The thickening that occurs throughout the tissue layer will progressively add layers of aragonite over the initially deposited material. This overgrowth can continue for more than 6 months, during which time the trace element composition of the deposited aragonite could change significantly in response to seasonal changes to environmental parameters (Barnes and Lough, 1993; Barnes et al., 1995; Taylor et al., 1993; Taylor et al., 1995). The sheath of overgrown aragonite will appear to be a smoothed average of the 6 months, although if the spatial resolution of an analytical technique is sufficiently high, it may be possible to resolve the changing composition of this thickened layer.

This smoothing may affect included trace element records (Barnes et al., 1995; Taylor et al., 1995). The shape of seasonal signals may be distorted, and their timing shifted slightly relative to the growth surface. The most significant effect, however, is the reduction in seasonal amplitude caused by the averaging of the thickening deposit (Barnes et al., 1995). Short sharp trace element signals, such as river floods, will not be distorted to the same degree. Their timing will remain faithful relative to the growth surface of the coral (but not necessarily to any seasonal trace element signals), although their shape and amplitude may be distorted by the thickening (Taylor et al., 1995).

Skeletal growth models, and their implications for high spatial resolution trace element signals are explored further in chapter 7.
CHAPTER 6: LITERATURE REVIEW: TRACE ELEMENTS IN CORALS

6.1 Introduction

Over the last 30 - 40 years there has arisen a considerable body of literature reporting the measurement of a wide range of trace elements in coral skeletons. Many authors have attempted to correlate these trace element signals with environmental or biological parameters; however, the literature is characterised by conflicting observations and ambiguity. Many authors have also attempted to provide mechanistic details on the partitioning of trace elements into coralline aragonite, and again these have generally failed to offer any clarity. Both environmental and mechanistic explanations are clearly hampered by the poorly understood physiological and inorganic basis for trace element partitioning (see chapter 5).

This chapter summarises the many observations made of the 5 major trace elements (B, Mg, Sr, Ba and U) in corals, the proposed mechanisms for their coprecipitation, and the environmental, chemical, and biological parameters that affect their partitioning into coralline aragonite. These observations are measured against current calcification models, to identify consistency and contradictions. It is hoped that this work will provoke critical examination of the current calcification theories, and provide a basis on which to build more consistent and reliable models of trace element partitioning into coral skeleton.

6.2 Strontium

6.2.1 Mechanism of Incorporation

Lattice Substitution

Strontium is arguably the best characterised trace ion coprecipitated into coralline aragonite. Sr$^{2+}$ is chemically very similar to Ca$^{2+}$, being close in size and electronic structure. The ionic radius of Sr$^{2+}$ is around 1.31 Å compared with 1.18 Å for Ca$^{2+}$,
and strontium can form an orthorhombic carbonate (Speer, 1983). Sr therefore readily substitutes for calcium in aragonite, to form a solid solution (Speer, 1983; Veizer, 1983). Amiel et al. (1973a) studied the phase in which Sr$^{2+}$ is incorporated into coralline aragonite and concluded that it is entirely lattice bound, except for a minor proportion (< 7%) present as organic material. Greegor (1997), however, identified domains of pure strontianite in corals. Although the technique gives no indication of the size of the domains, this may be evidence for a break-down in solid solution partitioning at high calcification rates. In this situation, supersaturations may lead to the precipitation of SrCO$_3$ as a phase in its own right rather than a trace phase coprecipitated with CaCO$_3$.

**Biogenic vs. Inorganic Aragonite**

Early research suggested that corals partition Sr into their skeleton in the same ratio as inorganic aragonite (as reviewed in Swart, 1981). Coralline aragonite, however, appears to be slightly depleted relative to inorganic values (Alibert and McCulloch, 1997; Beck et al., 1992; Beck et al., 1994; de Villiers et al., 1994; Shen et al., 1996; Swart, 1981). The initial confusion derives from the differences between inorganic and organic D$_{Sr}$ being small (approximately 1.10 for Porites, compared with the inorganic value of around 1.15 McCulloch et al., 1994 and see also table 6.1), and the fact that the magnitude of the inorganic temperature dependence found by Kinsman and Holland (1969) is similar to that found in coral (see summary in chapter 8, section 8.3.1).

**6.2.2 Factors Affecting Partitioning**

**Biological Factors**

The Sr depletions relative to inorganic aragonite clearly indicate that corals are not calcifying in equilibrium with seawater, and that biological enhancement of the calcification rate results in a discrimination against strontium in the skeleton. From the review of coral physiology (see section 5.3) and inorganic crystallisation processes (see section 5.5) it is possible for this to occur in several ways. Firstly, the enhanced calcification rate may cause a shift from equilibrium to some type of kinetic or transport controlled partitioning. Alternatively, the enzyme-mediated transport processes occurring in the coral could actively enhance the Ca$^{2+}$ concentration in the calcifying fluid, resulting in a 'dilution' of Sr relative to seawater.

The depletions observed for Sr in coral aragonite (around 5%) are smaller than would be expected, however. According to McConnaughey's models of calcification (section 5.6), cations should experience dilutions of between 11% and 53% for a pH of 8.5 - 9.0 in the calcifying fluid (McConnaughey, 1986). The low dilutions of Sr in corals may indicate that the pH of the calcifying fluid is not as high as predicted. Normal seawater
Trace Elements in Corals

has a pH of around pH 8.2, and a calcifying pH of below 8.5 may not generate sufficient supersaturation to account for the high precipitation rates observed in corals (table 5.1). Corals may therefore have other mechanisms for enhancing calcification, such as removing crystal poisons from the calcifying fluid (section 5.3.2).

Table 6.1 Distribution Coefficients in Coral and Inorganic Aragonite

<table>
<thead>
<tr>
<th>Element</th>
<th>D</th>
<th>Range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>0.0114</td>
<td>0.0092 - 0.0189</td>
<td>Davies Coral[1]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vengosh (1990)</td>
</tr>
<tr>
<td>Mg</td>
<td>0.89 x 10^{-3}</td>
<td>0.81 x 10^{-3}</td>
<td>Buddemeier et al. (1981), hermatypic</td>
</tr>
<tr>
<td></td>
<td>1.4 x 10^{-3}</td>
<td>0.7 - 1.8 x 10^{-3}</td>
<td>Flor and Moore (1977)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.64 - 0.82 x 10^{-3}</td>
<td>Meece and Benninger (1993)</td>
</tr>
<tr>
<td>Sr</td>
<td>1.0</td>
<td>1.1</td>
<td>Buddemeier et al. (1981), hermatypic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.1</td>
<td>Buddemeier et al. (1981), ahermatypic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.1</td>
<td>McCulloch et al. (1994)</td>
</tr>
<tr>
<td>Ba</td>
<td>1.1</td>
<td>1.4</td>
<td>Buddemeier et al. (1981), hermatypic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.27</td>
<td>Lea et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>1.24 - 1.30</td>
<td></td>
<td>Flor and Moore (1977)</td>
</tr>
<tr>
<td></td>
<td>0.95 ± 0.32</td>
<td>0.51 - 1.9</td>
<td>Swart and Hubbard (1982)</td>
</tr>
<tr>
<td></td>
<td>0.77 - 1.02</td>
<td></td>
<td>Chen et al. (1996)</td>
</tr>
<tr>
<td>U</td>
<td>1.035</td>
<td>0.857 - 1.317</td>
<td>Flor and Moore (1977)</td>
</tr>
<tr>
<td></td>
<td>0.95 ± 0.32</td>
<td>0.51 - 1.9</td>
<td>Swart and Hubbard (1982)</td>
</tr>
<tr>
<td></td>
<td>0.77 - 1.02</td>
<td></td>
<td>Chen et al. (1996)</td>
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</tbody>
</table>

Inorganic Distribution Coefficients

<table>
<thead>
<tr>
<th>Element</th>
<th>D</th>
<th>Range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>0.0011</td>
<td>1 - 15 x 10^{-3}</td>
<td>Kitano et al. (1978) experimental[1]</td>
</tr>
<tr>
<td></td>
<td>0.0177</td>
<td></td>
<td>Hemming et al. (1995b) experimental[1]</td>
</tr>
<tr>
<td></td>
<td>0.0047</td>
<td></td>
<td>Vengosh (1990) deep sea sediments[1]</td>
</tr>
<tr>
<td>Mg</td>
<td>0.16 x 10^{-3}</td>
<td></td>
<td>Kinsman (1970)</td>
</tr>
<tr>
<td></td>
<td>0.16 x 10^{-3}</td>
<td></td>
<td>Oomori et al. (1987)</td>
</tr>
<tr>
<td>Sr</td>
<td>1.15</td>
<td>1.1</td>
<td>Kinsman (1970)</td>
</tr>
<tr>
<td></td>
<td>0.9 - 1.12</td>
<td></td>
<td>Kitano et al. (1971)</td>
</tr>
<tr>
<td></td>
<td>0.9 - 1.12</td>
<td></td>
<td>Veizer (1983)</td>
</tr>
<tr>
<td>Ba</td>
<td>1.0</td>
<td>1.0 - 1.8</td>
<td>Kitano et al. (1971)</td>
</tr>
<tr>
<td></td>
<td>1.8 - 9.8</td>
<td></td>
<td>Veizer (1983)</td>
</tr>
<tr>
<td>U</td>
<td>0.3 - 1.12</td>
<td></td>
<td>Meece and Benninger (1993)</td>
</tr>
</tbody>
</table>

[1] Calculated from concentrations using B in seawater = 4.60 ppm (Alibert 1997, unpublished data), Ca in seawater = 10.3 mmol/Kg (Bruland, 1983)
Alternatively, the pCO₂ in tissues may be higher than the levels modelled (McConnaughey assumes atmospheric CO₂ levels), allowing lower pHs in the calcifying fluid to maintain high CO₃²⁻ concentrations. This would be possible if respiration rates were high, photosynthetic activity low, and/or acidity generated by calcification produced a shift in the HCO₃⁻/CO₂ equilibrium. Alternatively, Sr²⁺ may have some active transport mechanism (possibly sharing the Ca²⁺ transport pathway (McConnaughey, 1986)), thereby lowering the dilution by physiologically pumped Ca²+. There are also uncertainties associated with how representative the Kinsman and Holland (1969) distribution coefficients are of true inorganic equilibrium, and whether this is an appropriate value with which to compare coral strontium partitioning (McConnaughey, 1986; Morse and Bender, 1990).

The use of corals as reliable environmental proxies hinges on the assumption that the disequilibrium behaviour is constant between all corals. Some researchers report that the magnitude of the offset between biological and inorganic partitioning is not significantly different between genera and species of coral (Alibert and McCulloch, 1997; Shen et al., 1996; Swart, 1981). In contrast, a large amount of evidence exists to suggest that different genera, species and even individual corals may have differing physiological influences over the Sr partitioning (e.g. Cross and Cross, 1983; de Villiers et al., 1995; de Villiers et al., 1994; Schneider and Smith, 1982; Thompson and Livingston, 1970). This is most dramatically demonstrated by de Villiers et al. (1995) who observe Sr/Ca temperature offsets of 2 - 3°C between corals of the same species growing 20 m apart.

Temperature

Inorganic Temperature Dependence

The partitioning of Sr into coralline aragonite inversely tracks the temperature of the water in which the coral grows, and Sr/Ca has long been recognised as a potential paleothermometer (Smith et al., 1979). Early investigations were restricted to low precision analyses of bulk samples. Because the magnitude of Sr/Ca temperature dependence is small (Kinsman and Holland, 1969), there was initial confusion as to whether Sr displayed any temperature dependence (Thompson and Livingston, 1970). Recent developments in instrumentation, however, allow extremely high precision analysis of very small samples, making accurate subannual analysis of corals possible. Sr/Ca ratios have now been clearly demonstrated to display seasonal cycles, varying in antiphase with sea surface temperature (Alibert and McCulloch, 1997 and many others; Beck et al., 1992; Beck et al., 1994; de Villiers et al., 1994; McCulloch et al., 1994; Shen et al., 1996).

While it is plausible that Sr substitutes for Ca in aragonite, the mechanism responsible for its temperature dependence remains uncertain. Many researchers treat Sr/Ca as a
Trace Elements in Corals

'black box', and there is surprisingly little published on the thermodynamic and kinetic controls on Sr partitioning into aragonite. Recent investigations have concluded that there is still much that is not understood (de Villiers et al., 1995; de Villiers et al., 1994; Hart and Cohen, 1996; Hart et al., 1997). A general discussion of some of the factors affecting temperature dependence is given in appendix A (section A.6).

Pioneering work in this area was done by Kinsman and Holland (1969) who precipitated crystals of calcite and aragonite slowly from solution under a range of temperatures to study the temperature dependence of the Sr/Ca ratio. They found that the distribution coefficient for Sr in aragonite dropped from around 1.17 at 16°C to 0.88 at 80°C. Assuming equilibrium between the solution and the crystal surface (since solid-state diffusion is too slow to allow equilibration within the crystal) the distribution coefficient for Sr in aragonite is given by equation 6.1, which is similar to equation A17 derived in appendix A (Kinsman and Holland, 1969 quoting Holland et al. 1963).

\[
D_{Sr} = \frac{\gamma_{CaCO_3} \gamma_{Sr^{2+}}}{\gamma_{SrCO_3} \gamma_{Ca^{2+}}} \frac{K^{A}}{K^{S}}
\]

(6.1)

where:

\(0K^{A, S} = \) Solubility product for aragonite and strontianite respectively

\(\gamma_{CaCO_3, SrCO_3} = \) Activity coefficients for CaCO₃ and SrCO₃ in aragonite

\(\gamma_{Ca^{2+}, Sr^{2+}} = \) Activity coefficients for Ca²⁺ and Sr²⁺ in solution

In this expression, D depends on the ratio of aragonite to strontianite solubility, and the activity coefficients of Sr and Ca in aqueous and solid solution. The activity ratio of Sr and Ca in solution remains relatively constant with temperature, and the activity of Ca in aragonite is constant (because coralline aragonite is nearly pure CaCO₃). As temperature rises, the solubility of strontianite increases more than that of aragonite (because of a larger free energy change associated with strontianite deposition). The ratio of \(K^{A}/K^{S}\), therefore, decreases with increasing temperature. The activity of Sr in aragonite decreases with temperature, in response to the increasing tolerance of a crystal for 'foreign' ions, and the ratio of \(\gamma_{CaCO_3}/\gamma_{SrCO_3}\) consequently increases. Thus, Kinsman and Holland (1969) conclude the negative temperature dependence for \(D_{Sr}\) is a balance between the decreasing \(K^{A}/K^{S}\) and the increasing \(\gamma_{CaCO_3}/\gamma_{SrCO_3}\) with temperature.

The assumption of a constant ratio of Sr²⁺ and Ca²⁺ solution activities with temperature does not necessarily hold. Differences between the \(D_{Sr}\) obtained from sulphate-free seawater and normal seawater suggest that the sulphate speciation of Sr and Ca can play a significant role in the behaviour of the two ions (Kinsman and Holland, 1969). Swart (1981 and references cited therein) noted that the complexation of an ion is likely to increase with increasing temperature, causing a negative temperature dependence in the Sr/Ca solution activities.
The model used by Kinsman and Holland (1969) assumes equilibrium between the solution and the surface of the aragonite crystal. Equilibrium partitioning in corals is unlikely, however, because corals calcify many times faster than the inorganic precipitation experiments (de Villiers et al., 1994; Swart, 1981). Swart (1981) offers two alternative mechanisms for temperature dependent Sr incorporation into biogenic aragonite: temperature induced changes in the growth rate (i.e., the degree of disequilibrium - see appendix A), or temperature dependent changes to speciation (as mentioned above). Changing the relative amounts of free ion in solution, and speciation, may affect the relative rates of biological transport.

**Biological Temperature Dependence**

Although corals may actively modify the composition of their calcifying fluid, temperature may control Sr partitioning in the same manner as it would from an inorganic solution. This explains the similar temperature correlations for inorganic and coral aragonite, despite the presence of a Sr/Ca offset in corals.

Physiological factors, however, could also contribute to this temperature response. Enzyme systems often have a temperature optimum, (Krishnaveni et al., 1989) and any deviation will usually result in a decrease in the efficiency of an enzyme process. If Sr is enzymatically discriminated from Ca during physiological transport (see section 5.7), it is possible that the transported ratio of Sr to Ca will change as enzyme systems respond to temperature.

Physiological effects can certainly be of sufficient magnitude to produce the observed temperature dependence in corals. Hart and Cohen (1996) analysed a deep-sea solitary coral and found variations in the Sr to Ca ratio that were a similar amplitude to the variations observed for surface dwelling zooxanthellate corals. As the deep waters in which this coral was growing were of almost constant temperature, Hart and Cohen concluded that these Sr/Ca variations represented physiological variations. A number of other researchers similarly conclude that Sr/Ca variations are primarily a product of poorly understood variations in coral physiology (Cross and Cross, 1983; de Villiers et al., 1995; de Villiers et al., 1994; Hart and Cohen, 1996; Hart et al., 1997).

**Temperature Dependence Under Extreme Conditions**

If it is accepted that Ca$^{2+}$ is actively transported through the coral tissue to the calcifying site, but Sr$^{2+}$ is not (Ip and Krishnaveni, 1991; Ip and Lim, 1991), then under normal conditions the Sr/Ca ratio in the calcifying pool is lower than in seawater (see also McConnaughey, 1986). This matches the observations that $D_{SR}$ is lower in corals than inorganic aragonite. Under near-optimal temperature conditions, the enzyme responsible for the transport of Ca$^{2+}$ would be relatively unaffected by temperature variations, and precipitation would occur inorganically from a Sr-depleted calcifying fluid. Under these
circumstances, the temperature dependence may be largely influenced by the factors proposed by Kinsman and Holland (1969) (with the caveat that assumptions about equilibrium may not necessarily hold), resulting in a similar magnitude of temperature dependent partitioning.

If temperature conditions become more extreme, the Ca\(^{2+}\) transport enzymes would become less efficient, resulting in a lowering of the active Ca\(^{2+}\) transport and a decreasing calcification rate. The Sr\(^{2+}\) transport, being passive (Ipf and Lim, 1991), would not be affected to the same degree, and the Sr/Ca ratio of the calcifying pool would move closer to normal seawater values. This would introduce a nonlinearity in the Sr/Ca vs. SST record, with ratios changing disproportionately (giving apparently 'cooler' temperatures) as the coral shifted further away from its temperature optimum.

While evidence for this process at abnormally high temperatures is lacking (possibly because corals bleach when stressed in this manner), several studies of corals growing close to their cold-water extremes seem to support this hypothesis. McCulloch et al. (1994) noted anomalously cold Sr/Ca temperatures during the winter of 1982, when the cool water incursions during the strong 1982/83 El-Niño resulted in temperature anomalies of ~ -2°C along the Great Barrier Reef (Holbrook et al., 1993). Hart and co-workers (1996; 1997) observe an anomalously large range in strontium seasonality for a coral growing in Sodwana Bay, South Africa. They report that this coral grows close to the high latitude limit for corals, and that winter temperatures often drop below 20°C. A recent study by Fallon et al. (1998) examined the growth of a Japanese coral, growing near to the low temperature extreme. They noted both a significant slowing of growth (possibly shutting down completely below temperatures of 18°C), accompanied by anomalously high Sr/Ca values during winter.

**Growth Rate**

Changes in the growth (extension) rates of corals are often equated with calcification rates, which in turn relate to the degree of physiological modification that the coral is exerting over the calcifying microenvironment. Following this reasoning, slower growth would imply slower precipitation, lower supersaturations, and more 'equilibrium' kinetics. Similarly there would be less 'pumping' of Ca\(^{2+}\), and therefore less dilution of Sr. Generally, therefore, slower extension would be expected to be accompanied by higher skeletal Sr/Ca.

Kinsman and Holland (1969) predicted that D\(_{\text{Sr}}\) would be dependent on growth rate of a crystal; however, they failed to observe any link between growth and the Sr/Ca ratio in the precipitated CaCO\(_3\). Both Alibert and McCulloch (1997), and Shen et al. (1996) found no significant difference between Sr/Ca and growth rates in corals, over relatively wide ranges of extension rates. Oomori et al. (1982) did report a variation in the Sr/Ca
ratio with growth, but opposite to the expected result, with slow growing corals displaying a lower Sr concentration. They hypothesised that species preferentially accepted into the aragonite lattice (Sr, Ba, Pb) would similarly display a direct proportionality to growth rate, whereas smaller ions favoured by calcite would be inversely related to growth.

Extension and calcification are linked by the following relationship:

\[ E = \frac{C}{D} \]  

(6.2)

where:

\( E \) = extension rate
\( C \) = calcification rate
\( D \) = skeletal density

Extension and calcification rate covary only if skeletal density remains constant. Calcification rates are not, however, forced by extension, and changes in the extension rate may be accompanied by changes in density rather than calcification. Failure to observe a change in Sr/Ca with changes in extension may simply be because calcification rates have not changed.

Several workers do, however, report an inverse relationship between Sr/Ca and extension (Cross and Cross, 1983; de Villiers et al., 1995; de Villiers et al., 1994; Thompson and Livingston, 1970). Thompson and Livingstone attribute lower Sr/Ca ratios in zooxanthellate corals over non-zooxanthellate corals and corals growing without zooxanthallae as being a result of zooxanthallae-enhanced calcification rates. Several researchers also report that strontium concentrations are higher in the slower growing valleys between growth humps (Alibert and McCulloch, 1997; de Villiers et al., 1994), with Sr/Ca derived temperatures being up to 4°C cooler. Slow growth may result from a number of factors including greater sediment build-up, older coral polyps, poorer nutrition, and lower light intensity (Cohen and Hart, 1997). It is uncertain whether growth, or one of these other causative factors that is responsible for the lower Sr/Ca.

**Sr/Ca in Seawater**

Other possible factors affecting the Sr/Ca ratio in corals relate to changes in the Sr/Ca ratio of the water in which the corals grow. The residence time of Sr in the oceans is long relative to mixing (Beck et al., 1992; Broecker and Peng, 1982; Bruland, 1983), and concentrations of Sr are relatively uniform (Andersson et al., 1992; Anderssson et al., 1994). The oceanic strontium concentration is not perfectly constant, however. Slight surface depletions are reported relative to calcium, caused by the uptake of Sr into the calcareous tests of organisms in the surface ocean. For the Great Barrier Reef lagoon, Sr variations in the order of 0.6% are seen (Alibert, 1997 unpublished data).
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These surface depletions may not be constant with time: upwelling of deeper Sr enriched water may raise the Sr/Ca ratio, while the surface depletion may change seasonally as the balance between aragonitic, calcitic and celestite organisms varies throughout the year (de Villiers et al., 1994; Swart, 1981). Some authors suggest that surface water variations may ultimately limit the temperature resolution of high precision, high resolution Sr/Ca proxies (de Villiers et al., 1994; Shen et al., 1996).

Other Factors

Salinity

De Villiers et al. (1994) noted that the Sr/Ca ratios in fresh river-water were significantly lower than in the ocean, and suggested that river run-off may affect the Sr/Ca ratio of near-shore corals. McCulloch et al. (1994), however, found no such affect, despite clear oxygen isotope evidence for major fresh-water incursions. They pointed out that the absolute concentrations of both Sr and Ca are significantly lower in river waters, and therefore to a first approximation, flood plumes resulted in a conservative dilution of both Sr and Ca in seawater.

It is possible that physiological buffering of the pH in the precipitating microenvironment may lead to a salinity dependence for coral Sr/Ca ratios, and this mechanism is discussed further in chapter 5, section 5.7.4.

