Chapter 4 Effects of variation in tissue thickness and stress upon stable isotope records in coral skeletons

4.1 Introduction

Reconstruction of past climates and environments based on proxy records is an important task because instrumental records only go back (in the best cases) about 150 years. Earth's climate system can shift between dramatically different climate states in a matter of years and/or decades; climate cycles may vary on sub-decadal, decadal, centennial and even longer scales. For example, to determine the scale and severity of the current global warming episode (or, in fact, to identify it), it must put it in context with previous episodes from the distant past.

One of the first biological proxy-record instantiations was the use of varying width of tree rings to determine rainfall patterns in semi-arid Arizona (Fritts, 1976). Patterns in tree-ring widths, density, and isotopic composition reflect variations in climate. In the marine area, massive corals were immediately interesting because it was discovered that the skeletons of many massive corals are organized into annual density bands – which were straight away likened to tree rings. Similar techniques for extraction of proxy records from tree rings are used for coral records. The use of stable isotopes of oxygen and carbon from the carbonate of coral skeletons to reconstruct past environments has become increasingly popular over the last 20 years as the techniques and equipment allow higher resolution.

Techniques used to extract proxy environmental records from coral skeletons are based on correlations between observed variations in skeletal parameters and instrumental records of environmental variation. Despite early warnings against the assumption that corals are simple black box analogue recorders of environmental variation (e.g. Buddemeier and Kinzie, 1976) there have been no long-term or manipulative biological field experiments to test the validity of skeletal proxies. Likewise, there is an implicit assumption when using these tools that the stable isotope-environmental proxy relationship has remained stable throughout the history of the coral, without regard to possible biological changes in the meantime (Quinn & Sampson, 2002). There is a perceived need for these techniques to be ground-truthed against records of biological stress to determine the validity of reported phenomena.

Variations in the thickness of the tissue layer are likely to affect proxy environmental records stored in coral skeletons, both as annual density banding (see, Lough and Barnes, 1992; Taylor *et al*, 1993) and as inclusions within skeletal calcium carbonate (see, Barnes *et al*, 1995; Taylor *et al*, 1995). It has been shown here (Chapters 2 & 3) that tissue thickness varies seasonally and inter-annually, and that it is affected by stress. This chapter reports linkages between variations in tissue thickness and variations in $\delta^{18}O$ and $\delta^{13}C$ records recovered from the skeleton of *Porites*.

4.1.1 Proxy records from coral skeletons

Skeletal records in coral skeletons fall into two main groups: first, the annual density bands initially described by Knutson *et al* (1972); second, variations in

the composition of skeletons, dating of which was initially made possible by the discovery of annual density bands. Density bands in coral skeletons have been regarded as a much-needed marine analogue to tree rings (e.g., Knutson *et al.,* 1972; Hudson, 1981; Wellington and Glynn, 1983). X-radiography of sections cut from a growth axis of skeletons of massive corals reveals alternating bands of dense and less-dense skeleton. There is general agreement that each couplet represents one year's growth. Early research tended to link annual density banding with annual cycles in temperature and light. Later work invoked accessory factors such as reproductive cycles, turbidity and nutrient availability. Barnes and Lough (1993) and Taylor *et al.* (1993) argued that annual density bands in coral skeletons were likely to be due to temperature or light or both, since these were the only environmental factors which followed annual cycles. They suggested that light and temperature would more-or-less vary as a sine curve through the year. Hence, they used a sine curve annual forcing function in their numerical models of coral growth. Eventually, Lough and Barnes (2000) concluded that temperature is the dominant annual environmental forcing factor for *Porites* growing in shallow (< 6m) water.

The geographical distribution of large massive corals meant that researchers in the Atlantic Ocean used faviid corals (mainly *Montastrea*), while Indo-Pacific researchers concentrated on *Porites*. Other massive corals (mainly Agariciids) have been investigated as potentially useful proxy environmental recorders because of their slow growth, which means