Diagenesis

Secondary aragonite has a higher Sr/Ca ratio than biogenic aragonite (Schneider and Smith, 1982), and early diagenesis is associated with increases in the Sr/Ca ratio of coral skeletons (Cross and Cross, 1983). Schneider and Smith (1982) report finding quantities of calcite in coral skeletons, especially at weaknesses in the structure. High and low Mg calcite both have significantly lower concentrations of strontium, and diagenetic transformation of aragonite to calcite is accompanied by a reduction in the Sr/Ca ratio (Amiel et al., 1973a). Diagenesis could, therefore, be associated with both increases and decreases in the Sr/Ca ratio of corals, depending on the nature of the alteration.

Organic Inclusions

Organic material is found to have a higher Sr/Ca ratio than coral aragonite, and zooxanthallae are even more enriched over and above polyp tissue (Buddemeier et al., 1981). The absolute concentration of Sr in tissue, however, is still lower than the concentration in coral skeleton (Amiel et al., 1973a).
6.2.3 Fine-Scale Variability

As analytical techniques and technology have advanced in recent years, it has become possible to examine corals at very fine spatial resolutions. Ion microprobe and laser ablation now allow analysis on spots as small as 30 μm. All researchers who have examined coral chemistry at these high resolutions report that coral skeletons are not homogeneous over sub-mm scales (Allison, 1996a; Allison, 1996b; Allison and Tudhope, 1992; Hart and Cohen, 1996; Hart et al., 1997 and see chapter 7; Oomori et al., 1982). Conventional high-resolution milling techniques generally do not pick up these fluctuations, as the total mass of sample used can be around 3 orders of magnitude larger than the samples generated by micro-beam techniques. Fine-scale variations are significantly greater than analytical error (Allison, 1996a), often with Sr/Ca temperatures fluctuating by up to 4°C (Allison and Tudhope, 1992), and sometimes variations can be large enough to obscure seasonal Sr/Ca variations (Allison, 1996a).

Hart and Cohen (1996) suggested that short term excursions in seawater SST might be responsible for some of this variability, and later Hart et al. (Hart et al., 1997) reported that there were some seasonally repeating fine-scale features that appeared to correlate with spikes in the δ¹⁸O record. Allison (1996a), however, considered it unlikely that physical or chemical changes in the water could be responsible. An alternative suggestion is that short timescale fluctuations in the coral physiology could be responsible for this variability; however, no explanation for what might trigger these was given (Hart and Cohen, 1996).

Allison (1996b) found that Sr was not significantly enriched in micro-borings; however, centres of calcification had higher Sr concentrations. It was proposed that this may relate to the greater porosity of these structures, and therefore possibly higher concentrations of trapped organic material (Allison, 1996b). Several researchers tentatively conclude that the heterogeneous distribution of an organic matrix with different affinities for Sr²⁺ could explain the high resolution variability in coralline Sr/Ca ratios (Allison, 1996b; Allison et al., 1996; Hart and Cohen, 1996).

6.3 Magnesium

6.3.1 Mechanism of Incorporation

There has been considerable interest and debate regarding the affect that Mg²⁺ has on the precipitation kinetics of different CaCO₃ polymorphs, and there is abundant literature concerning the incorporation of magnesium into inorganic calcites. Surprisingly,
however, there has been little research undertaken into the incorporation of Mg$^{2+}$ into either inorganic or biological aragonite. As a consequence, the physicochemical factors responsible for the coprecipitation of Mg$^{2+}$ into coral skeletons are not well understood, and the mechanism has yet to be resolved (Katz, 1973; Oomori et al., 1987). Amiel et al. (1973a) examined the distribution of a number of ions in coral skeletons and concluded that there were 3 phases in which a trace element might be found: bound in some form in the aragonite lattice, bound to organics, or adsorbed (see also Dodd, 1967).

**Lattice Bound**

Several researchers report evidence suggesting that magnesium in coralline aragonite is associated with a mineral phase (Amiel et al., 1973a; Kinsman, 1970). Delaney et al. (1996), and Mitsuguchi et al. (1996) report that washing procedures employed did not result in the loss of magnesium, indicating that labile organic and adsorbed magnesium components are negligible relative to skeletal Mg$^{2+}$. Amiel et al. (1973a) conclude that around 75% of the magnesium present in corals is lattice bound.

While magnesium may be in a mineral phase, this does not necessarily imply that it substitutes for calcium in aragonite. Magnesium is a small cation, with an ionic radius of 0.72 Å, compared with calcium with an ionic radius of 1.18 Å (Speer, 1983). It is generally agreed that an ion differing by more than 15% in radius cannot substitute for each other in a crystal lattice (e.g. Amiel et al., 1973a). Magnesite is a rhombohedral mineral, and there is no orthorhombic magnesium carbonate that might form a solid solution with aragonite (Mitsuguchi et al., 1996; Speer, 1983; Veizer, 1983). Magnesium is therefore strongly discriminated against in aragonite compared with calcite (Okumura and Kitano, 1986; Oomori et al., 1987), and compared with other divalent cations, the distribution coefficient of Mg$^{2+}$ in aragonite is conspicuously low ($D_{Mg} \approx 0.00016$ cf. $D_{Sr} \approx 1.0$ (Oomori et al., 1987)).

Magnesium does not inhibit the precipitation of aragonite, although it strongly poisons calcite growth (Berner, 1975), and has a distinctly different influence over the coprecipitation of univalent alkaline earth cations in aragonite compared with calcite (Okumura and Kitano, 1986). All these observations indicate that the incorporation of Mg into coral skeletons is not simple, and Mg$^{2+}$ may not be substituting for Ca$^{2+}$ in the aragonite lattice. The differences between the partitioning of Mg into calcite and aragonite invalidate any attempt to extrapolate Mg behaviour in calcite to biogenic aragonite.

**Other Mineral Phase**

Ions may be included interstitially, in cracks, dislocations and other crystal defects, or form their own small mineral domains. Amiel et al. (1973a) found that the skeletal
component of magnesium was readily leached from the aragonite by demineralized water, in ratios significantly higher than found in the bulk skeleton. From this they concluded that Mg is bonded loosely in the aragonite structure or is present in a dispersed, high magnesian mineral of greater solubility than aragonite. These observations are supported by the findings of Cross and Cross (1983), who report that diagenetic alteration, especially exposure to fresh water, decreases the skeletal magnesium concentration. Swart (1981 and references cited therein) noted that traces of diagenetic calcite were found in live coral heads, and proposed that this may be the source of labile magnesium.

**Adsorbed and Organic Phases**

A significant component of Mg$^{2+}$ in coralline aragonite may be adsorbed (Amiel et al., 1973a; Cross and Cross, 1983). Adsorbed magnesium was observed directly in high spatial resolution ion-probe analyses performed by Allison (1996b), who found clear enrichments in Mg at the edges of coral structural elements.

While Amiel et al. (1973a) found only 0.10 - 0.15% total organics in the skeleton, they concluded that a reasonable proportion of the 25% non-mineral magnesium was bound to organic material. Organic material was found by Buddemeier et al. (1981) to be have a significantly higher Mg/Ca ratio than the skeleton.

**6.3.2 Factors Affecting Partitioning**

**Temperature**

Experiments with inorganic calcite have revealed a clear temperature dependence in the distribution coefficient (e.g. Katz, 1973; Oomori et al., 1987) and this has been investigated as the basis of a calcite-based paleothermometer. There has, however, been no equivalent research into the inorganic temperature-dependent partitioning of Mg$^{2+}$ between solution and aragonite (Oomori et al., 1982). Kinsman (1970) reports a tentative positive correlation for $D_{Mg}$ in aragonite; however, the results are variable, and apparently no follow-up work has been conducted. Oomori et al. (1987), report a 'negligible' temperature dependence on the trace element partitioning into inorganically precipitated aragonite; however, their analytical techniques (XRD) may not have the precision to detect changes in $D_{Mg}$ at the low concentrations involved.

Early coral investigators reported cautious correlations between temperature and the skeletal Mg/Ca ratio (Chave, 1954; Weber, 1974); however, the correlations were not high, and many coral genera failed to demonstrate a clear temperature dependence (Weber, 1974). This may partly have been due to the high biological variability inherent in magnesium uptake into corals (see below). Only as techniques have improved, and it
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has become possible to subsample within a single year's growth, has clear evidence of a seasonality become apparent. The earliest observations of a seasonal cycle for magnesium were made by Goreau (1977). Subsequently a number of researchers have reported clear, large amplitude seasonal variations in the Mg/Ca ratio (Hart and Cohen, 1996; Mitsuguchi et al., 1996; Oomori et al., 1982). The seasonal cycle is well correlated with variation in the coral Sr/Ca ratio (Hart and Cohen, 1996; Mitsuguchi et al., 1996), and is around 4 times the magnitude of the Sr/Ca thermometer (Mitsuguchi et al., 1996).

Mechanism

While the strong seasonal anti-correlation between Sr/Ca and Mg/Ca suggests that Mg2+ incorporation is temperature dependent, there is no clear explanation why this might be so. The lack of experimental work on the inorganic partitioning of Mg2+ into aragonite hampers any attempt to put an understanding of the temperature dependence on a firm scientific basis. Similar arguments to those proposed by Kinsman and Holland (1969) for Sr might be used (see previous section); however, magnesium varies positively with temperature, rather than inversely. A positive correlation for Mg2+ is hard to rationalise under the equilibrium solid solution model, and such a model is invalid if Mg2+ does not substitute for Ca2+ in aragonite.

The tolerance of any crystal lattice to distortions (and hence foreign ions) increases with increasing temperature (Kinsman and Holland, 1969), and this would tend to produce a positive temperature correlation if Mg2+ is included as either a lattice or an interstitial substituent. Temperature dependent changes to the activity of Mg2+ in solution may also be responsible for a positive temperature dependence. As temperature increases, the packing of water molecules around an ion loosens, allowing greater complexation of the ion by other species in solution. Solution activities, therefore, tend to decrease as temperatures rise (Swart, 1981). If the activity of Ca2+ decreases more than the activity of Mg2+, then the Mg/Ca solution activity ratio would increase, allowing a greater proportion of Mg2+ to be incorporated into the growing crystal. While Swart (1981) concluded that the activity ratio would increase, it seems unlikely that it could change by as much as 50% over a 10°C range, which is what would be required to explain the observed Mg temperature dependence.

Transport limiting kinetics may also produce a positive temperature dependence if calcification rates increase with temperature (appendix A, section A.5). Because $D_{Mg}$ is $<<1$, the Mg/Ca ratio of the solution at the surface of a precipitating crystal tends to be higher than the bulk solution. If calcification rates increase with temperature, this fractionation will be enhanced and will lead to an increase in the Mg/Ca ratio in the depositing crystal. Also, Mg2+ is a small ion with a high charge density, and it is therefore more highly hydrated than Ca2+. The large hydration shell for Mg2+ imparts
considerable drag on the ions, and their diffusion is slow compared with Ca\(^{2+}\). As temperature increases, and this shell relaxes, the transport rate of Mg\(^{2+}\) may increase more than Ca\(^{2+}\). If the crystal growth is transport limited, then this would result in an increase in the aragonite Mg/Ca ratio with increasing temperature.

Oomori et al. (1987) suggest that the inorganic temperature dependence of Mg partitioning is very small, and cannot explain the relatively large variation observed in corals. This would imply a biological temperature control, with enzymatic Mg\(^{2+}\) transport strongly linked to temperature. This is pure speculation, and a review of the calcification physiology literature (chapter 5) did not reveal evidence to support this idea; however, it is not inconsistent with the strong biological influence seen for Mg\(^{2+}\) partitioning (see below), and the phylogenetically variable temperature dependence (Duckworth, 1976).

**Biological Factors**

Mg\(^{2+}\) is conspicuously variable in skeletal aragonite, varying between individuals, locations, and taxa. Different genera and species have been found to display distinct ranges of magnesium concentration (Chave, 1954; Cross and Cross, 1983; Dodd, 1967; Mitsuguchi et al., 1996; Oomori et al., 1982; Swart, 1981; Weber, 1974). These concentrations span a factor of 4, ranging from roughly 700 ppm to 2400 ppm. Individuals of the same species from the same reef location have been found to vary by a factor of two in their Mg content (Allison, 1996a). This variability cannot easily be correlated with differences in temperature, or water chemistry (Allison, 1996a; Cross and Cross, 1983), and most researchers therefore attribute it to physiological influences over the Mg incorporation by the coral (Cross and Cross, 1983; Hart and Cohen, 1996; Weber, 1974).

Duckworth (1976) reports that biological Mg partitioning does not agree with experimental inorganic values. Coralline aragonite, however, appears to precipitate magnesium close to inorganic 'equilibrium', with inorganic experimental concentrations of around 1000 ppm determined at 15°C (Kinsman, 1970). The range of inorganic values summarised in Veizer (1983 p 269) spans nearly an order of magnitude, however, making a precise comparison with biological Mg/Ca ratios impossible. It appears that corals tend to precipitate magnesium towards the lower end of the inorganic range, consistent with the hypothesis that physiologically pumped Ca\(^{2+}\) dilutes the other cations in the calcifying pool. The degree of physiological Ca pumping may therefore be responsible for the large range of Mg/Ca ratios observed; however, by this reasoning, an equivalent (proportional) change should be seen for all other species competing for calcium in the aragonitic lattice. Barium values do display a variability between genera; however, strontium values are remarkably uniform (see debate in section 6.3.3).
hypothesis would also require that increases in calcification rate would result in lower Mg/Ca ratios. As seen below, the correlation between growth rate and Mg partitioning is tenuous at best.

The exact link between Mg/Ca incorporation and coral physiology remains uncertain, with calcification rate, number or zooxanthallae, levels or organics trapped in the skeleton, or a suite of other genetic or physiological factors potentially affecting the magnesium partitioning (Allison, 1996a). This issue is discussed further in chapter 7.

**Growth Rate**

Kinsman (1970) reported experimental data from inorganic precipitation experiments, and suggested the high degree of variability observed in the partitioning of Mg into corals may have been due to a possible growth rate effect. Weber (1974) found no clear link between calcification rate and Mg content in a wide variety of coral genera. Swart (1981) reported a tentative positive correlation between Mg incorporation and growth rate in corals, although this may have been confounded by the effects of habitat or species. In contrast Oomori et al. (1982) found that Mg was preferentially incorporated into the slower growing coral genera. Again this result may be caused by taxa specific variability, rather than growth. Oomori et al. (1982) also suggested that the seasonality for Mg might be an effect of seasonal changes in growth rate.

**Other Factors**

**Seawater Mg/Ca**

In general, the long residence time of magnesium in the ocean (about 1.3 x 10^7 years: see review in Chester, 1990) results in a relatively uniform Mg^{2+} distribution (Bruland, 1983; Quinby-Hunt and Turekian, 1983). Variability in the seawater Mg/Ca would be expected to be less than 1%, corresponding to less than 0.3°C error in the temperature record derived from corals (Mitsuguchi et al., 1996). Conservative changes to salinity do not appear to change the Mg^{2+}/Ca^{2+} activity ratio (Mitsuguchi et al., 1996), and changes in seawater concentration were not linked with coralline Mg partitioning (Swart, 1981).

**Habitat**

No consistent patterns were seen for corals growing in different habitats (Swart, 1981), and no clear change in Mg/Ca could be found for corals growing at different water depths (Weber, 1974). Both of these factors, however, may have been obscured by the large taxonomic variability.
6.3.3 Fine-Scale Variability

Magnesium is very heterogeneously distributed on spatial scales of a few hundred microns (Allison, 1996a; Allison, 1996b; Allison and Tudhope, 1992; Oomori et al., 1982). This variability cannot be correlated with SST or water chemistry (Allison, 1996a; Allison, 1996b), and adjacent crystals, separated by no more than 2 days growth, can differ in concentration by up to 230 ppm (Allison, 1996b). Allison (1996a; 1996b) rules out the possibility of diurnal temperature fluctuations, and different aged overgrowths caused by tissue 'smoothing' of the temperature signal. Although adsorbed magnesium is seen to be enriched at the edges of skeletal elements, careful placement of the microprobe beam, and 'burn-in' of the beam before measurement would have avoided these enrichments (Allison, 1996a). No significant differences were found between 'bulk' skeleton and microborings (Allison, 1996b; Amiel et al., 1973a). Although other elements display some gross skeletal segregation, magnesium is not found to be significantly different in concentration between different skeletal elements (Amiel et al., 1973a).

Centres of calcification were found to be enriched in magnesium (Allison, 1996a; Allison, 1996b; Allison and Tudhope, 1992), where high concentrations of Mg-rich organic material may be trapped. These enriched domains account for around 5 to 25% of the bulk skeletal magnesium (compared with about 1% for strontium), which is enough to explain most of the variability observed (Allison, 1996b). Centres of calcification are porous structures, and may also trap small amounts of magnesium rich sediment (Allison, 1996b; Amiel et al., 1973a).

A dispersed organic matrix with variable metal-binding affinity may be responsible for high resolution variability (Allison, 1996a), and different amounts of these trapped organics could be responsible for the taxa-specific bulk Mg/Ca ratios observed. Allison (1996a), however, could not rule out the possibility that weekly to monthly variation in the coral or zooxanthallae physiology was responsible for the high resolution Mg heterogeneity.
6.4 Barium

6.4.1 Mechanism of Incorporation

Phase

Barium forms an orthorhombic BaCO$_3$ (witherite), and can therefore form solid solutions with CaCO$_3$ (aragonite) (Speer, 1983). Some authors therefore propose that barium is lattice-bound in coral aragonite (Lea et al., 1989). While the 9 co-ordinate cation site in aragonite is tolerant to large ions [Speer, 1983 #426], the ionic radius of Ba$^{2+}$ is significantly larger than Ca$^{2+}$ (1.47Å cf. 1.18Å), and a miscibility gap exists in the BaCO$_3$ - CaCO$_3$ solid solution at room temperatures (Pingitore et al., 1989; quoting Speer, 1983). A strong temperature and rate dependence on the distribution of Ba in aragonite was found by Gafford (1969 as cited in Pingitore, 1989) who concluded that Ba was not substituting for Ca$^{2+}$ in aragonite. Pingitore et al. (1989) propose an occlusional mechanism for Ba incorporation, noting, however, that this is not inconsistent with some lattice-inclusion of Ba$^{2+}$.

While it is likely that some Ba$^{2+}$ is lattice bound in corals, barium is also enriched in a number of non-calcitic phases such as detrital clays, organic material, authigenic iron/manganese phases, and barite particles (Lea and Boyle, 1993). The barium concentrations in these phases can range from 200 to 600,000 ppm (see Lea and Boyle, 1993 and references cited therein). In contrast, coral skeletons have around 2 - 15 ppm Ba. Clearly, contaminants could dominate the bulk Ba composition of coral skeletons, and rigorous cleaning methods are necessary to remove any contaminant phases before analysis (Lea and Boyle, 1993).

There is evidence that a proportion of barium is adsorbed to the surfaces of skeletal elements. Dissolution experiments suggest about 20% of the barium is labile and possibly surface bound (Pingitore et al., 1989). Allison (1996b) directly observed enrichments of barium on the outsides of structural elements, which may be adsorbed or authigenic overgrowths.

Organic material contains high concentrations of Ba; enriched by between 10 and 100 times over skeletal barium (Buddemeier et al., 1981; Flor and Moore, 1977; Lea and Boyle, 1993). Organic material can make up 1% of coral skeletons (Lea and Boyle, 1993), and may therefore represent a significant source of Ba contamination. This organic material is intimately dispersed throughout the coral skeleton, and is often inaccessible by solution, so its removal by wet chemical techniques is difficult, and impossible by micro-beam methods.
The extreme enrichments of Ba seen in the tissue layer of coral skeletons demonstrate the potential for organic contamination to affect skeletal Ba/Ca (Allison, 1996a; Hart and Cohen, 1996; Tudhope et al., 1996). Even coral samples that have been bleached in concentrated peroxide or hypochlorite are found to have extremely large enrichments in barium for the few years in and below the tissue layer (Tudhope et al., 1996). SEM analysis of the top few years of coral growth reveal the presence of small barite crystals and high Ba concentrations associated with small cracks and pores in the skeleton (Tudhope et al., 1996). The barite was interpreted as being a decay product of organic tissue residues, some of which still appeared to be bound in small fractures and pores (such as between trabeculae or surrounding individual fibres) where cleaning solutions could not penetrate.

The tissue layer Ba enrichment decays away to more ambient skeletal Ba/Ca levels over a period of several years (Allison, 1996a; Hart and Cohen, 1996; Tudhope et al., 1996 and see also chapter 8). It appears, therefore, that organic Ba inclusions are not stable with time, and the decay of organic material is accompanied by loss of Ba. Because Ba in the bulk skeleton may be associated with dispersed organic phases (Allison, 1996a; Allison, 1996b; Allison and Tudhope, 1992; Hart and Cohen, 1996; Hart et al., 1997), the stability, and therefore reliability, of bulk skeletal Ba measurements is questionable. Several authors report Ba levels decreasing with time (Hart and Cohen, 1996 quoting Lea pers. comm.; Lea and Boyle, 1993). Hart and Cohen (1996) found that Ba enrichments and strong seasonality in young coral skeleton had decayed away to lower 'ambient' levels after 30 years. They also found that features present in the 30-year old skeleton could not be seen in a 13,000 year old fossil coral. Lea et al. (1993) observed that both skeletal concentration and variability between samples decreased as skeletons got older. These observations led Hart and Cohen (1996) to propose that 3 phases exist in coral skeletons. Firstly, a short lived labile organic barium phase that is responsible for strong seasonality and enrichments in the top few growth bands. Secondly, a more durable 'background' organic phase that decays over centuries to millennia (which may be responsible for some of the Ba variability seen between individuals and genera). Thirdly, a stable lattice-bound component that contains a low-level seasonal signal. Further discussion is presented in chapters 7 and 10.

**Biogenic vs. Inorganic Aragonite**

The distribution of barium between seawater and coralline aragonite is found to be surprisingly indiscriminate (Shen and Sanford, 1990). $D_{Ba}$ is generally very close to 1.0 (table 6.1), although values of up to 1.41 have been reported (Buddemeier et al., 1981; Lea et al., 1989). Distribution coefficients around 1.0 are often taken as evidence for kinetic control over trace element partitioning; however, in this case inorganic
distribution coefficients are also found to be around 1.0 or a little higher (Kitano et al., 1971 and table 6.1), and thermodynamic considerations suggest that \( D_{Ba} \) should be around 1.3 (Shen and Sanford, 1990). Barium partitioning in coral aragonite could, therefore, be close to thermodynamic equilibrium (Lea et al., 1989). It should be noted, however, that organic inclusions in coral skeletons may have led to an overestimation of the distribution coefficient (Allison, 1996b). It therefore cannot be clearly determined whether Ba partitioning between solution and skeleton is controlled by kinetics, thermodynamics, (or, for that matter, physiology).

### 6.4.2 Factors Affecting Partitioning

#### Biological Factors

Barium concentrations in corals are not constant between individuals or taxa. The literature contains several reports of different barium incorporations and partitioning behaviours which are attributed to differences between coral genera (e.g. Allison, 1996a; Buddemeier et al., 1981; Pingitore et al., 1989; Tudhope et al., 1996). Allison (1996a) found that barium concentrations varied by a factor of 3 between individuals of the same species, and different individuals seemed to have different degrees of scatter within their skeletons. Pingitore et al. (1989) found that *Montastrea annularis* displayed a lower and more consistent range of Ba concentrations than *Acropora palmata*, and discounted post-depositional modification of the skeletons, secondary calcite, sediments, or adsorption as being causative factors. Both papers report that variations in environment or temperature could not account for the observed variability, and concluded that physiological or genetic factors were instead responsible.

Pingitore et al. (1989) suggested that differences and variability may relate to the different growth rates, since the fast, variable growth of *Acropora* was accompanied by higher and more variable barium. This agrees well with their proposed occlusion mechanism for barium incorporation, as fast growth would be accompanied by greater trapping of the occluded ion. Lea and Boyle (1993), however, suggest that the results of Pingitore et al. (1989) can be explained by differing amounts of organic material trapped in the skeleton. The amount and distribution of organics may be under genetic control, explaining the taxonomic differences and the variability.

Allison (1996b) found that centres of calcification and microborings contain significantly enriched concentrations of barium. Different coral genera have different skeletal architectures, varying in the numbers of these features. This may explain some taxonomic variability; however, Allison (1996b) calculated that this could only be responsible for about a 10% variation.
It is clear that taxa cannot account for all Ba variability, however, as two corals of the same species growing at the same location displayed distinctly different Ba partitioning behaviour (Allison, 1996a). Differences in the health, number of zooxanthallae, calcification rate, or other genetic effects are all possibilities (Allison, 1996a). Tudhope et al. (1996) also suggested that nutritional factors, and ingestion of particulate barium may affect Ba uptake. Given that organic tissues tend to be rich in barium, the diet of the coral may play a role in contributing to the barium input into the skeleton. The source of fluid and ions to the calcifying surface is via the coelenteron. Corals feed at night, but spend most of the day (when calcification rates are highest) with their polyps closed. During these times there is unlikely to be any fluid recharge of the coelenteron. Any digesting material may therefore be releasing trace elements into a small, static pocket of fluid. This may significantly raise the Ba composition of the coelenteric fluid and hence the coral skeleton. Skeletal Ba concentrations may therefore be influenced by the feeding habits or type and availability of food for the coral.

Because of the roles that organics and physiology play in controlling skeletal compositions, barium needs to be treated with caution as an environmental tracer, especially if habitats and growth rates vary significantly between sample locations (Pingitore et al., 1989).

**Temperature**

Increases in the Ba/Ca ratio are reported during cold water upwellings in a number of locations including the Galapagos (Lea et al., 1989; Shen et al., 1992), the Arabian Sea (Tudhope et al., 1996) and Japan Sea (Fallon et al., 1999). Barium enrichment associated with upwelling is caused by different Ba concentrations in the vertically displaced water masses (Lea et al., 1989). The apparent correlation with temperature is due to the association of deep cold waters with enrichments in barium.

In the absence of changes to water chemistry, Ba/Ca ratios appear to be relatively uniform (Shen and Boyle, 1988), although several researchers report the possibility of a 'true' temperature component, related to the Ba distribution coefficient (Lea et al., 1989; Shen et al., 1992). This temperature signal is small, contributing around $1/5$ to $1/3$ of the Ba/Ca change associated with upwelling. The barium distribution coefficient decreases with temperature in inorganic aragonite (quoting Gafford, 1969; Pingitore et al., 1989), but because the mechanism for Ba incorporation into aragonite is not well understood, it is difficult to explain the physical basis for this temperature dependence.

**Water Chemistry**

Because the Ba concentration in seawater is variable, environmental parameters that cause shifts in water masses may be recorded in coral skeletons as varying Ba/Ca ratios
Barium has a nutrient distribution in the ocean, with surface waters being depleted relative to deep ocean (Bacon and Edmond, 1972; Bruland, 1983; Lea et al., 1989; Quinby-Hunt and Turekian, 1983). Barium is, therefore, a strong indicator of oceanic upwelling (Fallon et al., 1999; Lea et al., 1989; Shen et al., 1992; Tudhope et al., 1996; Tudhope, ), which can be associated with El-Niño events (Shen et al., 1992), and monsoonal climate (Fallon et al., 1999; Tudhope et al., 1996).

Significant differences between Ba behaviour, despite similar oceanographic settings (Tudhope et al., 1996), suggest that barium may be sensitive to other microgeographic effects.

Barium is enriched in fresh waters relative to seawater, and strongly desorbed from riverine particulates in the high ionic strength, alkaline environment in the ocean (see chapter 9). Corals that experience fresh water from river discharge, therefore, record elevated barium in their skeletons. Shen and co-workers (Shen and Sanford, 1990) measured Ba/Ca ratios in a coral from Barbados and linked the annual variations seen to discharge from the Amazon River. The coastal marine environment is richer in barium than oceanic settings because of fresh-water discharge and high concentrations of terrestrial particulates. Corals growing close to land therefore record higher background barium concentrations (Shen and Boyle, 1988).

**6.4.3 Fine-Scale Variability**

Barium is heterogeneously distributed over small spatial scales (<100 μm) within coral skeletons (Allison, 1996a; Allison, 1996b; Allison and Tudhope, 1992; Hart and Cohen, 1996; Hart et al., 1997 and see also chapter 7). In general, Ba is relatively uniform between adjacent crystals, varying by around 1% (Allison, 1996b; Allison and Tudhope, 1992). Microborings, centres of calcification and the edges of skeletal elements, however, are found to be greatly enriched in barium. The magnitude of this barium enrichment is significantly larger than for other elements (Allison, 1996b). Organic material is rich in barium (Buddemeier et al., 1981; Flor and Moore, 1977; Howard and Brown, 1986; Howard and Brown, 1987), as is sedimentary detrital material (see Lea and Boyle, 1993 and references cited therein). The large enrichments are therefore consistent with organic material, or sedimentary detritus infiltrating into the porous microborings and centres of calcification (Allison, 1996b; Allison and Tudhope, 1992). These enriched regions of barium can have a significant effect on the bulk skeletal composition (Allison, 1996b), and contaminant phases could generate significant small-scale variability that can obscure some environmental signals (Allison, 1996a; Allison, 1996b).
6.4.4 Ba Spikes

Some of the most interesting and enigmatic features to appear in the Ba records of corals are short, regular, large amplitude spikes of barium. These Ba spikes have been observed by two research teams (Hart and Cohen, 1996; Hart et al., 1997; Tudhope et al., 1996). The spikes are examined in detail in chapter 10; however, a brief review of what has been observed follows.

Tudhope et al. (1996) analysed corals from two locations near Oman. The two corals were both collected from embayments, and the two sites were only 20 km apart. In both corals, the south-west monsoon was marked by a distinct negative spike in $\delta^{18}$O in August. A fluorescent band occurred 1-2 months later in October-November. The Wadi-Ayn coral displayed Ba peaks approximately synchronous with the fluorescence, and the intensity of these peaks was consistent with an upwelling source. In contrast, the Marbat coral barium record appeared to have a double-spike: a peak in October synchronous with fluorescence (the same as for the Wadi-Ayn coral), and an earlier peak correlating strongly with the negative spike in the $\delta^{18}$O. The intensity of the second peak in the Marbat coral is consistent with upwelling (again like the Wadi-Ayn coral); however, the first peak is of significantly larger amplitude, much larger than could be realistically attributed to upwelling.

Tudhope et al. (1996) tentatively interpreted the October-November barium spikes as being upwelling induced by the SW monsoon, although the lack of correlation with oxygen is an anomaly. The large spikes in the Marbat coral were attributed to either some micro-geographic effect, or to the incorporation of particulate biological barium, which was associated with increased productivity during upwelling. The presence of this bio-barium 1-2 months ahead of fluorescence (which was also linked to marine productivity) is a little puzzling.

Hart and Cohen (1996) observed large amplitude barium spikes in a coral from Sodwana Bay in South Africa. These spikes were present in young growth bands, 30-year old growth bands, but not in a 13,000 year old coral from Tahiti. These spikes regularly occurred in March or April (boreal summer), slightly after the high density bands. The spikes represented a continuous horizon in the coral, although in a companion paper (Hart et al., 1997) this horizon was not found to be perfectly parallel to the dissepiments in the coral, which Hart et al. took to be an indication that the Ba horizon was not isochronous. Despite an initial organic-inclusion hypothesis, there was no apparent correlation between the barium spike and skeletal organics, nor was there any trace of barite particles (greater than 1 µm), or any correlation with micro-borings (Hart et al., 1997). While Hart et al. (1997) conclude that this spike remains a mystery, they suggest that a biologically mediated factor triggers an inclusion of high-Ba material in the
skeleton, possibly as an unusually enriched organic phase trapped in the microscopic interstices.

6.5 Boron

6.5.1 Mechanism of Incorporation

The incorporation of boron into coralline aragonite is complex and still poorly understood. Boron is apparently included as an anion complex and solution speciation may play a role in determining its partitioning. Boron cannot be occurring as a fluid inclusion as the crystal B concentration is higher than the parent solution (Hemming et al., 1995b; Sen et al., 1994). There are 3 major ways in which boron can become bound into a growing crystal (Ichikuni and Kikuchi, 1972). Boron can precipitate as a distinct calcium-borate phase, $\text{BO}_3^{3-}$ can substitute directly for $\text{CO}_3^{2-}$, or boron can adsorb to the crystal surface. Ichikuni and Kikuchi (1972) dismiss the first two possibilities, because solutions are rarely supersaturated with respect to calcium borates, and free $\text{BO}_3^{3-}$ occurs in negligible quantities at normal pH ranges.

Adsorption to Crystal Surface

It is, therefore, believed that the first step in boron coprecipitation with $\text{CaCO}_3$ is adsorption of boron species to the surface of the growing crystal. This was demonstrated by Ichikuni and Kikuchi (1972) who showed that boron incorporation into travertines followed the Freundlich adsorption isotherm. Kitano et al. (1978) did not observe any crystal-poisoning behaviour for B, and B did not appear to influence the $\text{CaCO}_3$ phase precipitating, as might be expected for a strong surfactant (e.g. $\text{Mg}^{2+}$, phosphates, sulphates and organics - see appendix A, section A.8.1). Hemming et al. (1995b), however, observed a change in crystallographic behaviour in calcite that was consistent with boron being a strongly surface-bound species.

In seawater there are two major B species that might adsorb to a crystal surface: the neutrally charged boric acid ($\text{B(OH)}_3^0$), and the negatively charged borate ion ($\text{B(OH)}_4^-$). Ichikuni and Kikuchi (1972) tentatively suggested that both $\text{B(OH)}_4^-$ and $\text{B(OH)}_3$ adsorbed to the surface in approximately equal proportions, with borate binding to the crystal through ionic interaction with cations on the surface and boric acid forming hydrogen bonds with carbonate groups on the crystal. Kitano et al. (1978) proposed that $\text{B(OH)}_4^-$ adsorbed more to aragonite, while $\text{B(OH)}_3$ favoured calcite. More recent work, however, strongly suggests that only the negatively charged $\text{B(OH)}_4^-$ is adsorbed to calcite or aragonite surfaces (see below). This is consistent with the fact that $\text{CaCO}_3$
crystal surfaces are positively charged under normal seawater conditions (Given and Wilkinson, 1985; Lahann, 1978).

**Isotopic Evidence**

Evidence for B(OH)$_4^-$ adsorbing to the crystal surface comes from isotopic studies. Seawater has a $\delta^{11}B$ of around 39.5‰ (Spivack and Edmond, 1987). This is a balance of the isotopically heavy B(OH)$_3$ ($\delta^{11}B$ of about 42‰), and the isotopically light B(OH)$_4^-$ ($\delta^{11}B$ of approximately 20‰; Hemming and Hanson, 1992; see also Vengosh, 1990; Vengosh et al., 1991 and references cited therein). As pH increases, the $\delta^{11}B$ of both B(OH)$_4^-$ and B(OH)$_3$ increase (Vengosh, 1990; Vengosh et al., 1991).

The isotopic composition of boron precipitating from solution is quite insensitive to both the phase precipitated, and mechanism by which precipitation occurs. The range of isotopic compositions of biogenic aragonite is narrow (Hemming, 1990; Hemming and Hanson, 1992; Hemming et al., 1995b; Vengosh, 1990; Vengosh et al., 1991), even though they have a wide range of B concentrations (which suggests that a number of biological processes may be occurring: Gaillardet and Allegre, 1995; Gaillardet and Allègre, 1995; Hemming, 1990; Hemming and Hanson, 1992; Vengosh, 1990; Vengosh et al., 1991). Aragonite and calcite have also been found to have similar isotopic compositions, despite very different B concentrations (Fürst et al., 1976; Hemming et al., 1992; Hemming et al., 1995b; Kitano et al., 1978; Vengosh, 1990; Vengosh et al., 1991).

The $\delta^{11}B$ of carbonates precipitated from seawater are around 22‰ (e.g. Hemming and Hanson, 1992 and many others), which is very close to the isotopic composition of the free B(OH)$_4^-$ in solution at seawater pHs. Researchers, therefore, conclude that only the borate ion binds to the surface of the growing crystal (Hemming, 1990; Hemming and Hanson, 1992; Hemming et al., 1995b; Vengosh, 1990; Vengosh et al., 1991), and becomes cemented into the lattice without further isotopic fractionation (Hemming and Hanson, 1992). This indiscriminate incorporation is taken by some researchers to be an indication that disequilibrium exists between the crystal surface and the parent solution (Sen et al., 1994).

**Incorporation into the Crystal**

The anion site in aragonite is smaller than in calcite (37.77 Å vs. 40.87 Å; Hemming et al., 1995b). HBO$_3^-$ has B-O bonds that are around 7% larger than the C-O bond length (Hemming et al., 1995b). It might therefore be expected that the larger borate anion would prefer calcite over aragonite. Similar behaviour is seen for sulphate anions which substitute preferentially into calcite (Hemming et al., 1995b and references cited therein). The concentration of boron in aragonite precipitated inorganically from seawater is
higher than calcite, however, (around 75 ppm compared with 15 ppm precipitated with calcite Hemming et al., 1995b; and see also Kitano et al., 1978; Vengosh, 1990; Vengosh et al., 1991).

Using $^{11}$B NMR techniques, Sen et al. (1994) report that the coordination environment of boron in aragonite is tetrahedral, whereas in calcite it is trigonal (planar). It therefore appears that the tetrahedral $\text{B(OH)}_4^-$ ion adsorbed to the surface of an aragonite crystal maintains its tetrahedral coordination as it is cemented into the crystal. In contrast, adsorbed $\text{B(OH)}_4^-$ must change geometry to become trigonal before being included into calcite. If this transformation is sterically hindered, or otherwise energetically disfavoured, then there will exist an additional activation energy barrier for B incorporation into calcite, which may explain the lower abundances observed (Hemming et al., 1995b; Sen et al., 1994).

Hemming and Hanson (1992) proposed that boron was incorporated into CaCO$_3$ crystals as a planar HBO$_3^{2-}$ ion substituting directly for the planar CO$_3^{2-}$. The results of Sen et al. (1994) appear to contradict this in the case of aragonite, raising a number of questions about how a tetrahedral BO$_4^{5-}$ ion could fit into the aragonite lattice. Oxygen atoms form the bulk of a carbonate ion, and a 4-oxygen tetrahedral borate would be significantly larger than the 3-oxygen carbonate. The incorporation of a large ion into the small anion lattice site in aragonite might be expected to create a significant distortion of the lattice. This would raise the energy of the lattice, increasing its solubility and therefore poisoning the growth (cf. Lahann, 1978). As no such poisoning was observed for aragonite, (Kitano et al., 1978) it is possible that boron does not substitute for carbonate. This result remains puzzling, and the implications should be considered further.

**Biogenic vs. Inorganic Aragonite**

Inorganic B determinations have been carried out by Kitano et al. (1978) and Hemming et al. (1995b). Extrapolating their results to seawater composition fluids, Kitano et al. (1978) proposed an 'inorganic' boron composition in aragonite of 5 ppm. Hemming et al. (1995b) suggest a much higher value of around 75 ppm. The discrepancy is attributed to differences in the carbonate ion concentrations for the two experiments (Hemming et al., 1995b). It is difficult to compare coral B concentrations to theoretical compositions because these two estimates vary by so much. Assuming the results of Hemming et al. (1995b) are correct, then corals are precipitating close to, or slightly depleted relative to, inorganic values. On the other hand, surficial deep sea sediments analysed by Vengosh et al. (1991) lie at concentrations of between 5 and 20 ppm, indicating that corals are significantly enriched relative to these carbonates. Until more experimental determinations of inorganic B compositions are carried out, it is not
possible to resolve whether corals precipitate close to inorganic equilibrium with respect to B.

6.5.2 Factors Affecting Partitioning

Biological Factors

Physiological Control over pH

Corals precipitate B at conspicuously higher concentrations than other calcareous organisms (50 - 80 ppm cf. 2 - 3 ppm for gastropods Vengosh, 1990; Vengosh et al., 1991). This implies that some vital effect in corals is responsible for a boron enrichment in the skeleton. It has been suggested that a high pH in the coral calcifying fluid (as proposed by McConnaughey, 1989b and summarised in section 5.4; McConnaughey, 1986) is responsible for an increase in the amount of B present as B(OH)$_4^-$ in the precipitating microenvironment, which would result in an enrichment in the aragonite (Vengosh, 1990; Vengosh et al., 1991). This explanation, however, does not consider the simultaneous variation in boron and carbon species in solution as a function of pH.

Boron exists in solution almost exclusively as B(OH)$_3$ or B(OH)$_4^-$ (Cotton and Wilkinson, 1988; Hershey et al., 1986). At seawater concentrations and pH, B(OH)$_3$ is dominant (Kitano et al., 1978), and accounts for approximately 81% of the boron in solution, in comparison with 19% for the borate ion (calculated using a value of pH = 8.2, and pK - the boric acid dissociation equilibrium constant - of 8.83). The dissociation of boric acid is clearly pH dependent (equation 6.3) and at there is a steep change in B(OH)$_4^-$ abundance with pH at normal seawater pHs (Hemming and Hanson, 1992 and figure 6.1).

$$B(OH)_3 + H_2O \leftrightarrow B(OH)_4^- + H^+ \quad (6.3)$$

Small changes in solution pH can therefore have a significant effect on the ratio of B(OH)$_4^-$ to B(OH)$_3$.

The boron concentration in an aragonite crystal is proportional to the activity of boron in the precipitating solution (Hemming et al., 1995b; Kitano et al., 1978; Vengosh, 1990; Vengosh et al., 1991). Because B(OH)$_4^-$ is preferentially incorporated into aragonite, changing the speciation of boron in solution will change the concentration in the crystal. B(OH)$_3$ and B(OH)$_4^-$ are sensitive to pH, and raising the pH in the precipitating microenvironment shifts this equilibrium towards B(OH)$_4^-$ (equation 6.3). While this might be expected to raise the boron concentration in the crystal, B(OH)$_4^-$ competes for anion sites with CO$_3^{2-}$, and the simultaneous effect of pH on the carbonate ion concentration must also be considered.
Figure 6.1 Boron Speciation in Seawater with pH

Activity of boron species in a seawater-composition solution as a function of pH. Details of the models used to generate this data are presented in appendix B. The data is graphed on both a linear and a logarithmic axis. The grey dashed line represents normal seawater pH (8.2). The shaded region spans the range of pHs in the precipitating fluid predicted by McConnaughey's calcification models (1986). The heavy line represents the activity of B(OH)$_4^-$, which is believed to be the species incorporating into coralline aragonite. Note that the activity of this species increases with pH over the range of pHs likely to occur in the calcifying fluid.

It is possible to model the B/CO$_3^{2-}$ system, assuming equilibrium speciation for C and B in solution. Two models are presented, and their details can be found in appendix B. Model A assumes that the calcifying fluid is in equilibrium with atmospheric CO$_2$ (after McConnaughey, 1986). Model B assumes that the precipitating microenvironment is isolated from atmospheric CO$_2$ (as might happen if reaction rates are too high for diffusion and equilibration with CO$_2$ to occur). In model B the total dissolved carbon pool remains constant, with C speciation changing in equilibrium with pH. In reality, the
behaviour of carbon in the coral's precipitating environment may fall somewhere between these two models.

As pHs increase, the speciation of boron shifts towards B(OH)$_4^-$ (figure 6.1). The CO$_3^{2-}$ concentration, however, increases more steeply, and the B(OH)$_4^-$/CO$_3^{2-}$ ratio decreases (figure 6.2(a) and (c)). Boron may compete for both CO$_3^{2-}$ and HCO$_3^-$, however. At the pHs being considered, CO$_3^{2-}$ + HCO$_3^-$ more or less represents the total dissolved carbon in the system. If CO$_2$ is free to diffuse into the calcifying fluid (model A), then the total ΣC increases, and still produces a decrease in the B(OH)$_4^-$/ΣC ratio (figure 6.2(b)). Only in the case where the ΣC in the solution is fixed (model B) does the B(OH)$_4^-$/ΣC ratio increase (figure 6.2(d)), driven by the shift in boron speciation.

If corals raise the pH of their precipitating microenvironment, B/C ratios in the skeletal aragonite are most likely to be lower than aragonite precipitated inorganically from seawater. In essence this represents 'dilution' of B(OH)$_4^-$ by physiologically generated CO$_3^{2-}$, analogous to the dilutions predicted for cations through physiological pumping of Ca$^{2+}$. The more the coral raises the pH in the calcifying fluid, the greater this dilution will be. This prediction contrasts with the observations of higher B in corals compared with other organisms and inorganic aragonites (Vengosh, 1990; Vengosh et al., 1991). The mechanisms proposed for B incorporation into skeletal aragonite, or the models for coral calcification, obviously need to be reconsidered if an explanation is to be found for the observed B partitioning into coralline aragonite.

**Taxonomic Effects**

Because pH controls the isotopic composition of the B(OH)$_4^-$ ion, the δ$^{11}$B of any CaCO$_3$ mineral precipitated can be used to calculate the pH of the original solution, stimulating research into the possible use of marine carbonates as paleo-pH proxies. Despite this interest, and the scrutiny with which the inorganic mechanism for B incorporation has received, there have been surprisingly few analyses of B in corals reported in the literature.

The broad range of concentrations seen in corals (Hemming and Hanson, 1992; Vengosh, 1990; Vengosh et al., 1991), despite a relatively constant oceanic B composition (Spivack and Edmond, 1987), suggests that some biological factors are responsible for mediating bulk skeletal compositions. Few studies compare different coral genera, species, or environment to ascertain whether these variations have a consistent pattern.
Figure 6.2 Boron to Carbon Ratios with pH
Models of B(OH)$_4^-$ ratio to CO$_3^{2-}$ and (CO$_3^{2-}$ + HCO$_3^-$) in seawater as a function of pH. Details of the models are presented in appendix B. The grey dashed line represents normal seawater pH (8.2). The shaded region spans the range of pHs in the precipitating fluid predicted by McConnaughey's calcification models (1986). B(OH)$_4^-$ is the species believed to incorporate into aragonite, competing with carbonate and/or bicarbonate for anion lattice sites. Thus the ratio of B(OH)$_4^-$ to carbon anion controls the B/CO$_3^{2-}$ ratio in the precipitating aragonite. Model A (graphs (a) and (b)) assumes that solution carbonate is in equilibrium with atmospheric CO$_2$. Model B (graphs (c) and (d)) assumes that the calcifying fluid is isolated from the atmosphere, and that the total dissolved carbon is constant. Graphs (a) and (c) present the ratio to carbonate. Graphs (b) and (d) present the ratio to carbonate plus bicarbonate. Note that only in scenario (d) does the ratio increase with increasing pH.
Of the corals studied by Hemming and Hanson (1992), the two *Porites* specimens appeared to have a significantly lower B concentration than the *Montastrea, Siderastrea,* or *Acropora* samples (52.8 ppm vs. 59.8 ppm). A possible growth rate effect could be responsible, with the slower growing coral incorporating a lower B concentration. Gaillardet and co-workers (Gaillardet and Allegre, 1995; Gaillardet and Allègre, 1995) noted significant variations between individual corals, but could not find a correlation with species.

**Temperature Dependence**

The ratio of $\text{B(OH)}_4^-\text{ to B(OH)}_3$ decreases with increasing temperature as the equilibrium constant controlling their speciation decreases (Hemming and Hanson, 1992). The only clear evidence that temperature affects the boron partitioning in corals comes from the high resolution ion-microprobe analyses of B carried out by Hart and Cohen (1996). They demonstrate a clear seasonal signal in the B/Ca ratio, closely correlated with Sr/Ca and therefore inversely following temperature. The B/Ca ratio varies by approximately 20% for a 7°C change in water temperature. The cause of this temperature dependence is not known. A decreasing boric acid dissociation constant may be responsible. Alternatively, the solubility of Ca borate may increase more than Ca carbonate for an increase in temperature. If increasing temperatures are associated with an increase in calcification rates, then physiologically enhanced pH may be responsible for 'dilution' of the $\text{B(OH)}_4^-$ by $\text{HCO}_3^-$ and $\text{CO}_3^{2-}$.

**Other Factors**

**Salinity**

While Fürst et al. (1976) observe that mollusc shell B is sensitive to salinity, no similar effect has been demonstrated in corals. Kitano et al. (1978) found that concentrations of boron in aragonite decreased with increasing NaCl concentration, dropping by about 5% as the NaCl concentration doubled (calcite displayed the opposite trend). They proposed that increasing the ionic strength of the solution decreased the activity coefficient for $\text{B(OH)}_4^-$. The changes in salinity that a coral might be exposed to are small, however, and this effect should be insignificant.

**Lattice Defects**

Hemming et al. (1995b) noted that the distribution coefficient for boron increased dramatically at low boron concentrations, apparently caused by high B affinities for lattice defects in aragonite. At seawater B concentrations, $D_B$ was still significantly nonlinear. These experiments were not carried out in seawater, however, and because the number of these defects is low, and would be quickly saturated by other ions in seawater, this may not be a significant effect.
Authigenic and Diagenetic Phases

Gaillardet and Allègre (1995) observe a decrease in concentration and variability of B in corals from the last interglacial compared with modern corals. They propose that a secondary aragonite of lower B concentration may be forming, or that a labile B-rich biomineralization present in modern corals may be decaying with time, resulting in lower, less variable aragonitic compositions.

Seawater Chemistry

Boron has a long residence time in the ocean (as reviewed in Chester, 1990), and its distribution is therefore conservative (Bruland, 1983). An unpublished study by Alibert and McCulloch demonstrated that B displayed a conservative profile with salinity in the Great Barrier Reef lagoon. Boron also has a very low concentration in river water (Chester, 1990), and variations in oceanic water masses are unlikely, therefore, to have a significant effect on coralline B concentrations.

6.5.3 Fine-Scale Variability

Two studies report that corals appear to have significant high-resolution variability in their B concentrations and isotopic character. Hemming et al. (1995a) analysed small (1-5 mg) chip fragments from within one coral, while Beets and Deloule used an ion-probe to investigate B heterogeneity in different structural elements (K. Beets, pers comm.).

Hemming et al. (1995a) report isotopic variations of 2‰ (equivalent to 0.2 pH units) between chips, which is roughly the magnitude of the changes expected between glacial and Holocene oceans. Since this is significantly larger than pH variations expected from one location over short timescales, they propose that fluctuations in the pH of the precipitating microenvironment associated with changes in growth rate may be responsible for the large shifts in δ¹¹B.

The variations observed by Beets and Deloule were more extreme, with δ¹¹B varying up to 25‰ over small distances (K. Beets, pers comm.). The isotopic variability was confirmed by wet-chemical analyses of small coral fragments. They were able to reproduce δ¹¹B variations along different structures, and believe that vital processes are responsible for this fractionation.
6.6 Uranium

6.6.1 Mechanism of Incorporation

Uranium is incorporated into coral in three main phases (Amiel et al., 1973b). A very small proportion (<0.1%) is complexed to organic material at a concentration around 40 - 70 ppm, between 2% (Amiel et al., 1973b) and 6% (Shen and Dunbar, 1995) is adsorbed; however, the bulk of uranium is bound in the aragonite lattice at a concentration of about 3 ppm.

The mechanism by which uranium incorporates into the aragonitic lattice remains an issue of debate in the literature. Uranium forms an orthorhombic carbonate Rutherfordine (UO$_2$CO$_3$), and substitution of UO$_2^{2+}$ for Ca$^{2+}$ in CaCO$_3$ was considered possible for aragonite (Christ et al., 1955), and foraminiferal calcite (Russell et al., 1996; Russell et al., 1994).

The uranyl cation is quite large, however, being 3.15 Å along its linear axis compared with 1.18 Å for Ca$^{2+}$ (Min et al., 1995). The small cation site in calcite is intolerant to large cations (Russell et al., 1994), although the large 9 coordinate cation site in aragonite is more accepting of larger species. U is observed to be heavily favoured in aragonite over calcite (Meece and Benninger, 1993; Min et al., 1995; Shen and Dunbar, 1995), and this is taken by Min et al. (1995) to be an indication that U occurs as UO$_2^{2+}$ in the aragonite lattice.

In controlled coral growth experiments Swart and Hubbard (1982) observed that the ratio of U to Ca in coral aragonite was independent of the Ca$^{2+}$ concentration of the water in which the corals grew, and deduced that U did not compete with calcium for the aragonite cation site. At seawater pH and carbonate concentration, the levels of UO$_2^{2+}$ in sea water are very low and the speciation of U is largely dominated by the carbonate anion complexes UO$_2$(CO$_3$)$_2^{2-}$ and UO$_2$(CO$_3$)$_3^{4-}$ (Langmuir, 1978). On the basis of this, Swart and Hubbard (1982) postulated that U incorporated into aragonite as a carbonate anion complex competing with CO$_3^{2-}$ for lattice sites.

Shen and Dunbar (1995) examined several possible incorporation mechanisms including UO$_2^{2+}$ substituting for Ca$^{2+}$, UO$_2$(CO$_3$)$_2^{2-}$ substituting for CO$_3^{2-}$ and direct incorporation of UO$_2$CO$_3^0$. On the basis of observed variation in carbonate concentration during upwelling events, they concluded that the likely substituting species is UO$_2$(CO$_3$)$_2^{2-}$. They commented, however, that it was hard to envisage the large UO$_2$(CO$_3$)$_2^{2-}$ species substituting into the small CO$_3^{2-}$ lattice site in aragonite. It is possible that the large uranyl carbonate binds to several lattice sites with a substituent CO$_3^{2-}$ binding first to an anion site, with the uranyl portion occupying what will become
a normal cation site in the growing aragonite crystal. The kinetics of U incorporation would therefore be as an anion competing with \( \text{CO}_3^{2-} \), even though the \( \text{UO}_2^{2+} \) substitutes for \( \text{Ca}^{2+} \).

**Biogenic vs. Inorganic Aragonite**

Coralline aragonite and aragonite precipitated inorganically from seawater have very similar distribution coefficients (table 6.1). This would imply that the calcifying environment within the coral polyp is very similar to that of ambient seawater. In the review of cited distribution coefficients for uranium by Swart and Hubbard (1982), the values for coralline aragonite were not consistently above or below the inorganic distribution coefficient of 1.1 found by Thompson and Livingston (1970). It is generally stated, however, that corals precipitate marginally less U than inorganic aragonite (Meece and Benninger, 1993; Min et al., 1995; Thompson and Livingston, 1970).

**6.6.2 Factors Affecting Partitioning**

**Carbonate Concentration, pH, and pCO\(_2\)**

**Solution Equilibria**

In seawater, uranium exists mostly as various carbonate species. The speciation is strongly dependent on the concentration of carbonate in the solution (equations 6.4 to 6.6), hence both pH and pCO\(_2\) affect the distribution of U species.

\[
\begin{align*}
\text{UO}_2^{2+} + \text{CO}_3^{2-} & \rightleftharpoons \text{UO}_2\text{CO}_3^0 \quad (6.4) \\
\text{UO}_2\text{CO}_3^0 + \text{CO}_3^{2-} & \rightleftharpoons \text{UO}_2(\text{CO}_3)^2^{2-} \quad (6.5) \\
\text{UO}_2(\text{CO}_3)^2^{2-} + \text{CO}_3^{2-} & \rightleftharpoons \text{UO}_2(\text{CO}_3)^3^{4-} \quad (6.6)
\end{align*}
\]

Raising the solution pH raises the carbonate ion concentration (equations 5.5 and 5.6), and figure 6.3 presents the activity of the various U species in solution as a function of pH. At ambient seawater the distribution of uranium is roughly 68% \( \text{UO}_2(\text{CO}_3)^2^{2-} \), and 32% \( \text{UO}_2(\text{CO}_3)^3^{4-} \) (Swart and Hubbard, 1982), with the balance shifting towards \( \text{UO}_2(\text{CO}_3)^3^{4-} \) species as pH and pCO\(_2\) increases.

**pH Model**

If U incorporates into aragonite as \( \text{UO}_2(\text{CO}_3)^2^{2-} \) substituting for \( \text{CO}_3^{2-} \), factors that change the pH or carbonate ion concentration of the precipitating solution would be expected to change the U concentration in the precipitated aragonite. As with boron, changes in the speciation of U must be considered together with changes in the carbonate ion concentration, as the ratio of \( \text{UO}_2(\text{CO}_3)^2^{2-}/\text{CO}_3^{2-} \) controls the incorporation of U into the aragonite crystal. Figure 6.4 presents the U to carbon anion ratios derived from
the two speciation models (see appendix B for details). As before, model A assumes equilibrium between the solution and CO_{2} in the atmosphere, while model B assumes that the precipitating solution is isolated and that the \Sigma C remains constant. Figures 6.4(a) and (c) display the UO_{2}(CO_{3})_{2}^{2-/CO_{3}^{2-}} ratio, while figure 6.4(b) and (d) display the UO_{2}(CO_{3})_{2}^{2-/CO_{3}^{2-} + HCO_{3}^{-}} ratios.

### Seawater Uranium Speciation with pH

![Uranium Speciation in Seawater with pH](image_url)

**Figure 6.3 Uranium Speciation in Seawater with pH**

Activity of uranium species in a seawater-composition solution as a function of pH. Details of the models used to generate this data are presented in appendix B. While this data is calculated using model A (solution in equilibrium with atmospheric CO_{2}), the results are qualitatively similar for model B. The grey dashed line represents normal seawater pH (8.2). The shaded region spans the range of pHs in the precipitating fluid predicted by McConnaughey's calcification models (1986). The heavy line represents the activity of UO_{2}(CO_{3})_{2}^{2-} which is believed to be the species incorporating into coralline aragonite. Note that the activity of this species decreases with pH over the range of pHs likely to occur in the calcifying fluid.
Figure 6.4. Uranium to Carbon Ratios with pH
Models of $\text{UO}_2(\text{CO}_3)^{2-}/\text{CO}_3^{2-}$ ratio to $\text{CO}_3^{2-}$ and $(\text{CO}_3^{2-} + \text{HCO}_3^-)$ in seawater as a function of pH. Details of the models are presented in appendix B. The grey dashed line represents normal seawater pH (8.2). The shaded region spans the range of pHs in the precipitating fluid predicted by McConnaughey’s calcification models (1986). $\text{UO}_2(\text{CO}_3)^{2-}$ is the species believed to incorporate into aragonite, competing with carbonate and/or bicarbonate for anion lattice sites. Thus the ratio of $\text{UO}_2(\text{CO}_3)^{2-}$ to carbon anion controls the $\text{U}/\text{CO}_3^{2-}$ ratio in the precipitating aragonite. Models A and B as described for figure 6.2. Graphs (a) and (c) present the ratio to carbonate. Graphs (b) and (d) present the ratio to carbonate plus bicarbonate. Note that in all cases the ratio decreases sharply with increasing pH.

In each case the U/anion ratio decreases as pH increases. Increasing pH shifts the C speciation towards higher $\text{CO}_3^{2-}$ concentrations, which has the effect of 'diluting' the uranium. In addition, at the alkaline pHs considered here, increasing $\text{CO}_3^{2-}$-
concentrations shifts the solution speciation from $\text{UO}_2(\text{CO}_3)_2^{2-}$ to $\text{UO}_2(\text{CO}_3)_3^{4-}$ (see figure 6.3). Thus the concentration of the species believed to incorporate into aragonite is decreasing as the concentration of carbonate increases. The $\text{UO}_2(\text{CO}_3)_2^{2-}$/anion ratio therefore decreases strongly as pHs rise in all of the models.

**Oceanic pH and Alkalinity**

Many researchers predict that oceanic pH may affect U incorporation into corals. Because pH is relatively constant in seawater, however, the only reports of U variation with pH come from inorganic precipitation experiments. Meece and Benninger (1993) report an inverse relationship between U/Ca and pH, consistent with the notion that the incorporating species is $\text{UO}_2(\text{CO}_3)_2^{2-}$, and that increasing pH shifts the equilibrium in favour of $\text{UO}_2(\text{CO}_3)_3^{4-}$. In these experiments, however, increasing pH was confounded with an associated increase in growth rate.

While generally predicted, few people report seeing an unambiguous variation of U/Ca with carbonate ion concentration in the oceans. Russell et al. (1994) failed to observe the expected difference between U/Ca in benthic foraminifera collected from the Pacific ([CO$_3^{2-}$] about 80 μmol/mol) and Atlantic ([CO$_3^{2-}$] about 110 μmol/mol). In a later study, Russell et al. (1996) observed lower foraminiferal U/Ca ratios during the last glacial maximum when CO$_3^{2-}$ levels were higher than interglacial levels (consistent with UO$_2^{2+}$ decreasing as equilibria shift more towards carbonate species). A parallel shift was observed in Mg/Ca, however, and because the speciation of Mg is believed to be independent of carbonate concentration, the U/Ca changes were attributed to temperature effects. Min et al. (1995) failed to see variations between modern and fossil corals that were anticipated on the basis of higher atmospheric pCO$_2$ today compared with the last deglaciation.

**Biological Factors**

Swart and Hubbard (1982), Cross and Cross (1983), and Meece and Benninger (1993) all conclude that coral U/Ca is primarily under physiological control, and Russell et al. (1994) propose that foraminiferal U/Ca may be controlled by the pH of the organism's calcifying fluid. Physiological models for calcification (McConnaughey, 1989b and see chapter 5, section 5.4; McConnaughey, 1986) suggest that enzymatic ion pumping by ATPase controls pH and Ca$^{2+}$ concentrations in the calcifying fluid, while the raised pH and the CO$_2$ diffusing into the calcifying fluid controls the CO$_3^{2-}$ concentration. Thus it is possible that the concentrations of all these species are buffered or controlled by the coral's physiology, and that changes to the pH or carbonate ion concentration in the seawater are 'overwritten' by the effects of coral biochemistry. A similar idea was proposed by Min et al. (1995) to resolve the paradox between their thermodynamic model, which relies on temperature dependence of carbonate speciation reactions to
explain the overall temperature dependence, and their failure to observe any difference between corals growing in waters of different carbonate concentration.

If enhanced calcification in corals is associated with raised pH in the precipitating microenvironment, then McConnaughey's models (1989b; 1986) and the two U speciation models presented above predict that coralline U should be strongly diluted relative to aragonite precipitated inorganically. This dilution should become greater as calcification rates in corals increase. It appears, however, that the degree of dilution predicted is not observed in corals. McConnaughey's model predicts that physiological pumping accounts for between 49% and 89% of skeletal carbon assuming a precipitating micro-environment with a pH of 8.5 to 9.0. This would dilute skeletal U by between 2 and 9 times, (not counting any changes in speciation). The lowest distribution coefficient presented in the summary by Swart and Hubbard (1982) is 0.51 (table 6.1), which is a little over 50% of the inorganic value of 1.1. This would indicate that the pH in the calcifying fluid can be no greater than 8.5. As with boron, it appears that the current theories regarding trace element incorporation and chemical calcification cannot explain the U partitioning observed in corals.

The lack of dilution may indicate that some U species competes with Ca\(^{2+}\) rather than CO\(_3^{2-}\). In this case a distribution coefficient of 0.51 would suggest a calcifying pH approaching 9.0 according to McConnaughey's models.

**Calcification and Growth Rate**

In general coral U/Ca is remarkably constant across species (Shen and Dunbar, 1995; Thompson and Livingston, 1970), and taxonomic effects are not great. A number of authors, however, have called upon taxonomic or physiological effects to explain discrepancies in the U concentrations between one coral and another. Different rates of calcification have been proposed as one factor contributing to differences in U/Ca between different coral species. Thompson and Livingstone (1970) suggested that zooxanthallae-enhanced calcification rates were responsible for lower U/Ca seen in zooxanthellate corals compared with the slower growing non-zooxanthellate corals and zooxanthellate corals growing without zooxanthallae. These findings support a statement by Meece and Benninger (1993) that lattice compatible 2+ cations tend to decrease in concentration in crystals as crystallisation rate increases.

Several researchers have attempted to correlate U/Ca ratios with changes in skeletal extension rate, assuming that extension and calcification are linked. Schroeder et al. (1970) attributed high U/Ca bands, found subparallel to the growth surface, to periods of slow skeletal growth. In contrast, Swart and Hubbard (1982) found no consistent inverse correlation between growth and U/Ca and noted several cases where the opposite trend was seen. Cross and Cross (1983) similarly noted lower U/Ca ratios associated
with slower growth. Shen and Dunbar (1995) measured the U/Ca along different growth axes in individual colonies and concluded that growth rate influences on coral U/Ca are small at best, even between transects where growth rates vary by a factor of 3. These ambiguous results may derive from the fact that while extension and calcification are linked through skeletal density, they may vary independently of each other (see equation 6.2). Thus, extension is not necessarily a good analogue for calcification rate.

Temperature

Thompson and Livingstone (1970) saw no difference in the U/Ca between corals taken from deep water and shallow water, and concluded that there was no temperature dependence on the partitioning of U and Ca in aragonite. Recent work, however, has demonstrated a clear seasonality which appears to vary inversely with SST (Min et al., 1995; Shen and Dunbar, 1995). Min et al. (1995) model U incorporation and conclude that the temperature dependence derives from changes to the equilibrium equations governing seawater carbonate speciation. Shen and Dunbar (1995), however, caution that seasonality may be associated with different carbonate concentrations in upwelled deep-sea water. It should also be remembered that at the rates corals calcify, equilibrium behaviour may not be valid (appendix A).

Salinity

Shen and Dunbar (1995) observed variations in U/Ca in a coral from Tarawa atoll that were not associated with seasonal or natural variability in the local seawater U/Ca ratio (which is limited to ~3%). Their explanation called upon results from the controlled growth experiments of Swart and Hubbard (1982), in which it was found that U/Ca in coralline aragonite varied in proportion to the absolute concentration of U rather than the ratio of U to Ca in the water. Thus, Shen and Dunbar (1995) postulated that salinity variations of up to 10% associated with heavy rainfall were responsible for lowering the U concentration in the seawater, and hence the U incorporated into the corals.

Swart and Hubbard (1982), however, did not test the U/Ca growth of corals at different salinity. They doubled Ca\(^{2+}\) in the seawater in which the corals were growing and saw no change in the U/Ca ratio of the corals. It is incorrect of Shen and Dunbar (1995) to extrapolate this same behaviour to changes in salinity, where all species in solution are diluted proportionally. If it is accepted that U incorporates as UO\(_2\)(CO\(_3\))\(_2\)^{2-}\), substituting for CO\(_3^{2-}\) in an anion site, then doubling the Ca\(^{2+}\) concentration in a solution will 'dilute' trace cations by a factor of two, but will not affect the U/CO\(_3^{2-}\) ratio. Because Ca is still the major cation constituent of aragonite, its absolute concentration in the crystal is relatively uniform despite addition of Ca\(^{2+}\) to the solution. The U/Ca ratio in the crystal will remain almost unchanged, even though the U/Ca\(^{2+}\) ratio in the solution halves. This is what Swart and Hubbard observed, and what led Shen and Dunbar (1995) to
conclude that the absolute concentration of U controlled the incorporation rather than the U/Ca ratio. If this were true, then a two-fold conservative dilution of seawater would halve the concentration of U, and therefore halve the U in the crystal. In reality, conservative dilution will leave the U/CO$_3^{2-}$ ratio unchanged and the partitioning of U into the aragonite will not vary. This treatment ignores the complexities of changing speciation, calcification rate, or activity coefficients as seawater is diluted conservatively; however, dilutions from rainfall or river floods are expected to be at most about 25%, and these effects are therefore likely to be minor.

Physiological buffering of the saturation state in the coral may, however, lead to a salinity dependent U partitioning, as described in section 5.7.4.

### 6.6.3 Fine-Scale Variability

Fine-scale heterogeneities in the U/Ca distribution in some corals have been reported. Using fission-track analysis, Schroeder et al. (1970) found 3 types of heterogeneities: an enrichment along the outside surface of structural elements that may relate to an adsorbed U phase, a series of enriched U bands sub-parallel to the growth surface that were believed to relate to periods of slower coral growth, and finally a random heterogeneity inside structural elements on a scale of about 100 μm. Swart and Hubbard (1982) did not find the zonal heterogeneity reported by Schroeder et al., but noted the tendency for U to exchange with, and be precipitated along, skeletal margins. They also reported small-scale uranium enrichments associated with boring sponges in the skeletons of dead corals. Shen and Dunbar (1995) observed that traces of inorganic carbonate cements found in corals contained up to 3.5 times more uranium than the skeletal aragonite, while Amiel et al. (1973b) report that traces of organic material in coral skeletons contain between 13 and 23 times more U than the aragonite. Min et al. (1995) noted significant small-scale heterogeneity and attributed this to localised concentrations of a high U phase species such as organic material.
CHAPTER 7: TRACE ELEMENTS IN CORALS AT HIGH RESOLUTION

7.1 Introduction

It is evident from the review presented in the preceding two chapters that there is much that still remains unknown about the physiology and chemistry of trace element incorporation into coral aragonite. The coral literature contains many contradictory reports about the environmental and biological factors controlling trace element incorporation, and the dominant models of calcification chemistry do not account for a number of critical observations of trace element behaviour. Resolving the underlying chemistry of trace element coprecipitation is therefore important, and will lead to a greater understanding of calcification physiology, and the environmental factors that might influence a trace element signal.

Seasonal-scale variations in skeletal trace elements have been recognised for several decades. Shorter time-scale variations, however, are only now being considered as significant, despite being reported in the literature as early as 1970 (e.g. Schroeder et al., 1970). Interest in what causes these fine-scale variations has increased with the advent of micro-beam analytical techniques, such as ion microprobe and laser ablation.

'Fine-scale' trace element variations are arbitrarily defined as fluctuations occurring on distance scales corresponding to less than one month's coral growth. These features tend not to be reproducible in analytical traverses separated by small distances (> 1.0 mm). It is therefore easy to dismiss fine-scale variations as 'noise', and to remove them by smoothing or filtering the signal. While these fluctuations may only carry limited information about environmental factors, they are ubiquitous in corals, can be very large in magnitude, are resistant to cleaning, and are intimately reproducible in analytical traverses over the same track (figure 7.1). This implies that fine-scale variations are inherent to the aragonite, and represent real compositional or physical heterogeneities in the coral skeleton.

There are a number of factors that may contribute to fine-scale variations, and these have been summarised in Chapter 6. The most thorough investigations (e.g. Allison, 1996a; Allison, 1996b; Allison and Tudhope, 1992; Hart and Cohen, 1996; Hart et al., 1997) have identified randomly dispersed phases (such as detrital particles, organic material,
microborings, and centres of calcification) as being significant contributors to the variations. It has been suggested, however, that some fine-scale fluctuations are reproducible in different structural elements within a corallite (K. Beets, pers. comm.), which suggests that an environmental or physiological factor induces polyp-wide trace element variations. Investigating the fine-scale variations will therefore help to elucidate the underlying chemical and physiological processes that are occurring within a coral polyp.

Fine-Scale Reproducibility

![Graph showing B/Ca profiles for two replicate laser analyses.](image)

**Figure 7.1: Reproducibility of Coral Trace Element Profiles**

This figure presents two replicate laser analyses over the same section of coral. The B/Ca profiles for the two traverses match in detail right down to their highest resolutions (one data point, or $1/30$ mm). This reproducibility clearly demonstrates that the fine-scale fluctuations are not random noise, but are linked to compositional or structural features in the coral skeleton. The laser spot used here was a large slit, 600 x 50 μm; however, the results are similar when using a smaller laser beam.

A number of experiments offer insight into the behaviour of trace elements at very high resolutions. Fractionation of trace elements when ablating material from holes in the coral structure is studied in section 7.2. Sections 7.3 and 7.4 present investigations of fine-scale variation in relation to coral structure. These analyses employ a small (70 μm) diameter laser spot that is steered around holes and pores in the coral structure. In
Section 7.5 the fine-scale data is combined with some of the observations of trace element variation at seasonal scales presented in Chapter 8, to provide insights into the chemical and biological basis of fine-scale variability and coral calcification processes.

### 7.2 Ablation at Depth

Individual structural elements in *Porites* can range in size from less than 100 µm for columellae to greater than 400 µm for corallite walls, while pores between synapticulae are around 300 µm diameter. The coral is not impregnated with a void-filling matrix, and due to the long focal-length optics, the laser can ablate material from the bottom of pits and holes. Eggins et al. (1998) showed that transport efficiency to the ICP decreased, and fractionation effects associated with differential condensation of refractory and volatile elements increased, when ablating material from holes with high aspect ratios. It is therefore possible that fine-scale variability in the trace element signals is a function of the coral topology and porosity.

Although care was taken to navigate the laser around holes and pores in the coral skeleton during the high resolution analysis presented in the next sections, this was not always possible. The large laser spot used for other analyses (see following chapters) was, by design, bigger than a single structural element. All of these analyses would therefore be affected by depth ablation effects. An experiment was therefore set up to examine the effects of ablation from holes, and to assess the degree of element fractionation that might result.

#### 7.2.1 Experimental

Three fine holes with a diameter of around 300 µm and 500 µm deep were drilled into the surface of one of the coral composition CaSiO₃ glass standards to simulate a hole within a coral. The glass was cleaned by ultrasonic agitation in dilute Decon-90, followed by 18 MΩ water and then clean ethanol, and then preablated by laser to expose a fresh surface prior to analysis. The laser was scanned at 1/30 mm·s⁻¹ over the top of the three holes, and the major trace elements were analysed (¹¹B, ²⁵Mg, ⁴³Ca, ⁸⁴Sr, ¹³⁸Ba and ²³⁸U). Several beam sizes were used: the data presented here used the 50 µm x 600 µm beam; however, analyses using the 70 µm spot are qualitatively similar.

#### 7.2.2 Results and Discussion

The trace element profiles from the analyses of the holes is presented in Figure 7.2. Raw count rates decreased by around 50% when ablating material from the bottom of the
hole. When normalised to calcium, however, the profiles show essentially no perturbation except for U/Ca which increases by around 6% when ablating into the hole. This is consistent with the work of Eggins et al. (1998), who found that during ablation, refractory elements (such as uranium) can condense onto the sides of holes with a large aspect ratio. Hot ablation plasmas can subsequently re-mobilise these condensed debris leading to relative enrichments in refractory elements.

Ablation at Depth

![Graph showing trace element variations associated with depth ablation.](image)

Figure 7.2 Trace Element Variations Associated with Depth Ablation. Raw and calcium-normalised profiles generated by tracking the laser across three holes drilled into the wollastonite glass. The raw counts for each element (black line) drop to nearly half their maximum when ablating from depth. When normalised to calcium, however, these variations are almost zero, with the exception of U/Ca which increases by about 6%.

While straight-sided holes drilled into glass are not a particularly accurate model for coral porosity it is nonetheless concluded that with the exception of uranium, fractionation of elements ablated from depth is not a significant factor in contributing to the fine-scale variability in the coral trace element profiles. This result indicates that the trace element signal intensity should faithfully represent the composition of the material being ablated at any point in time.
This does not mean that depth ablation does not cause some high resolution variations. If the material at the bottom of a hole is a different composition to the material at the top of a hole (e.g. because it is a different age), then ablating into a hole could be associated with a sudden change in the intensity of the trace element signal.

7.3 Single Structural Elements

To eliminate the possibility that holes might be the cause of high resolution variability, a number of coral analyses were made using a small diameter (70 μm) laser spot which was steered around pores and holes in the coral structure. In addition to eliminating topographic effects, the small spot resulted in considerably less smoothing of the fine-scale variations compared with the large laser spot.

7.3.1 Experimental

Samples

Two Porites corals were analysed with the small laser spot. The first came from Davies Reef, and details of the location are presented in chapter 8. This coral is labelled Davies 1B, and should not be confused with the coral analysed in chapter 8 which is labelled Davies 2. The second coral was collected from Orpheus Island, and details of this coral can be found in chapter 10.

Preparation

The Davies 1B coral was carefully sectioned parallel to the direction of corallite growth, to expose linear structural elements representing cross sections through individual corallites. On this carefully prepared section it was possible to follow single structural features over distances of 6 - 8 mm (see figure 7.3). This section was cut close to the top of the coral, approximately 3-4 cm below the tissue layer. The piece of the Orpheus coral analysed was taken from a slab cut by the Australian Institute of Marine Science (AIMS), which was fortuitously sectioned very parallel to corallite growth. Similar linear structures to the Davies coral could be seen in this sample. Two parallel columellae were found, extending over about 3 mm (figure 7.4). The Davies and Orpheus sections were cleaned by high intensity ultrasonic probe in 18 MΩ water, and dried in a clean oven overnight.
Figure 7.3 Structural Elements Analysed in Davies 1B Coral. These images show the structural elements that were analysed at high spatial resolution. A 70 μm laser spot was tracked along the structures, avoiding pores and holes in the skeleton whenever possible. The tips of the white arrows indicate the beginning and end of each laser traverse. Traverses 1 & 2, and 6 & 7 are pairs of analyses made on different structural elements within a corallite. The image at the bottom right shows the section of coral analysed (note the different scale bar).
Figure 7.4 Structural Elements Analysed in Orpheus Island Coral. These two adjacent septa within the one corallite were analysed using a 70 µm laser spot. The tips of the white arrows indicate the beginning and end of each laser traverse. The width of the laser spot is indicated at the bottom of the figure as a small circle. The laser was steered around holes wherever possible in tracks 8 and 9. Track 10 ran down the line of holes, and the data is not presented here.
Chapter 7

Photography

The Davies coral section was photographed using a macro lens under a strong light-source shining obliquely across the surface to reveal as much detail as possible of the surface topology (figure 7.3). This image was scanned at the maximum possible resolution (resulting in around 120 pixels per millimetre on the coral skeleton) using a Nikon slide scanner and Adobe Photoshop software. Images of the Orpheus Island coral traverses were captured directly from the CCD video camera at the time of analysis (figure 7.4).

The digital image of the Davies coral was spatially calibrated against measurements taken from the edges and corners of the coral section using NIH image. After each laser analysis, the XY co-ordinates at the beginning and end of each traverse were recorded relative to the cut edges and corners of the coral. This allowed each laser analysis to be located on the photograph to within ± 5 - 8 pixels (42 - 67 μm).

Two types of linear structure can be distinguished in the coral. The thicker structures, which are relatively free of bumps, are likely to be corallite walls. Sometimes these structures form wide, relatively featureless expanses of coral (e.g. see run '5' in figure 7.3), which are interpreted as being oblique sections of the calyx wall. In contrast, the narrow features with a spongy or knobbled appearance are probably sections of septa and cross-connecting synapticulae (e.g. figure 7.4). Both structures are illustrated schematically in figure 7.5. The coral sections are not cut perfectly parallel to growth, and individual corallites seldom follow a perfectly linear growth vector. As a consequence, septa will often merge into a corallite wall, or 'run out' into a series of humps (synapticulae). No attempt was made to systematically sample one or other type of structural element, although corallite walls were often the easiest structures to track over any great distance.

Laser Analysis

The coral sections were analysed for $^{11}$B, $^{25}$Mg, $^{43}$Ca, $^{84}$Sr, $^{138}$Ba and $^{238}$U using a 70 μm diameter circular laser spot pulsing at 10 Hz. The laser was tracked over a single structural element, taking care wherever possible to avoid ablating into holes or pores in the coral structure. A linear motor attached to one axis scanned the laser at a constant rate in one dimension (usually $1/30 \text{ mms}^{-1}$), while the other dimension was adjusted by hand to prevent the laser beam from straying off the selected structural element.

On the Davies coral, seven linear structural elements were analysed, and these are labelled 1 - 7 in figure 7.3. Of these 7 analyses, there were 3 sets of paired analyses (runs 1 & 2, 4 & 5, and 6 & 7), where structural features within 1-3 mm of each other were compared. Runs 1 & 2 were separated by about 0.9 mm and both structures

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High Resolution Analysis of Corals

appeared to be walls of the same corallite. Similarly runs 4 and 5 were both on corallite walls; however, at a separation of 2.9 mm they were clearly not the same corallite. Runs 6 & 7 were 0.5 mm apart and while run 7 was along a corallite wall, run 6 traversed the lattice of septae and synapticulae that occurred within the corallite.

Cross Section Through a Corallite

![Cross Section Through a Corallite](image)

Figure 7.5 Schematic Cross Section Through a Corallite. This sketch illustrates the different structures exposed during a section through a corallite cut parallel to the growth direction. Wide structures are corallite walls, thin structures are septae, and 'knobbled' structures are sections through cross-connecting synapticulae.

On the Orpheus Island coral, three high resolution analyses were made on linear structures, labelled 8 - 10. In contrast to the Davies analyses the laser was scanned at half the speed (around $\frac{1}{60}$ mms$^{-1}$). The structures examined were approximately 3 mm long, and 330 μm apart. They seem to occur within one corallite, and appear to be two septae, separated by cross-connecting synapticulae (see figure 7.4). One analysis was made along each septa, and the third parallel analysis tracked over the synapticulae separating the septae.
7.3.2 Results

An example of the type of data collected is presented in figure 7.6. Data is collected once each second. During this time the laser has traversed 33 µm, corresponding to approximately one day's coral growth. High resolution analysis of the coral sections reveal considerable fine-scale variation in the trace element profiles over small distance scales. The high-resolution structure in the data appears to the eye to occur on several relatively distinct widths, although it is not clear whether these variations are periodic. By far the most common 'width' of trace element feature was around 260 µm. This represents approximately 6 - 7 days of coral growth (an extension rate of 12 - 15 mmyr⁻¹ was estimated from density banding). More significantly, however, this appears to correlate with the average spacing occurring between synapticulae (see figure 7.4 and discussion below). Variations ranged up to about 450 µm or 11 days growth. There are also a range of broader-scale features in the trace element profiles, and variations centred at approximately 600 µm (15 days), and 1.1 mm (28 days) were the next most common features seen.

7.3.3 Discussion

Periodicity

Initial observations of the data identified occasional regions of quasi-periodicity. These cycles appeared to range from roughly a week to a month. A more careful visual inspection, however, failed to reveal any consistent periodicity. The data was objectively analysed for cyclicity using autocorrelation (the individual time-series were too short for windowed fourier analysis). Autocorrelation confirmed what was apparent by eye: while small regions of approximately regular periodicity occurred in the coral, these patterns did not extend for more than a couple of millimetres, and both phase and wavelength were not constant with distance.

There are several possible explanations for this inconsistent periodicity. It is possible that these variations are periodic in time (cued by some environmental or endogenous parameter), but are distorted by a non-uniform growth rate. Corals display a seasonally varying extension (e.g. Alibert and McCulloch, 1997), that can sometimes be extreme, with corals shutting down growth completely (Fallon et al., 1998). The poor correlations would require that variation in extension occurs over timescales of weeks to months. Alibert and McCulloch did observe subannual variability in extension in a Davies Reef coral (Alibert and McCulloch, 1997).
Figure 7.6 Example of a Fine Scale Trace Element Signal.
This figure demonstrates the type of data collected during the high resolution analysis, and the width-scales of the variations observed. Note the variations over distances of 250 - 1000 µm which correspond to approximately 1 week to 1 month growth.

A second possibility is that some of the variations seen are artifacts of the smoothing caused by the laser spot: for a coral growing at about 13 mm yr⁻¹, the average daily growth increment is about 35 µm. The 70 µm diameter laser spot therefore spans approximately 2 days growth; however, data points are collected each 33.3 µm. Now, diurnal variations in feeding behaviour and calcification rate may generate large daily TE/Ca variations. If the laser spans several of these cycles, then it will smooth the signal, but its passage across the coral may also generate 'beat' cycles of a different frequency. The weekly cycles of trace elements could be explained this way, although it would be harder to produce the monthly variations. The variability seen in the signal may be caused by slight variations in the daily extension rate. An analysis at a resolution higher than a single day's growth (e.g. 20 µm spot, 1/60 mm s⁻¹) may prove enlightening.

The final possibility is that the variations are associated with the presence of adjacent structural features, which display some regularity in their spacing, but no consistent cyclicity.

Correlation with Structure
Attempts were made to visually correlate features in the trace element profiles with structures visible on the images. In some cases broad-scale (defined loosely as features spanning more than 1/2 mm) shifts in trace element composition were tentatively associated with regions where septae merged with the corallite wall. There was,
however, no consistent relationship between the chemistry of the coral and the presence of adjacent structural features. Many significant changes in the trace element profiles appeared to be associated with absolutely featureless regions of the coral skeleton. It should be noted, however, that with the sample preparation procedures used here it is not possible to distinguish the presence of microborings or centres of calcification as other high-resolution coral researchers have done (e.g. Allison, 1996b; Hart and Cohen, 1996).

Very fine scale fluctuations (< 0.5 mm) in the trace element composition were even harder to assign to skeletal features. The only case where such variations could be linked to particular structures was in the analyses of the two closely spaced septae in the Orpheus Island coral. There it was possible to make out some regions of approximately weekly periodicity (260 μm) in the Ba and U signals, that seemed to correlate with the presence of adjacent synapticulae (see figure 7.7). Although similar scale fluctuations were ubiquitous in other analyses, they did not clearly occur in association with synapticulae, and again sometimes featureless regions of coral displayed variations. This poor correlation is not caused by an inaccuracy in the alignment of the laser traverses on the image, as it was possible to precisely correlate large drops in the raw-count signal for $^{43}\text{Ca}$ with the appearance of holes along the structure being analysed.

In general it has not been possible to unambiguously assign trace element variations to structural features observed in the coral, although careful selection of sample sections and higher resolution analysis in the future may reveal a more consistent behaviour.

**Correlation Within a Corallite**

Beets and Deloule (K. Beets, pers. comm.) reported that fine-scale features in the $^{81}\text{B}$ and Sr/Ca in a coral were reproducible on adjacent skeletal structures. Inspection of the pairs trace element profiles (1 & 2, 6 & 7, 8 & 9) did not reveal clear features in common between analyses taken from the same corallite. Cross-correlation analyses were therefore carried out in an attempt to identify common structures within their data sets. The phase-lags determined from these cross-correlations were then compared with the offsets between analyses estimated by measuring the relative positions of each laser traverse on the images.

In general the cross correlations were weak, confirming the lack of any clear correlation seen by eye. It was usually possible to detect a broad trend towards a phase-lag roughly corresponding to the expected vertical offsets between pairs of analyses. This correlation, however, appeared to result primarily from very broad (3 - 4 mm) trends in the data, rather than alignment of fine-scale (weekly to monthly) features. Often each trace element within a pair of analyses would indicate contradictory phase-lags, and it was difficult to find an unambiguous alignment of the pairs of analyses. Sometimes
subsections of a data set displayed cross-correlation (e.g. figure 7.8); however, there were no cases where a good correlation was found across an entire data set, even in the two Orpheus Island analyses.

**Correlation with Structural Features**

![Diagram](image)

*Figure 7.7 Relationship Between Structure and Trace Element Profiles.*

Ba/Ca variations in the Orpheus Island coral. Barium appears to vary on width-scales similar to the spacing of the synapticulae (approximately 260 μm - see figure 7.4 for scale). Troughs in Ba/Ca often appear to correspond to the presence of adjacent synapticulae (dashed vertical lines), but the correlation is not perfect. Other coral analyses do not reveal any consistent trend.

Tracks 6 and 7 represent structures within the same corallite; however, run 6 traversed the spongy septa/synapticulae mesh, and as a consequence the laser was not ablating a smooth flat growth surface. The variability that this introduces into the data may be confounding any trace element fluctuations in common with run 7. Runs 1 and 2 were taken from the same corallite, and were largely free from interference from holes in the structure, yet cross-correlations of the paired trace element profiles were still poor. The failure to see any clear correlations between these pairs is taken to be an indication that trace element fluctuations are not occurring on a consistent horizon in a corallite.

Beets and Deloule (K. Beets, pers. comm.) attributed their correlations between fine-scale features in adjacent coral structures to synchronous changes in the chemistry or physiology of a whole coral polyp. The data presented here does not appear to support this observation. In each of the pairs of analyses presented here there were features that could be matched up between the profiles; however, these appear to be chance alignments, as other features in the data-sets did not match. The correlation observed by Beets and Deloule may therefore have been a fortuitous occurrence, and conclusions based on their isolated observation should be treated with caution.
Correlation Between Parallel Tracks

Figure 7.8 Trace Elements Correlated Between Parallel Structures. Trace element profiles measured on parallel structural elements (tracks 1 and 2: see figure 7.3). These profiles have been aligned using cross-correlation analysis. The resulting offsets reasonably matched the offsets predicted from the geometry of the two traverses (approximately 0.6 mm). The correlations between the traverses are generally poor for each trace element, and while some regions appear to display a good correlation (e.g. between 4 and 6 mm), others do not.

Summary

The high resolution trace element variations in the coral structures occur on several regular width-scales, but show no clear periodic behaviour or persistence within one corallite. The most common width of feature corresponded roughly to the spacing of synapticulae, but the presence or absence of synapticulae did not always correlate with
the presence or absence of these trace element features. Some sharp changes in trace element composition appeared to be associated with the traverse of the laser from one type of structural element onto another; however, corallite walls and septae did not appear to show any consistent differences in trace element composition, and some large changes appeared to occur in featureless regions of coral. The cause of these changes remains unexplained; however, a number of possibilities are explored in section 7.5.

7.4 Calyx Cross Section

7.4.1 Experimental

A section of the Davies 1B coral was cut perpendicular to the direction of growth to expose an end-section through the corallites. Cleaning, photographic, and analytical procedures are as described in section 7.3.1, with the exception that the photographic resolution was approximately 180 pixels per mm. A single calyx cross-section was selected, and two traverses (scan 1 and scan 2) were made across the diameter of the corallite (see figure 7.9).

7.4.2 Results

The trace element profiles across the corallite displayed some features that appeared to be symmetrical on either side of the centre of the calyx (see figure 7.9). In particular, Ba and Mg appear to peak somewhere between the division and the corallite wall, and again in the centre of the calyx. Uranium displays similar variation but in anti phase. Some symmetric variation across a calyx might be expected: the laser traverse to the centre of the calyx must have tracked across the corallite wall, septa, pali and synapticulae, with the same happening in reverse on the other side.

7.4.3 Discussion

The growth-surface of a coral is not perfectly flat, and each polyp grows in a U-shaped calyx. Any horizontal cross-section through a corallite will therefore expose zones of different age. Figure 7.10 illustrates a typical growth profile for a Porites calyx (from Veron, 1986). In a cross-section, the central pali structures, and the walls of the coral calyx will form the oldest material, with two rings of younger material representing the division and the synapticulae. If the trace element composition of the aragonite is changing with time, the different ages of the various structures will give rise to a 'bulls-
eye' pattern of concentrically varying composition. This may explain the symmetric variation seen in the trace element profiles.

Scans Across Calyx

Figure 7.9 Scans Across an End Section of a Corallite.
Two analyses were carried out across a single coral calyx using a 70 μm diameter laser spot, avoiding holes and pores. Note that the axes are uncalibrated TE/Ca ratios. The profiles of Mg and Ba are presented. The shaded regions on the graphs correspond to the position of corallite walls, and columellae. While the profiles appear to display a symmetry across the calyx in scan 1 (bottom), closer inspection reveals that the trace element variations do not correlate with the same structures on either side of the calyx centre.

The concentric symmetry of the trace element profiles is not especially good when matched to the image of the calyx, however. The peaks in Ba and Mg do not correspond to the same structures on either side of the calyx in scan 1 (see figure 7.9). This may be partly due to a number of holes encountered by the laser during scan 1, or it may represent inaccuracy in the alignment of the laser traverses on the image. Two independent attempts to locate the laser profiles on the image came up with alignments that were essentially identical however, indicating that the errors in the alignment are minimal.
Growth Profile of a *Porites* Coral Calyx

Calyx Diameter
\[ \sim 1.0 \text{ mm} \]

Calyx Depth
\[ \sim 0.5 \text{ mm} \]

Figure 7.10 Growth Profile of a Coral Calyx.

The growth surface of a coral is not perfectly flat, and the polyp sits in a U-shaped depression called a calyx. The horizon of constant age is therefore convoluted, and horizontally adjacent structures may not be exactly the same age. If the composition of the aragonite being laid down by the coral is changing with time, then a horizontal section through a corallite should reveal concentrically varying patterns of different composition.

The magnitude of the fluctuations observed is too large for the model to account for. If a coral is growing at approximately 15 mm per year, and the calyx is about 0.5 mm deep, then the youngest material encountered in a vertical cross section would be only about two weeks younger than the oldest material. Assuming a 6°C seasonal temperature cycle, the youngest material would have a Mg composition that is (at most) about 3% different from the oldest material (using the temperature calibrations presented in chapter 8). The range of magnesium variation seen in the profiles is close to 50%.

It therefore appears as though the trace element variations observed are not solely caused by the U-shaped calyx profile and seasonal variations in the composition of the aragonite. It also appears that the trace element fluctuations are not synchronous across a calyx, in agreement with the results presented in section 7.3. If corals display high frequency (daily to fortnightly), large amplitude fluctuations in their trace element profile in the direction of growth (section 7.3), any cross section perpendicular to growth will be likely to encounter similar variation.
7.5 Inter-Element Correlations

While it appears as though fine-scale trace element variations are not well correlated with visible structures in the coral, and are not synchronous across a single polyp, the high degree of reproducibility (figure 7.1) indicates that they represent real chemical fluctuations within the coral structure. If a single chemical mechanism is responsible for these variations, then it may fractionate one element consistently with respect to another. Examining the correlation of elements with each other may therefore provide valuable clues as to the nature of this chemical mechanism, and therefore to the underlying physiological process controlling trace element partitioning into coral skeletons.

Two data sets are examined in this section. The data collected for runs 1 - 7 in the Davies 1B coral (see section 7.3) have been spliced together to produce a single 1400 data-point composite data set. The second data set is raw (unfiltered) data from one of the Davies 2 analyses presented in chapter 8. Note that a large laser slit (600 x 50 μm) was used during this analysis in contrast to the 'composite' data set which was analysed using the 70 μm diameter circular spot.

7.5.1 Processing

Trace element profiles were all background subtracted and normalised to $^{43}$Ca. No attempt was made to calibrate the data; instead all data-series were converted into a proportional deviation from the mean by normalising the profile to its average, and subtracting 1.0 (equation 7.1).

$$D' = \frac{(D - \mu)}{\mu} = \frac{D}{\mu} - 1.0$$  \hspace{1cm} (7.1)

where:

- $D$ = raw data profile
- $D'$ = transformed data profile
- $\mu$ = mean of data profile

For this investigation, seasonal scale variations and fine-scale variations were investigated separately. Each trace element signal was therefore split into a coarse (seasonal) component and a fine component using wavelet-filtering (fourier filtering was not appropriate as the autocorrelation analysis demonstrated that the fine-scale fluctuations were not strongly periodic). The wavelet filtering provided an effective separation of components smaller than 1.07 mm or approximately 26 days growth (see figure 7.11).
In the Davies 2 data sets, the Ba signal increased dramatically in the tissue layer (see chapter 8). The elevated Ba region was therefore excluded from the Ba data set for the analyses presented in this section. The composite data set also displayed 10 extreme outlier points in the Ba signal, that have been excluded from the correlation plots.

Figure 7.11 Example of Wavelet Filtering.
Seasonal and fine-scale components of the U/Ca profile for the Davies 2 (large laser spot) analysis. The seasonal and fine scale components of a trace element profile are separated using wavelet filtering. Variations of less than 1 month are included in the fine-scale component, while sinusoidal seasonal variations are isolated in the coarse component.

7.5.2 Observations

Magnitude of Variation

The 70 μm laser spot used for the high resolution analysis results in significantly less smoothing than the 600 x 50 μm spot. The fluctuation in the high-resolution trace element profiles is therefore large. Using the temperature calibrations presented in chapter 8 it is possible to convert these variations into temperature equivalents, which are presented in table 7.1. These temperature equivalents are much larger than the seasonal range of SST experienced by the Davies coral. Mg/Ca ratios vary over the equivalent of 21°C, whereas seasonal water temperatures change by 6°C.

These observations are similar to those made by other researchers who report that small scale fluctuations in included coralline tracers can often exceed the plausible range of an

**Table 7.1** Magnitudes of Trace Element Variation in the Davies 1B Coral.

<table>
<thead>
<tr>
<th>Element Ratio</th>
<th>Compositional Range (% of Mean)</th>
<th>Temperature Equivalent (°C) [1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/Ca</td>
<td>53</td>
<td>15</td>
</tr>
<tr>
<td>Mg/Ca</td>
<td>80</td>
<td>21</td>
</tr>
<tr>
<td>Sr/Ca</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Ba/Ca</td>
<td>100</td>
<td>- [2]</td>
</tr>
<tr>
<td>U/Ca</td>
<td>80</td>
<td>19</td>
</tr>
</tbody>
</table>

[2] No temperature dependence is available for barium.

**Histograms**

Histograms of the fine component of the high-resolution ('composite') data set are presented in figure 7.12. These histograms illustrate the scatter of the data around the seasonally detrended trace element signal. If the scatter in trace element variation is even (or random), then a symmetric gaussian-shaped histogram should result. A non-symmetric, or skewed, distribution indicates an abundance of one sign or magnitude of trace element fluctuation. A positive skew suggests the presence of large positive excursions in the trace element distribution, such as might be caused by a scattered phase that is enriched in a particular trace element.

In general the histograms are all reasonably symmetric, and gaussian curve-fits appear to match the data well (figure 7.12). This implies that the process or phase that causes the fine-scale variability results in an even, homogeneous distribution of trace element compositions. The histograms for B and Sr have no significant skewness. In contrast, the histograms for both U and Ba are skewed slightly towards positive values, consistent with the presence of small contaminant phases enriched in U and Ba. A small number of Ba observations (which are not included in the analysis) display extreme enrichments, with values up to 3x the bulk aragonite concentration. The histogram for Mg is very broad, and may be slightly skewed towards negative values.
Figure 7.12 Histograms of Fine Component of Trace Element Variation. The histograms of composition vs. abundance for the fine-scale component of the 'composite' data set are presented here. The black line is a symmetric gaussian fit to the data. The histograms of Ba/Ca and U/Ca appear to be positively skewed, which may represent an abundance of large positive spikes in the data set. This would be consistent with the presence of discrete domains of trace element rich contaminants. Note that the skew tail on Ba extends beyond the edge of the histogram, up to values 3 x enriched relative to the bulk aragonite.
Correlations

The inter-element correlations for the fine and coarse components of the high resolution composite, and the coarse component of the Davies 2 data set, are presented as scatter-plot matrices in figures 7.13, 7.14, and 7.15. These matrices compare each pair-wise combination of the 5 trace elements investigated. Because each of the individual analyses in the composite data set were analysed in parallel, this data does not contain a whole seasonal cycle. The coarse component of the Davies 2 data set is therefore included to give information on the correlation of each trace element in the cyclic seasonal component of the data. Selected correlation statistics are presented in table 7.2.

Boron

In the Davies 2 coral, B shares a strong seasonal signal with Mg, Sr, and U (see chapter 8). Strong correlations are therefore observed in the scatter plots for the coarse component of the Davies 2 data set (figure 7.15). In contrast, the fine-scale B components of the Davies 2 data set (not shown) and the composite of high resolution analyses (figure 7.13) display a conspicuous lack of correlation with any other element. It therefore appears that any correlation between B and other trace element is entirely due to a mutual forcing at seasonal scales.

Table 7.2 Selected Inter-element Correlation Statistics

<table>
<thead>
<tr>
<th>Element Ratio</th>
<th>Coarse Component</th>
<th></th>
<th>Coarse Component</th>
<th></th>
<th>Fine Component</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>err</td>
<td>R²</td>
<td>Slope</td>
<td>err</td>
</tr>
<tr>
<td>B/U</td>
<td>0.8</td>
<td>0.1</td>
<td>0.64</td>
<td>0.09</td>
<td>0.2</td>
</tr>
<tr>
<td>Mg/U</td>
<td>-0.8</td>
<td>0.1</td>
<td>0.80</td>
<td>-1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Sr/U</td>
<td>0.18</td>
<td>0.01</td>
<td>0.89</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Ba/U</td>
<td>-0.8</td>
<td>1</td>
<td>0.093</td>
<td>1.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Mg/Sr</td>
<td>-4.0</td>
<td>0.7</td>
<td>0.67</td>
<td>-2.3</td>
<td>1</td>
</tr>
<tr>
<td>Mg/Ba</td>
<td>0.07</td>
<td>0.2</td>
<td>0.027</td>
<td>0.05</td>
<td>0.06</td>
</tr>
</tbody>
</table>

[1] R² (as a decimal fraction) quantifies the proportion of variance accounted for by the correlation
Figure 7.13: Scatter Plot Matrix: Fine Component of Composite Data Set. All of the inter-element correlations for the fine scale component of the 'composite' (small laser spot) data set are presented here. Note the low correlations between B and all other elements, and the very high correlation for Mg and U. Ten extreme outlier points have been removed from the Ba scatter plots for the sake of clarity.

Barium

In general, Ba displays poor correlations with other trace elements. There are tentative suggestions of a correlation between Ba and both Sr and B in the coarse component of the composite data set (figure 7.15); however, these are not considered to be meaningful and will not be discussed further here. The fine component of the composite data set is similarly characterised by poor correlations, although there appears to be a slight inverse correlation between Ba and U, and a moderate positive correlation between Ba and Mg (figure 7.13).
Figure 7.14: Scatter Plot Matrix: Coarse Component of Composite Data Set. All of the inter-element correlations for the coarse (seasonal) scale component of the 'composite' (small laser spot) data set are presented here. Correlations are generally low because of the small component of seasonal variation in the short data sets that were spliced together to make the composite data set. Note, however, the very high correlation for Mg and U.

Strontium

The correlation between Sr and B in the seasonal signal of the Davies 2 data set has been discussed above. Strontium also shows a strong seasonal correlation with Mg and U in the coral (figure 7.15 and chapter 8). These correlations are evident but weaker in the coarse component of the composite analyses (figure 7.14). The correlation between Sr and Mg is moderate in the fine component of the composite data set; however, a moderate to strong correlation is observed between Sr and U, and these two elements share around 41% of their variance. The slopes of the Sr and U correlation are relatively similar between the coarse and fine components, and the correlation coefficients are also similar. This implies that similar physicochemical processes may be operating at both distance scales.
Figure 7.15: Scatter Plot Matrix: Coarse Component of Davies 2 Data Set. All of the inter-element correlations for the coarse (seasonal) scale component of the Davies 2 (large laser spot) data set are presented here. Note the high correlations between B, Mg, Sr, and U, and the low correlations between Ba and other elements. Nearly 1/3 of the Ba signal is affected by tissue-layer enrichments, and this section of the data has been removed from the scatter plots.

Magnesium

The correlations between Mg and Sr, B, and Ba have been discussed above. Magnesium and uranium display a strong inverse correlation (figures 7.13, 7.14, 7.15 and see also figure 7.16). This correlation is the strongest of all the element pairs in all of the data sets, and accounts for more than 60% of the variance in Mg for both fine and coarse components of the composite data set. The correlation between seasonal signals for U and Mg is considerably higher, accounting for nearly 80% of the variance in the coarse component of the Davies signal. The slope of the Mg/U correlation for the fine-scale component of the composite data set is very similar to the slope of the coarse-scale component, and they are not significantly different within experimental error. As with the Sr/U correlation, this implies that similar processes are fractionating Mg and Sr on both distance scales.
Uranium

Observations of the U correlations have been presented in the preceding paragraphs.

**Magnesium and Uranium Inverse Variation**

![Graph showing inverse correlation between Magnesium and Uranium](image)

**Figure 7.16: Inverse Correlation Between Magnesium and Uranium.**
Magnesium and Uranium display a strong inverse correlation in all data sets, with around 60% of the fine-scale variance for these elements in common.

### 7.5.3 Discussion

Fine-scale trace element heterogeneity in corals has been attributed to a range of factors by different authors. These factors are reviewed in chapter 6 (sections 6.2.3 - 6.6.3), and can be broadly grouped into 4 categories based on the physical origin of the effects.

1) **Structural Features**, which arise as a consequence of the patterns of growth and 3 dimensional geometry of the coral.

2) **Contaminants**, which represent mineral and chemical phases that are distinct from the coral aragonite.

3) **Physiological Effects**, where the chemistry of the trace element coprecipitation is influenced by the biology and biochemistry of the coral.

4) **Environmental Effects**, where variations in the physical and chemical environment in which the coral is growing affect the partitioning of trace elements into the skeleton.

In categories 1 and 2, trace element variations are structurally imposed on a background skeletal composition that is varying on a seasonal scale. These factors do not implicitly require that the bulk composition of the aragonite deposited by the coral skeleton varies...
on short distance or time scales. Factors in categories 3 and 4, however, directly influence the composition of the deposited aragonite on short timescales.

Within each category are a number of possible physicochemical mechanisms which may be responsible for the heterogeneous trace element partitioning. Each of these models is examined below, and evaluated against the observations of distribution and covariance of trace elements presented in previous sections.

**Correlation Theory**

The partitioning of each trace element may be sensitive to some or all of the factors described above, and each factor may fractionate trace elements differently. The observed profile for a trace element is therefore a linear combination of the effects of all of the different component factors.

If a trace element profile is dominated by a single factor that does not affect another trace element, then its correlation with other elements will be low. Likewise if a trace element is equally sensitive to a number of factors, then its signal will be complex, and may result in poor correlations with other elements. If one factor results in a positive fractionation for two elements, but a second factor results in an inverse fractionation, then it is possible for the factors to cancel each other out, resulting in zero correlation. Only in the cases where one factor dominants the signal for two trace elements, or several factors produce very similar fractionations, will a pair of trace elements display any significant correlation.

**Noise**

If a trace element signal contains a significant component of random noise, then its correlation with other elements will be poor. Excluding variations within the coral itself, the only identifiable source of random noise in the data comes from counting statistics.

The trace element signals that are most susceptible to random noise are boron, which had large counting statistics errors, and strontium which had low counting statistics errors, but a small compositional range. To examine the effect that counting statistics might have, the component of variation due to random noise was modelled, using the predicted variance computed from counting statistics, and the range of trace element compositions observed in the trace element profiles.

The correlation coefficients derived from this model are presented in table 7.3. These correlation coefficients are high, and the variance associated with this noise is therefore low, ranging from about 0.5% for Mg/U correlation up to 12% for the B/Sr correlation. Clearly, random noise does not contribute significantly to the poor correlations seen for
some elements, and this is confirmed by the reproducibility for replicate trace element profiles (figure 7.1).

Table 7.3 Modelling Counting Statistics Errors

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Mg</th>
<th>Sr</th>
<th>Ba</th>
<th>U</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.5</td>
<td>-0.96</td>
<td>0.94</td>
<td>-0.96</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>-0.98</td>
<td>0.996</td>
<td>-0.97</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>8.6</td>
<td>0.73</td>
<td>5.6</td>
<td>-</td>
<td>0.996</td>
<td></td>
</tr>
<tr>
<td>8.3</td>
<td>0.54</td>
<td>5.2</td>
<td>0.87</td>
<td></td>
<td>U</td>
</tr>
</tbody>
</table>

These correlations statistics were generated by creating sinusoidal signals and adding a noise component calculated from the average number of ion counts per data point. The upper triangle tabulates correlation coefficients (R). The lower triangle tabulates the % variance introduced by the noise component (= (1 - R^2) x 100%).

7.5.4 Structural Features

Thickening

Coral skeletons have been inferred to thicken progressively throughout the width of the tissue layer (Barnes and Lough, 1993; Barnes et al., 1995; Taylor et al., 1993; Taylor et al., 1995). Up to 50% of the aragonite in a structural element is deposited as it forms at the surface of the coral, while the remainder is deposited progressively over a number of months. The composition of the aragonite deposited by the coral varies on a seasonal basis, so material laid down in thickening deposits will be an average of several months variation. In essence a coral structural element will have a core of one ('instantaneous') composition, and a concentric sheath of another composition representing an average of the material laid down during the following few months.

Any section through a skeletal element (such as occurs when a coral is sliced, or the surface layer is subjected to an intense pre-abilation) will expose a cross-section comprising the unsmoothed core, and smoothed 'thickening' deposit (see figure 7.17). Very high resolution techniques such as laser ablation may be able to partially resolve these concentric layers, resulting in variability in the trace element signal at fine scales.

All but the highest resolution sampling methods will average both the initial and thickening deposits, resulting in a seasonal signal with a magnitude that is attenuated with respect to the actual composition of the instantaneously deposited aragonite. Even
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the laser analyses of the Davies 2 coral will produce significant averaging of both initial and thickening deposits because of its large slit (chapter 8).

**Cross Section Through Structural Element**

*Figure 7.17: Schematic Cross Section Through a Structural Element.*

Coral structural elements are compositionally zoned, with an initial core of aragonite laid down at the outside edge of the coral skeleton, surrounded by a sheath of aragonite laid down progressively over the time that the structure is surrounded by coral tissue. The composition of this sheath will be an average of up to 6 months variation. Cutting and pre-ablating may expose regions of the initial (unsmoothed) core, and these zones may be resolved by the small laser spot leading to fine-scale variation in the trace element composition. Coral structures may also be surrounded in a rind of adsorbed contaminant material, which may be partially removed during cleaning and pre-ablation.

It is possible to model the difference in composition between the smoothed seasonal signal and the 'instantaneous' aragonite. The model assumes 50% thickening on top of 50% instantaneous deposit, and assumes that thickening is even throughout the tissue layer, which occupies the equivalent of 6 months of coral growth. The instantaneous composition is assumed to be a constant amplitude sinusoid. The 'average' composition (i.e. what was measured in the low resolution analysis of the coral) is therefore 0.5 x the instantaneous composition, plus 0.5 x a running average of the following 6 months of
instantaneous composition. The resulting 'bulk average' signal has an amplitude that is 63% of the variation of the instantaneous sinusoid (figure 7.18).

**Tissue Smoothing Model**

![Graph of Tissue Smoothing Model](image)

**Figure 7.18:** Output from the Tissue Smoothing Model.

Bulk milling and large laser spots will average over both 'instantaneous' and 'smoothed' deposits, resulting in a smoothed signal with a seasonal amplitude that is attenuated with respect to the instantaneous aragonite. The small laser spot may be able to resolve instantaneous from smoothed aragonite, resulting in fine-scale variations in the trace element profiles. The maximum amplitude of these variations is defined by the instantaneous aragonite, and is equivalent to 10.3°C for a 50% smoothing over 6 months.

If the fine-scale variability is produced only by the small laser tracking over patches of instantaneous aragonite and smoothed aragonite, then the maximum range of composition possible is 158% of the seasonal range observed for the low resolution analysis. This would translate to a maximum apparent temperature range of 10.3°C (assuming a 6°C range for the water at Davies Reef). The fine-scale variations in Mg, Sr, and U, however, span the equivalent of 20°C (table 7.1), more than twice the calculated maximum.

Examining the assumptions used in the model: 50% thickening is likely to be an overestimate of the degree of smoothing. Less thickening will result in an even smaller unsmoothed range relative to the 'bulk average'. The 6 month tissue layer is reasonable for corals. To account for the large range of variation seen, the tissue would need to be thickening over the equivalent of 9 months of coral, or more if thickening is less than 50%. The Davies 2 coral grows by 13.5 mmyr⁻¹ and has a tissue layer thickness of 4.5 mm: equivalent to 4 months only.
It is therefore concluded that the 'thickening' model of fine-scale trace element heterogeneity is insufficient on its own to explain the large magnitude fine-scale variability observed in the corals analysed here. While these results do not exclude this model as being a partial contributor to the trace element variability, other evidence suggests a minor role at most. Under this model, the fine-scale variability is a consequence of the seasonal variation in the trace element composition of the deposited aragonite. It would therefore be expected that the correlations between elements at fine and seasonal scale should be the same. Although this may be true for Sr vs. U and Mg vs. U, B displays almost no correlation with uranium at fine-scales (figure 7.13), despite a strong seasonal-scale correlation (figure 7.15). Clearly other factors must be dominating the fine-scale variation, in order to produce the poor correlation. This could also indicate that the estimate of 50% thickening by Barnes and co-workers (e.g. Barnes and Lough, 1993) is too large.

**Calyx Architecture**

The growth surface of a coral calyx is not flat, but U-shaped. Adjacent points on neighbouring structural features within a corallite may therefore not be the same age. Because the composition of the aragonite is changing with time, neighbouring structures may have slightly different compositions. If the laser tracks from one structural element to another, then variation in the trace element profile may result.

The calices of *Porites* corals are approximately 0.5 mm deep (Veron, 1986), compared with extension rates of around 10-15 mm yr\(^{-1}\). The calyx therefore spans a range of time equivalent to approximately 12 - 18 days. This equates to at most a change of 0.56 - 0.95°C (assuming 6 °C annual temperature range). Clearly the observed variations are much larger than this, with TEs changing by the equivalent of 10 - 14 °C over distances of 300 µm.

If neighbouring corallites are offset relative to each other, then jumps in composition might result if the laser crosses from one corallite to another. Graphical models of offsets between adjacent corallites, however, fail to reproduce the magnitude of the observed fine-scale structure. In addition, *Porites* is a perforate coral genus, where the corallite walls are not sealed, and adjacent polyps are in intimate contact (Barnes, 1970). Perforate corals must co-ordinate their tissue uplift, and therefore adjacent corallite structures are usually very similar in age, reducing the likelihood that significant fine-scale variations could be produced in this manner.

This mechanism also suffers from the same problems as the 'thickening' model in that it cannot explain a range of compositions greater than the calculated 'instantaneous' aragonite. It is therefore concluded that calyx architecture is not a significant contributor to fine-scale trace element variations.
Chapter 7

Structural Element Composition

In the high resolution analyses of the Davies 1B coral, large changes in trace element composition were cautiously correlated with regions where the laser moved off one type of structural element, onto another (e.g. from synapticulae to septa to corallite wall - see section 7.3). Similarly, the width and placement of some of the trace element variations suggests a link with the spacing of adjacent synapticulae. As demonstrated in the previous section, the different age of adjacent structures is not likely to result in significant compositional differences. If the fine-scale variations are indeed due to ablation of material from different structures, then the intrinsic composition of these structures must differ significantly.

While dissepiment and coenosteal structures may be less affected by smoothing (Taylor et al., 1995), in order to explain the large differences observed in the Porites corals, it is necessary to require that different structures are actually deposited at different compositions. This could only occur if the coral physically and/or chemically discriminated between the different types of structure.

The presence of distinct types of structural elements within a coral skeleton is an indication that corals have some physiological discrimination over at least the shape of these different structures. Different regions of the calcifying tissue also appear to display some independent physiological character, with only specific zones displaying calcification activity at night (Marshall, 1996 and references cited therein). Amiel et al. (1973a) examined the exterior, and interior of coral septae, and the interior of the calyx wall, and concluded that Sr and Mg do not differ significantly between these structures. Several other trace elements, however, did appear to be heterogeneously distributed. It is therefore possible that tissues may display some degree of chemical discrimination between structural elements. At the very least, structures may calcify at different rates, leading to different trace element partitioning (see chapter 5, section 5.5.2).

These suggestions are highly speculative, and the correlations between trace element variation and structure are not strong (see sections 7.3 and 7.4). The possibility that structures are chemically discriminated by the coral nevertheless remains an idea that should be investigated further.

7.5.5 Contaminants

A number of contaminant phases may be present in corals and these have been proposed by authors as a possible cause of fine-scale trace element variations (chapter 6). Contaminant phases are characteristically low in abundance and small in size, although they can sometimes be extremely enriched in one or more trace elements. If domains of
these contaminant phases are distributed heterogeneously in the coral on spatial scales similar to the laser spot, then they may introduce variability into the trace element signal.

The following sections document several possible contaminant phases listed in the literature, and their characteristic trace element compositions. These are discussed in reference to the observed correlations in the trace element profiles taken from the Davies 2 and Davies 1B corals.

**Different Types of Contaminant**

**Organic Material**

Coral tissue, and organic material is reported to be enriched in a range of trace elements over coral skeleton. Magnesium is enriched by a factor of 3 to 5 (Amiel et al., 1973a; Buddemeier et al., 1981; Flor and Moore, 1977; Howard and Brown, 1986; Howard and Brown, 1987), uranium by up to a factor of 40 (Amiel et al., 1973b; Flor and Moore, 1977), and barium by between 11 (Flor and Moore, 1977) and 60 (Lea and Boyle, 1993) times (see also Amiel et al., 1973a; Buddemeier et al., 1981; Flor and Moore, 1977; Howard and Brown, 1986; Howard and Brown, 1987). Boron may be slightly enriched in organic material, but only by about 50% (Leeman and Sisson, 1996). Although the Sr/Ca ratio in coral tissue is higher than in the skeleton (Buddemeier et al., 1981), its absolute concentration is lower (Amiel et al., 1973a; Flor and Moore, 1977; Howard and Brown, 1986; Howard and Brown, 1987), and organic material will not therefore produce any perturbation in the Sr signal.

Individual organic inclusions are small. Allison and Tudhope (Allison and Tudhope, 1992) found organic material trapped in small sub-micron cracks or pores in the skeleton, while Wainright (1963) reports the presence of a network of 1 µm fibres, and a spongework of 20 µm chitin fibrils. The concentration of organic material in corals is usually low (0.1% - 0.01% Wainright, 1963), however, the high concentrations of some trace elements may still influence the skeletal composition.

**Mineral Phases**

Different mineral phases may also be enriched in some trace elements. Berner (1975) noted that Mg is enriched in calcite relative to aragonite (by a factor of around 30 - 100 Veizer, 1983), and Shen and Dunbar (1995) found that traces of inorganic carbonate cements found in corals contained up to 3.5 times more uranium than the skeletal aragonite. Amiel et al. (1973a) propose the existence of an unidentified labile mineral phase of high Mg/Ca to account for observations in their leaching experiments, while Greegor et al. (1997) report evidence for discrete strontianite domains in Montastrea and Acropora aragonite. Calcite has been reported in some corals (Constantz and Meike, 1988), and calcite is characteristically enriched in Mg (Veizer, 1983).
While the size and concentration of different mineral domains is not well characterised by the authors quoted, such phases are likely to be very small with a low abundance, as they have not been documented as being major features of coral aragonite.

Particulates
Coral skeletons trap particulate material in their porous skeletal structure (Brown et al., 1991; Budd et al., 1993; McConnaughey, 1986; Neil et al., 1994). The amount of trapped material varies a lot; however, it appears to be around 0.2 weight%, possibly dominated by smectite clays (Budd et al., 1993). Ultrasonic cleaning may remove loose particulates; however, the dense architecture and high porosity of Porites corals make it difficult to remove all particulate and detrital material from the coral (Shen and Boyle, 1988). Sometimes these particulates can be calcified into the aragonite (e.g. by the mechanism proposed by Brown et al., 1991 for Fe inclusion), and barite may even form in situ as a byproduct of decaying organic material trapped in the skeleton (Dehairs et al., 1980).

Clay particles are the most likely source of sediment, and would be rich in magnesium, adsorbed boron (factor of 4-5 Leeman and Sisson, 1996; Spivack et al., 1987), and barium (enriched up to 300 times Lea and Boyle, 1993), while barite is enriched in barium by a factor of 170,000 over skeletal aragonite.

Centres of Calcification
Aragonite crystals deposited by the coral grow radially outwards from a centre of calcification (see chapter 5). Allison and co-workers (Allison, 1996b; Allison and Tudhope, 1992) found that significant enrichments in Ba (factor of 110), Mg (40%) and Sr (6%) were associated with these structures, possibly due to the presence of organic material or calcite (e.g. Constantz and Meike, 1988) in the higher porosity centres. Centres of calcification appear to account for about 3 - 10% of coral aragonite (Allison, 1996b), and could therefore be a dominant cause of trace element variability.

Overgrowths/Adsorption
Some trace elements have been found to be concentrated in the outside margins of coral structural elements. Amiel et al. (1973a) concluded that 25% of all Mg in coral skeletons was adsorbed, and authigenic overgrowths of Fe/Mn oxides have been found to be enriched in barium by a factor of 30 - 300 over coral aragonite (Lea and Boyle, 1993). Allison (1996b) observed enrichments in Mg and Ba at the outside edges of skeletal structures. Schroeder et al. (1970) found a coating of uranium around the margins of skeletal structures, and Swart and Hubbard (1982) noted the tendency for U to exchange with, and be precipitated along, skeletal margins. Authors conclude that between 2% (Amiel et al., 1973b) and 6% (Shen and Dunbar, 1995) of all U in the coral is adsorbed.
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For some elements - notably magnesium - this sheath of adsorbed material may be the single largest contributor to the observed coral heterogeneity.

The ultrasonic cleaning in water used to clean the corals may remove some of the more soluble adsorbed species; however, without adopting more sophisticated cleaning procedures (e.g. Lea and Boyle, 1993; Shen and Boyle, 1988) it is unlikely that more resistant authigenic overgrowths will have been removed. Pre-ablation has the effect of 'planing off' a 10 μm layer of coral, exposing a cross-section through these different compositional zones (analogous to figure 7.17). During high resolution analyses, the laser would traverse over these zones, resulting in fine-scale variation in some trace elements.

Microborings

Small endolithic boring organisms are ubiquitous in coral skeletons. Two types of organism are most common: filamentous lime-boring algae (Veizer, 1983) which produce holes up to 15 μm in diameter and fungi which have holes around 1-4 μm diameter (Allison, 1996b). Around 0.5 - 3% of any coral-aragonite surface is affected by these features (Allison, 1996b).

The holes of these organisms have been associated with localised enrichments of Ba (approximately a factor of 9 Allison, 1996b), and U (Swart and Hubbard, 1982), although not Mg or Sr (Allison, 1996b). These enrichments may be associated with the presence of residual organic detritus (either deposited by the borers, or the remains of dead organisms), or sheaths of secondary mineralisation that line some of the holes (Allison, 1996b).

Trace Elements and Contaminants

Coral skeleton is relatively pure aragonite, and contaminant phases make up only a very small volume proportion of the material ablated per unit time. If the contaminant phases are very highly enriched in a trace element, the signal may be perturbed, resulting in a positive excursion or spike. If, however, a contaminant phase is depleted in a trace element relative to skeletal aragonite, the signal will be dominated by the bulk aragonite, and will remain more or less constant as the laser traverses across the contaminant domains. It is not possible therefore for a contaminant phase to introduce a negative excursion into a trace element signal. This makes it almost impossible for contaminants to result in a strong inverse correlation between two elements.

There is no reason to expect that the trace element fractionations associated with a contaminant phase should be the same as the fractionation caused by changes to the SST. Inter-element correlations should therefore be significantly different at coarse and fine resolutions. Several contaminants may be enriched in one element, but may have
different enrichments in other elements. In this case, inter-element correlations in the fine-scale signal could be poor. Some contaminant phases are enriched in just one element, again leading to poor inter-element correlations.

**Boron**

Almost none of the fine-scale scatter in boron correlates with other trace elements. While it is possible that a contaminant phase enriched only in boron is responsible for the fine-scale variation, the histogram for boron is symmetric, and does not display significant scatter towards high values. The only contaminants likely to affect B are clay particles, or organic detritus, both of which are characteristically small in size, low in abundance and not especially enriched in B over coral aragonite. It is unlikely, therefore, that the variability in B is caused by the presence of a contaminant phase.

**Magnesium**

Magnesium is potentially enriched in a wide range of contaminant phases, and the fine-scale correlations with other elements might be expected to be very complex. The strong inverse correlation with uranium is therefore surprising. The inverse correlation may indicate the presence of a contaminant phase that is enriched in Mg relative to coral aragonite, but depleted in U (such as calcite Veizer, 1983). As discussed above, however, a contaminant phase that is a small volume fraction of the material ablated by the laser, may produce a positive spike but could not cause a negative one, making a strong inverse correlation impossible. It therefore appears that the negative correlation with uranium is not caused by a Mg-rich U-depleted contaminant phase. This is supported by the observation that the slope of the Mg/U correlation at fine-scales is almost indistinguishable from the Mg/U slope of the seasonal variation, and the fact that the Mg histogram is relatively symmetric.

The correlation with U in the fine-scale component accounts for 60% of the Mg variation. Of the remaining 40%, around 3-5% can be attributed to counting statistics and machine noise. This leaves 35% that may represent variability introduced by contaminant phases. Barium shares about 27% covariance with magnesium and almost none with uranium, so a phase rich in Ba and Mg may be responsible for much of the unaccounted variation in Mg. Many contaminant phases are rich in both Mg and Ba. The relatively small enrichment of Mg in organic material, and low abundance of organic inclusions suggests that organics are not the source of this variation. Calcite is not enriched in barium, and microborings are not enriched in magnesium. This leaves clay particulates, centres of calcification, and/or adsorbed phases or overgrowths as possible factors. Although there is no evidence to confirm this, it is the opinion of this author that the adsorbed component of magnesium may be the primary contaminant responsible for fine-scale Mg variation.
Uranium

As with magnesium, 60% of the variability in U at fine-scales is linked to an anti-phase correlation with Mg, and cannot therefore be attributed to contaminant phases. Most of the 35% residual variation appears to be tied up in correlations with Sr. Strontium and uranium share about 41% of their variance; however, up to 14% of this may be in common with magnesium, and is therefore included in the 60%. This leaves at least 25% variation in Sr and U that is independent of Mg, and approximately 10% that is unique to U.

The U histograms are distinctly skewed towards high values, indicating an abundance of positive excursions in U in the data set. This is consistent with the presence of an enriched contaminant phase; however, there has been no phase identified thus far that is enriched in both Sr and U, which means that either these positive excursions are limited to the 10% variation that U does not share with Sr, or there exists a contaminant phase that has not yet been identified.

There is no way to determine exactly which contaminant phases might be responsible for the uranium variability (if any); however, the most likely candidates are adsorbed phases, or organics.

Strontium

The histogram for strontium is normally distributed with no indication that a broad tail exists towards positive values. Strontium is generally poorly correlated with most of the trace elements examined, with about 10% inversely correlated with Mg, and around 41% positively correlated with U. The inverse correlation with Mg is difficult to explain and is probably not a contaminant. The fine-scale component of the Sr/U correlation is very similar to the coarse-scale component, suggesting seasonal and fine variations are caused by the same factor. In this case it is unlikely that contaminant phases are responsible.

About half of the variation in strontium at fine-scales is not correlated with any of the other trace elements. Of the contaminants thus far examined, only strontianite could be responsible for significant variation in Sr independently of other trace elements. The very large enrichment of Sr in strontianite over aragonite would be very evident as large spikes if this phase was present in domains of any significant size. Such variations are not detected in the coral record, and clearly the domains of strontianite detected by Greegor et al. (1997) must be significantly smaller than the scale of the laser-ablation technique, behaving essentially as part of the bulk aragonite. It is therefore concluded that most of the fine-scale variation in Sr cannot be accounted for by contaminant phases.
Barium

Like strontium and boron, barium does not display especially great correlations with other trace elements. The 27% variance shared with Mg is the exception, and this has been discussed above. The histogram for Ba is distinctly skewed with clear evidence of quite large positive excursions. There are also an abundant contaminant phases rich enough in barium to be responsible for the fine-scale variation in this element. It is conceivable therefore that much of the variation in the Ba signal is caused by contaminants.

It is not possible to isolate a single contaminant phase that may be responsible for most of the Ba variation. Allison and Tudhope (1992) could not find evidence of barite particulates in coral skeletons; however, given the huge Ba enrichment, a barite particle could be a small fraction of 1 μm in diameter and still have a significant effect on the Ba signal. It is likely, however, that organic material and centres of calcification dominate the variability in this element. Barium behaves semi-independently of other trace elements in these phases due to its much greater enrichment compared with other elements.

7.5.6 Physiology

The strong inverse correlation between magnesium and uranium is not consistent with a dispersed contaminant phase. It therefore appears as if something affects the bulk aragonite composition at high resolutions. In this section, a number of physiological and biochemical mechanisms are examined and discussed with reference to the observed trace element variations. The effect of environmental parameters is discussed in the following section.

Physiological

Coral calcification is clearly an energy-dependent process. Metabolic energy is probably required for maintaining ATPase mediated active ion transport at a number stages in the calcification process, especially in maintaining the high pH, and the elevated Ca²⁺ concentrations that produce the CaCO₃ supersaturations responsible for rapid calcification (e.g. McConnaughey, 1989b and see review in chapter 5; McConnaughey, 1986).

In order to maintain rapid calcification rates, the coral polyp must obtain energy from the metabolism of certain types of organic material (mostly low molecular weight carbohydrates). Zooxanthellate corals have two sources of these organics: ingestion, or translocated products from their symbiotic photosynthetic algae. There are therefore a
number of processes that can act to affect the nutritional status of the coral and hence the amount of metabolic energy available for calcification.

The efficiency of the coral in utilising metabolites to produce energy may be adversely influenced by any factor that stresses the coral such as disease, fresh water, extreme temperatures, etc. In addition, any competing physiological process, such as the production of gametes for coral spawning, will divert resources away from calcification.

The basic calcification models predict that lowering the energy available for calcification will lower the ability of the coral to transport ions, both to the coelenteron and the calcifying fluid, resulting in lower Ca\(^{2+}\) concentrations, and a lower pH in the calcifying fluid. This will in turn lower carbonate ion concentration, leading to lower supersaturations and decreased calcification rates.

Changes to the pH, calcification rate, Ca\(^{2+}\) or CO\(_3^{2-}\) concentrations in the precipitating microenvironment can independently affect each trace element. This is in addition to any effects that changing the physiological state of the polyp may have on the many different enzyme and metabolic pathways that are implicated in calcification (see chapter 5).

Linking a physiological parameter to a particular trace element fluctuation is therefore a task that is fraught with difficulty. Patterns of trace element covariance help in this process; however, too much is still unknown about coral calcification physiology to make any clear conclusions.

**Biochemical**

There are 5 principle chemical factors that may affect trace element partitioning. These processes are not independent of each other and often occur together in response to changes in the physiological state of the coral (see above).

**pH and [CO\(_3^{2-}\)] in the Calcifying Fluid**

The pH of the coral microenvironment is believed to be highly variable (K. Beets, pers. comm.), and circumstantial evidence for this is given by Kühl et al. (1995) who observed changes in pH from around 7.3 to 8.5 in the surface tissues of corals as a function of light intensity.

Because pH affects carbonate ion concentration and the speciation of both U and B in solution, equilibrium models for C, B, and U speciation were set up as a function of pH (see appendix B for details. No attempt has been made to include the precipitation of aragonite in this model; however, inferences are drawn from changes to the boron and uranium activity compared with the carbonate ion activity in solution. Two models were created, and are discussed in chapter 6 and appendix B. Model A assumes that solution carbonate species are in equilibrium with a constant atmospheric pCO\(_2\). Model B is based on the possibility that pH change happens rapidly so that gaseous equilibrium
does not occur, but solution speciation is reorganised while total DIC remains constant. Both models produce qualitatively similar models for the behaviour of B and U. Full details of these calculations are presented in appendix B. An equilibrium model may not be realistic for this system, as there is plenty of evidence that corals precipitate rapidly and solutions may therefore be diffusionally limited (chapter 5). Equilibrium, however, is a useful starting point on which to base further discussion.

Both models predict that $\text{B(OH)}_4^-/\text{CO}_3^{2-}$ and $\text{UO}_2(\text{CO}_3)_2^{2-}/\text{CO}_3^{2-}$ will decrease with increasing pH (see chapter 6). If pHs fluctuate in the precipitating microenvironment, it might be expected therefore that these two elements would display a positive correlation in skeletal aragonite. This modelled correlation is not linear; although over the range of compositions found in the coral, the relationship can be approximated by a straight line (figure 7.19). The actual correlation between B and U in the coral at fine-scales, however, is almost zero (figure 7.13). This result is surprising, as pH was expected to be a dominant factor controlling the variation of these trace elements. It is possible that B and U are equally sensitive to two factors: one which results in a positive correlation (e.g. pH), and one which results in a negative correlation. If these factors are perfectly balanced, then a zero correlation could result.

It is possible that the mechanisms proposed for B and U coprecipitation into aragonite are wrong, and that one or both elements incorporate as cations rather than anions. Alternatively, theories of calcification chemistry may be flawed. The lack of correlation between B and U needs to be examined in more detail, for the conclusions that can be drawn from their behaviour could have major implications for the whole calcification process.

If proposed mechanisms are indeed correct, then the speciation model predicts that $\text{U}/\text{CO}_3^{2-}$ is very sensitive to pH. To account for a U variation of ± 40%, the pH of the precipitating microenvironment would need to change by only ± 0.05 pH units or ± 0.1 pH units for models A and B respectively (pH fluctuations of this magnitude would result in a B variation of ± 13% and ± 6% respectively, compared with the observed variation of ± 15-20%).

This would seem to suggest that the pH in the calcifying fluid is very stable, varying by less than a factor of 1.12. This is significant, as much larger pH variations are implied by other researchers' results (Kühl et al., 1995 and K. Beets, pers. comm.; Marshall, 1996), especially during the diurnal cycle, when metabolic energy from the zooxanthallae is no longer available. Clearly there is much that is still not understood about the biochemistry of B and U precipitation in corals.
Figure 7.19: Output from the U and B Speciation Models.

pH and carbonate ion concentration control the speciation of U and B in solution and therefore their incorporation into coralline aragonite. The ratio of both elements to carbonate decrease with increasing pH, and a positive correlation is expected to result. Model A (equilibrium with atmospheric CO$_2$) predicts a ±16% variation in B for a ±40% variation in U. This is close to that observed in the coral; however, the B and U signals display essentially zero correlation. It appears that other factors may obscure any pH based correlation, or that the incorporation of one or both elements into coral aragonite is insensitive to pH. Note that the range of U values observed in corals can be accounted for by a very narrow range of pHs, suggesting that the pH of the precipitating microenvironment is extremely stable.

**Ca$^{2+}$ Concentration in the Precipitating Microenvironment**

Elevated pH in the calcifying microenvironment is maintained by pumping Ca$^{2+}$ in exchange for H$^+$ (chapter 5, section 5.4). Fluctuations in pH would therefore be expected to be accompanied by fluctuations in the Ca$^{2+}$ concentrations, and hence the degree of 'dilution' that cations will experience when coprecipitating with CaCO$_3$. 
The degree of dilution should be the same for all species substituting for cations in the aragonite. Clearly, however, strontium, barium, and magnesium have different magnitudes of fine-scale variation. Barium varies more than magnesium, and this may be due to Ba rich contaminant phases (section 7.5.5). Strontium varies less than magnesium. Strontium and calcium are very similar ions, and may share the same enzymatic transport mechanisms, resulting in only partial discrimination or no discrimination between these ions (section 5.7). Thus variations in the Ca$^{2+}$ pumping would dilute Sr less than Mg, resulting in the lower magnitude of variation.

Dilution of cations by pumped Ca$^{2+}$ should result in positive correlations between Mg, Sr and Ba. Because CO$_3^{2-}$ concentrations are linked to Ca$^{2+}$ pumping, anion substituents would also be diluted by increasing Ca$^{2+}$ pumping, and should display a positive correlation. This is clearly contradicted by the results; magnesium and barium display only a weak positive correlation, Mg and Sr are weakly anti-correlated, and Mg and U are strongly anti-correlated. It must therefore be concluded that dilution of cations by Ca$^{2+}$ plays, at most, a minor role in controlling fine-scale trace element variations.

This conclusion is consistent with observations made in the previous section. The variability in U can be accounted for by very small changes in the pH of the precipitating fluid. The McConnaughey model (see chapter 5) predicts that the variation in Ca$^{2+}$ in the calcifying fluid is even smaller than the variation in pH, so the Ca$^{2+}$ concentration in the calcifying fluid would be almost constant.

**Precipitation Rate**

It is likely that coral physiology acts to increase calcification rate. As reviewed in chapter 5 and appendix A, calcification rate may intrinsically change the mechanistics and kinetics of trace element precipitation, resulting in changes to the partitioning of one element relative to another. Short timescale changes to calcification rate (especially diurnal variations) may therefore cause some of the fine-scale trace element variability.

Physiologically pumped Ca$^{2+}$, CO$_3^{2-}$, and pH may dominate the trace element chemistry if calcification rates are enhanced by changing the CaCO$_3$ supersaturation (see previous section); however, corals may enhance calcification in other ways such as removing crystal poisons (see section 5.3.2).

It might be expected that a decrease in reaction rate would be associated with a shift from kinetic or transport limited behaviour towards more equilibrium behaviour. As crystal growth is, by definition, a non-equilibrium process, the 'equilibrium' composition is usually taken from inorganic precipitation experiments or from inorganic crystals precipitated from seawater. In many cases, however, the composition of these 'equilibrium' phases is not well defined.
A review of the literature does not reveal a consistent pattern of Mg or Ba precipitation above or below inorganic values, and the inherent variability of these trace elements in corals makes it difficult to predict where 'equilibrium' lies in relation to their coralline values (chapter 6). Coralline uranium precipitates close to inorganic values, although neither consistently above or below. Corals precipitate Sr slightly below inorganic values, and lowering calcification rates would tend therefore to result in increases in the coral Sr/Ca. Although this effect has not been unambiguously reported in corals (section 6.2.2), higher Sr/Ca ratios are seen in the slower growing valleys between growth axes in corals (Alibert and McCulloch, 1997; de Villiers et al., 1994). The inorganic distribution coefficients for B determined by Kitano et al. (1978) and Hemming et al. (1995a) produce contradictory conclusions about whether B precipitates above or below equilibrium; however, boron is enriched in corals relative to inorganic deposits (Vengosh, 1990; Vengosh et al., 1991). It is tentatively suggested that lowering calcification rates might result in a lowering of the B/Ca ratio in the coral.

Strontium and boron might therefore be expected to vary in anti-phase; however, this is contradicted by the data which indicates almost zero correlation (figure 7.13). The poorly characterised equilibrium behaviour of Mg and U makes it impossible to comment further on the strong anti-correlation observed for these elements.

If solution transport is limiting calcification, increasing calcification rates would produce a positive correlation between all elements that have distribution coefficients on the same side of unity (appendix A, section A.4). Thus Sr and U, which both appear to have $D > 1.0$, would be positively correlated, while Mg which has a $D << 1.0$ would be anti-correlated. The positive Sr vs. U correlation and negative Mg vs. U correlation are consistent with this idea. This is an extremely simplistic argument, however, which ignores the complicating factors of diffusion coefficients and speciation. No firm conclusions can therefore be drawn. Transport limitation remains an area of calcification chemistry that should be examined in more detail.

Concentrations of TEs in the Microenvironment

The final factor that might affect trace element partitioning into the coral skeleton is the concentration of the elements in the precipitating microenvironment. Several factors may influence the composition of the calcifying fluid: dilution of ions by physiological pumping is discussed above, and changes in the trace element chemistry of the surrounding seawater are examined in the following section.

Coral trace elements are generally close to inorganic composition (table 6.1), indicating that corals probably do not exert a major control over the trace element composition of their calcifying fluid. Nonetheless, there may be enzymatic mechanisms that could fractionate trace elements during transport from seawater. Corals may withdraw
biochemically important ions from the calcifying fluid, add or remove trace species to control the form and phase of the precipitated CaCO$_3$, or 'dump' waste ions into the precipitating fluid (section 5.7.4).

Too little is known about the specifics of tissue and cellular transport of different ions to generate a physiological model that could explain inter-element correlations. These issues are currently being investigated (Clode and Marshall, 1998), and further insights will hopefully eventuate soon.

### 7.5.7 Environmental

The seasonal variations seen in the trace elements (see chapter 8) are a clear indication that an environmental parameter or parameters affects the partitioning of trace elements into corals. It is not certain whether this is a direct effect, or whether the environment mediates physiological processes that are in turn responsible for the trace element partitioning. It is also not clear whether environmental effects can be responsible for the fine-scale compositional fluctuations seen in corals. The following discussion examines a number of environmental parameters that may be directly responsible for the fine-scale variability seen.

**Temperature**

The slope of the Mg/U correlation at fine scales is very similar to the slope at seasonal scales (table 7.2), suggesting that the seasonal and fine scale variations are linked by the same physicochemical mechanism. A similar pattern is seen for the Sr/U correlation. Strontium, Mg, and U are all strongly correlated with SST at seasonal scales (chapter 6, chapter 8, and figure 7.15). It is therefore reasonable to suggest that some of the fine-scale variation seen in corals for Mg, Sr, and U is caused by short time-scale fluctuations in sea-surface temperature.

To test this, a section of the AIMS SST record for Davies Reef was obtained by Dr Alibert. This record was de-trended to remove the seasonal variation, and the magnitude of the daily to monthly variations was compared with the fine-scale variations in the coral record. The % variations in the coral data were converted into temperature variations using the calibrations presented in chapter 8. These results are graphed in figure 7.20, and it is clear that the amplitude of the fine-scale variation in the coral is significantly larger than the daily variations in the sea-surface temperature.

This means that temperature cannot be directly responsible for the fine-scale variations. If the fine-scale and seasonal-scale variations are caused by the same chemical mechanism, as deduced above, then it must also be concluded that the seasonal variation in the coral is not directly caused by temperature. Seasonal variations may still be forced
by temperature; however, temperature must act to mediate another physiological or chemical process that is responsible for the Mg and U fractionations. This process must also be sensitive to some other factor that causes the large magnitude fluctuations seen on daily to monthly timescales.

Residuals as Temperature Equivalents

![Residuals as Temperature Equivalents](image)

Figure 7.20: Fine Scale Variations as Temperature Equivalents. The fine scale component of the 'composite' data set has been transformed into equivalent temperature fluctuations using the correlations presented in chapter 8. These are graphed against the fine-scale (weekly to monthly) variation in the SST and light intensity signals. Clearly neither temperature nor light can account for the large magnitude of variation observed in the U, Sr, and Mg profiles.

This is a significant result that has implications for the use of trace elements as paleothermometers. If temperature controls Mg and U partitioning through an intermediate reaction, rather than by directly affecting thermodynamic parameters, then the Mg and U chemical thermometers may not follow the simple and rigorous rules that thermodynamics would impose. Because of the likelihood that an intermediate process is involved, there is also the possibility that a number of environmental, chemical or
physiological parameters will affect the process, complicating and confounding the temperature signal.

**Light Intensity**

Instead of temperature, Mg, Sr, and U may vary directly in response to another environmental parameter that displays a seasonal variation, and higher amplitude fluctuation on daily to monthly timescales. The obvious possibility is light intensity. Maximum light intensity varies sinusoidally on a seasonal basis, with a shape that closely matches sea-surface temperature. Cloudy days are characterised by large amplitude negative excursions in light intensity. Thus light could be directly forcing the trace elements.

Changes in light intensity will affect the photosynthetic symbiont, and therefore the degree of energy available for metabolic processes and enhanced calcification. The link between the photosynthetic zooxanthallae and enhanced calcification processes is not a simple matter, and remains poorly understood (section 5.3.2). It is likely that changes to light intensity will translate through to changes in physiological pumping, hence Ca\(^{2+}\), pH, and CO\(_3^{2-}\).

Light intensity clearly does have an effect on corals. Kühl et al. (1995) have demonstrated that the pH in the surface tissues of corals rises sharply when exposed to light, possibly because the zooxanthallae consume CO\(_2\) during photosynthesis. If the theories of McConnaughey and Whelan (1997) are correct, the increased demand for protons at the photosynthesising sites in the oral epithelium is met by protons pumped from the site of calcification, raising pH, and hence calcification rates.

It is not certain, however, to what degree daily fluctuations in light intensity might affect the average pH and calcification rate in a coral. Zooxanthallae in corals growing close to the surface of the water are generally light-saturated. This is demonstrated by Kühl et al. (1995), who find only small changes in pH over light intensities varying by nearly a factor of 10.

To compare the variations in light with the trace element signals, the sinusoidal maximum light intensity was converted into an equivalent temperature variation by matching summer and winter temperatures with their corresponding light intensities. The resulting calibrated light signal was then detrended to remove the seasonal component, and graphed as a residual on figure 7.20.

The variability in this light signal is larger than the temperature signal, with excursions of up to 11\(^\circ\)C. This is still not enough to account for the magnitude of fine-scale variations. It is also clear that the day to day variations in light intensity do not reproduce the week to month long trends seen in the trace element records.
In section 7.5.6 it was concluded that the observed trace element correlations were not consistent with large variations in Ca\(^{2+}\), pH, and/or CO\(_{3}^{2-}\). This means that if light is responsible for the fine-scale variability, it must act through another unknown physiological mechanism that produces different trace element fractionations.

\textit{pH}\

If the calcifying fluid is largely derived from unmodified seawater then there is a possibility that the ambient pH of this water may affect the precipitation by the coral. The response of trace elements to oceanic pH is reviewed in chapter 6, and authors have generally found no evidence for any effect. From section 7.5.6 it was concluded that pH may be remarkably stable, suggesting a strong metabolic control over the pH of the precipitating microenvironment. Seawater pH may therefore have little influence over the precipitating environment, and is unlikely to be responsible for fine-scale variations.

\textit{Salinity}\

Changes in salinity may have a range of effects including speciation, and changes to the saturation state of the seawater. If the coral buffers the saturation state of the precipitating microenvironment, then both Ca\(^{2+}\) and CO\(_{3}^{2-}\) must be physiologically adjusted as they become diluted in the seawater component of the calcifying fluid. Other ions that are derived from the seawater component and not physiologically mediated by the coral will change in proportion to the changes in salinity. The ratio of these ions to Ca\(^{2+}\), or CO\(_{3}^{2-}\) in the fluid (and hence their ratio in the deposited aragonite) will therefore change with salinity.

Again there have been few reports of any clear link between salinity and trace element composition in corals (with the exception of river run-off - see chapter 10), suggesting that the effect is minor. It is considered unlikely that salinity could be responsible for fine-scale variations in the trace element signals.

\textit{Carbonate Ion Concentration}\

A number of authors have examined the possibility that uranium incorporation in corals reflects carbonate ion composition in the oceans; however, there are no unambiguous reports that this is the case (section 6.6.2). Carbonate ion concentrations in the ocean are responsive to temperature and a number of other parameters that influence gaseous exchange. Upwelling of deep waters may change the surface carbonate ion concentration; however, in general the surface of the ocean is well mixed and equilibrated with the atmosphere, and fluctuations in the carbonate ion composition are probably too small to account for the magnitude of the fine-scale variations in the coral trace elements (B. Opdyke, pers. comm.).
Chapter 7

Seawater Chemistry

Because the calcifying solution is largely seawater (McConnaughey, 1986), short term fluctuations in the trace element composition of the seawater in which the corals are growing may translate to changes in the skeletal trace element concentration. While there is little doubt that seawater changes can affect skeletal concentrations (e.g. chapter 10), it is not clear whether these could be responsible for the fine-scale fluctuations. In general the trace and minor element chemistry of the surface ocean is buffered on short timescales, and concentrations would not be expected to change by the proportions observed in the coral skeletons.

River run-off may affect trace elements but in mid-shelf GBR locations such as Davies Reef, these effects are negligible. Upwelling may well influence the chemistry of the trace elements towards the edge of the outer reef (Wolanski, 1994), and these effects have clearly be seen in some coral records (Fallon et al., 1999; Lea et al., 1989; Shen et al., 1987; Shen and Sanford, 1990; Tudhope et al., 1996). Generally at mid-shelf locations individual upwelling events are averaged out into a broad 'upwelling' period over several months. Individual upwelling events, however, can be relatively short lived, and may be resolvable with high resolution techniques such as laser ablation (Fallon et al., 1999). It is therefore conceivable that upwelling may contribute to the variability in some trace elements in coral records.

Of the elements studied here, only Ba has been previously reported as being significantly enrichment in upwelled waters. Upwelled waters are colder than surface waters and intrusions of upwelled water masses are characterised by decreases in water temperature. Lea et al. (1989) observed upwelling in the Galapagos, and found that fluctuations in Ba/Ca of about ±10% were associated with ±2°C changes in the water temperature. The effects of upwelling are minor away from the edge of the outer reef (Wolanski, 1994), and the Davies Reef temperature record only shows relatively small temperature fluctuations (±2°C at most Alibert and McCulloch, 1997). If upwelling in the GBR followed the same pattern as in the Galapagos, Ba/Ca ratios in surface waters would change by at most 10%, which is only around 1/5 of the Ba variability observed.

The salinity-normalised concentrations of B, Ba, and U in the GBR are conservative with depth (Alibert 1997, unpublished data), and are unlikely to be affected by upwelling. Sr/Ca ratios showed a slight enrichment in deep waters, but this was equivalent to less than 1°C. Both Mg2+ and Ca2+ have conservative depth profiles (Bruland, 1983). It appears therefore that none of the elements has the potential to be significantly affected by upwelling, and this mechanism is not important in causing fine-scale trace element variability.
Nutrients and Food Sources

If the trace element variability in the coral is linked to physiology, then anything affecting the energy status and health of the coral could act to change calcification rates and ion transport. While corals derive a significant portion of their metabolic energy requirements from their \( \text{α}{\text{σ}}_{\text{α}} \) symbionts, the availability of food sources for the corals may have a significant effect on the polyp.

Nutrient distributions can be heterogeneous in the GBR reef both on spatial and temporal scales in response to a number of factors including resuspension, upwelling, season, temperature, eddies, etc. (Wolanski, 1994). It is possible that this variability may influence the coral, and thereby be responsible for some of the fine-scale trace element structure; however, at present it is not clear how significant this effect would be, or how it would fractionate different trace elements.

If it is accepted that the fluid that charges the calcifying microenvironment is drawn from the coelenteron, then the presence of digesting material may significantly affect the composition of the small pool of coelenteric fluid and hence the skeleton (see chapter 5 5.7). Organic material is characteristically enriched in a number of trace elements (section 7.5.5), and this therefore provides a potential link between organic enrichments and bulk skeletal composition without the presence of micro-scale contaminant domains. While nutrient availability is a 'global' parameter, affecting an entire coral colony, individual polyps may well feed independently of each other. If this feeding behaviour translates into trace element variations, adjacent polyps would not be expected to display synchronous variations in fine-scale trace element variability (see below).

Final Note

Environmental and physiological effects might be expected to affect an entire polyp, yet no clear correlations between different structures can be found within the same polyp (sections 7.3 and 7.4). This would imply that either variations are not general within a single polyp, or contaminant phases and overgrowths may contribute enough to the fine-scale variability to obscure any horizons of trace element variation.

7.6 Summary

The data presented in this chapter constrains the chemistry of the calcification process, and the range of factors that are responsible for trace element variation at fine scales. There is, however, still much that remains unknown about trace element incorporation into coralline aragonite. More research is required, especially into the physiology of ion
transport through tissues to the skeleton, and inorganic factors affecting trace element partitioning during rapid calcification from a supersaturated solution.

**General**

Coral trace element profiles exhibit large amplitude variation over fine distance scales. The amplitude of this variation was large, equivalent to more than 20°C for some trace elements, despite the waters changing by only 6°C. Profiles appear to display an abundance of features around 260 µm wide, equivalent to ~ 7 days growth; however, no clear periodicity could be found in the data. This width is similar to synapticulae spacing; however, fine-scale features were not convincingly correlated with the presence of adjacent structures. The trace element fluctuations did not seem to correlate between different structural elements within the same corallite. Fine-scale variations therefore do not represent a continuous 'horizon' within a corallite, or this horizon is obscured by contaminant phases or growth processes. Similarly, no clear symmetry could be found associated with traverses across an end-section of a corallite.

The large amplitude of variation cannot be accounted for by growth related architecture (such as smoothing, or the U-shaped calyx), and is too large to be directly caused by short-timescale variations in temperature, light intensity, or a range of other environmental parameters such as carbonate concentration, upwelling, or pH in the ocean. It is possible, however, that several factors act together to produce large amplitude variability. The similarity between the seasonal and fine scale inter-element correlations for Mg, U, and Sr suggest that the same chemical process controls their variation, and that temperature must mediate this process rather than act directly to fractionate the trace elements. Models of uranium and boron speciation suggest that the pH of the precipitating microenvironment must be remarkably stable.

**Boron**

Boron is characterised by conspicuously low correlations with other elements at fine-scales, in contrast to the strong correlations at seasonal scales. It therefore appears that the chemical processes responsible for seasonal variation are distinct from those responsible for fine-scale fluctuations. The poor correlation with uranium suggests that fluctuations in pH are not responsible for fine-scale features. While much of the boron heterogeneity could be caused by contaminant phases, the histogram is symmetric, and the contaminants identified thus far are not especially enriched in boron. It is possible, therefore, that an unidentified physiological process is responsible for the variations; however, this is speculation.
Magnesium

Magnesium has a strong inverse correlation with uranium, and most of the fine-scale variation cannot therefore be accounted for by contaminant phases, or mutual dilution by physiological pumping. The inverse correlation with U could be associated with changes in calcification rate under transport limiting kinetics, or with some unidentified physiological process. The variation in Mg that is not accounted for by U may be a contaminant phase, and correlations with Ba suggest either centres of calcification or adsorbed phases could be responsible.

Strontium

About half of the variance in strontium does not correlate with any of the other elements. This variation may represent strontianite domains in the skeleton; however, symmetric histograms and the absence of large variation suggests that such phases must be small and homogeneously dispersed on the scale of the laser spot. The rest of the Sr variance correlates with U and/or Mg. The slopes of the Sr/U correlation are similar on fine and seasonal scales, and it is therefore considered unlikely that contaminant phases can be the cause. The inverse correlation between Sr and Mg, and the different magnitudes of Sr and Ba variation are not consistent with mutual dilution by pumped Ca$^{2+}$. It is possible that an unidentified physiological process is responsible for the Sr and U variation.

Barium

Barium is generally poorly correlated with other elements, although both Mg and U share some variation. The histogram for Ba is distinctly skewed, consistent with the presence of contaminant phases. While much of the barium variability may be associated with contaminants, it is impossible to distinguish which contaminants are dominant. Centres of calcification, or adsorbed phases may account for the correlation with Mg, while organic phases may account for the correlation with U. Barite may also be a significant contaminant phase.

Uranium

A significant proportion of the U variation is correlated with Mg or Sr, and as described above, neither correlation is consistent with contaminant phases. These correlations may be caused by a physiological parameter; however, mutual dilution by pumped ions is not consistent with the inverse correlation, while the zero correlation with B does not support fluctuations in pH. The U histogram does display a positive skew, and the variance in U that is not correlated with Mg or Sr may be caused by an enriched contaminant phases, probably organics or adsorbed material.
Discussion

Uranium, strontium and magnesium appear to have the largest inter-element correlations. They each have similar correlation slopes between seasonal and fine scales, suggesting a mechanistic link. It is therefore possible that a single physicochemical factor dominates the variation for all three elements, influenced by temperature on seasonal scales, and some other physiological or chemical parameter at fine-scales.

The large inverse correlation between Mg and U is not consistent with an enriched contaminant phase, and implies that something alters the composition of the bulk aragonite. The physicochemical mechanism responsible for this intimate pairing is curious, especially considering that the correlation is in anti-phase, Mg and U concentrations differ by more than 3 orders of magnitude, they incorporate as oppositely charged species, presumably have different physiological transport paths, and would be expected to have very different responses to both pH and alkalinity in the precipitating microenvironment. Probably the only thing that these two elements have in common is that they are both significantly different in size from the lattice sites for which they both compete: Mg being too small and U too large.

It is possible that the presence of one of the elements directly affects the inclusion of the other in the skeletal aragonite. Magnesium has been known to affect the incorporation of other ions in calcite (Morse and Bender, 1990). The average Mg concentration in corals is around 0.4 mol% or around 3 - 5 ions per 10 x 10 x 10 cube of cations. The concentration of Mg in some of the more enriched domains may approach twice this, or around 1 ion per 5 x 5 x 5 cube of cations. At these concentrations, most of the anions in the lattice would be either a nearest neighbour or next-nearest neighbour to a magnesium ion.

Magnesium ions distort the CaCO₃ lattice around them because of their small size relative to calcium (Folk, 1974). If this distortion extends for several lattice points around each inclusion, then it is possible that the bulk chemical properties of the crystal would be significantly affected. This distortion may be enough to significantly disfavour the incorporation of the large uranyl carbonate anion, thereby providing a direct inverse link between magnesium and uranium incorporation. A similar idea may be extended to explain the inverse correlation between strontium and magnesium.

Under this mechanism, the magnesium concentration forces the variation of Sr and U. If an environmental or physiological parameter influences the bulk Mg concentration, then it would also induce a variation in U and Sr, even though there may not be an obvious chemical link between the parameter and the other elements. This parameter may also 'overwrite' other biochemical factors that would influence other ions, e.g. pH in the case of uranium.
It is most likely that Mg forces Sr and U. The chemical characteristics of Sr$^{2+}$ are very similar to Ca$^{2+}$, and would not be expected to induce a significant distortion in the aragonite lattice. The concentrations of uranium are probably too low (one ion per $100^3$) to influence other ions significantly.

It is still necessary to find an environmental, chemical or physiological parameter that causes the fine-scale variations in Mg, however.
CHAPTER 8: SEA SURFACE TEMPERATURE CALIBRATIONS

8.1 Introduction

The previous chapter focused on the fine-scale trace element variations present in coral skeletons. It was concluded that a number of factors affect trace element variation, and that physiology and contaminant phases may dominate the fine-scale signal. While it appeared that temperature was not the physicochemical factor that directly controlled trace element fractionation, it was apparent that coral records displayed a sinusoidal variation on seasonal distance scales. This seasonal variation may represent the influence of the sea-surface temperature (SST) over some other physiological or chemical process. If this process is stable over seasonal timescales, then corals may faithfully record SST in their skeletons.

The research presented in this chapter examines seasonal-scale variations in a coral from Davies Reef, and correlates them with instrumental SST records. The aims of this work are to investigate the behaviour of seasonal trace element records at high spatial resolution, to compare the seasonal behaviour of different trace elements, and to evaluate the potential for the laser ablation analytical method to rapidly and quantitatively extract paleo-SST information from coral skeletons.

8.2 Experimental

8.2.1 Samples

The coral analysed was a Porites mayeri collected from the central Great Barrier Reef (GBR) near the weather station tower at Davies Reef. This coral was previously analysed by Alibert and McCulloch (1997), who refer to it as 'Davies 2'. Further details of the sample can be found in that paper. This coral is an ideal test subject for evaluating SST variations. Davies Reef is a mid-shelf location that appears to offer optimal growing conditions for coral, and a large seasonal temperature range (approximately 6°C). Mid-shelf corals are not subjected to incursions of fresh-water from the mainland, and the
effects of deep-water upwelling are attenuated away from the outer reef. The Australian Institute of Marine Science (AIMS) has been operating a weather station at Davies Reef and consequently several years of high quality instrumental SST data are available. Finally, the high precision Sr/Ca record obtained by Alibert and McCulloch (1997) for this coral provides an ideal comparison for the laser results.

The samples were prepared in the manner described in chapter 2. Briefly; a 7 mm slab was cut from the coral core, and the tissue layer was bleached in 50% H₂O₂ to remove residual organic material. A 5cm x 3cm section was cut from the top of the coral for mounting in the ablation cell. This section spans from September 1990 to October 1993, and includes the tissue layer. Prior to analysis, the coral material was subjected to an intense ultrasonic cleaning in 18 MΩ water and dried at 40°C in a clean oven.

In addition to the laser analysis, a bulk sample of the coral was taken for isotope dilution analysis. This sample was shaved from the ledge that remained following the high-resolution milling performed by Alibert and McCulloch (1997), taking care to avoid the tissue layer. The bulk sample spanned from about September 1990 to October 1992 (see figure 8.1).

8.2.2 Methods

The Davies 2 coral was analysed using the fully quantitative laser-ablation method described in chapters 2 to 4. The standard used to calibrate the analysis was the old wollastonite glass. After cleaning ablations and conditioning steps, the coral sample was analysed for B, Mg, Ca, Sr, Ba, and U using a wide (600 µm x 50 µm) laser slit scanned at 1/30 mms⁻¹. Drift correction was performed by linear interpolation between analyses of the wollastonite standard made at the beginning and end of the analysis. A driftmonitor section was run as part of the routine analysis; however, secondary correction using the driftmonitor made no appreciable difference to the resulting trace element profiles. Three replicate analyses were made over the same section of coral, and for each trace element, the results were averaged into one profile.

The methodology for solution analysis is presented in chapter 2. To summarise, coral samples were dissolved directly in quartz distilled 2% nitric acid. Solutions were then diluted using 2% nitric acid, and splits were taken for Ca and Sr analysis by thermal ionisation mass spectrometry (TIMS). The analysis for Sr and Ca was carried out in a similar manner to the isotope dilution method reported by Alibert and McCulloch (1997), except that the mixed Sr Ca isotope spike used enriched ⁴³Ca only rather than ⁴²Ca and ⁴³Ca. The remaining solution was spiked with enriched ¹⁰B, ²⁵Mg, ¹³⁷Ba and ²³⁸U and analysed on the ICP-MS by isotope dilution methods.
This figure shows the location of the laser traverse (1) and the bulk milled sample (3) superimposed on an X-radiograph of the Davies 2 coral. Both the laser traverse and bulk sample are shifted to one side of the major growth axis (2) that was milled for the high precision Sr/Ca analyses carried out by Alibert and McCulloch (1997). The growth peak is broad, and therefore analyses on the side of the peak are not expected to suffer the geochemical anomalies associated with analyzing a growth margin.

Some of the fine-scale variability in the trace element signals was removed using data filters, in order to isolate the seasonal component of variation. The filtering regime used here was an 11 point running median followed by a running average. Full details of this digital filter are presented in chapter 2; however, it is important to note that the median filter removes variations on timescales of less than a fortnight, whereas the wavelet filter used in chapter 7 removes variations less than one month. The data presented in this chapter therefore contains what is described as 'mid-scale' variability. Mid-scale variability is arbitrarily defined as features in the data spanning the equivalent of 2 weeks up to 2 months of coral growth. Note that this definition includes some of what was defined as 'fine-scale' variability in the previous chapter (which was 1 day up to 1 month of growth). Mid-scale variability will include short-timescale fluctuations in temperature and other environmental forcing functions, but there also appears to be some component of this variation that does not relate to an environmental parameter, and does not represent a horizon of trace element fluctuation in the coral.
8.3 Results

Seasonal trace element cycling is clearly observed in the Davies 2 coral for B/Ca, Mg/Ca, Sr/Ca, and U/Ca (figure 8.2). While Ba/Ca displayed significant mid-scale variation in its profile, no regular seasonal trend could be detected. Although all profiles were affected by mid-scale variation, the data was suitable for determining a tentative calibration against sea surface temperature for B, Mg, Sr and U.

A weekly SST record was obtained from the AIMS monitoring station at Davies Reef (the same used in Alibert and McCulloch, 1997). The coral was growing at about 12 mmyr⁻¹ over the section analysed, and a broadening of the trace element signal during the summer months indicated an average summer growth rate of around 14.5 mmyr⁻¹ compared with 9.5 mmyr⁻¹ in winter (consistent with the observations of Alibert and McCulloch for the same coral). Using time series analysis software (Paillard et al., 1996), distance was transformed into time by matching the boron signal to the temperature record using a sinusoidal distance distortion (Martinson et al., 1987; as quoted by Paillard et al., 1996).

No attempt was made to match up high resolution fluctuations between the temperature and trace element records in the manner of Alibert and McCulloch (1997), because structural contributions to the mid-scale variability in the coral tended to obscure the more subtle temperature features. The data series were resampled at the same time-resolution as the temperature record and graphed. Figure 8.2 displays the element data superimposed upon the SST record, while figure 8.3 contains scatter plots of SST against elements. Variation in the trace element to calcium ratio with temperature is treated empirically and, following the precedent of other researchers, linear functions were fitted to the trace element vs. SST data. These equations are presented in table 8.1, and graphed in figures 8.4 - 8.7.

The results of the solution analysis and the averages of the laser profiles are presented in table 8.2. The region of the laser profile that was averaged was carefully matched to the region of the coral that was milled for the bulk analysis. Uncertainties associated with misalignment have been simulated, and are generally insignificant compared with other sources of error.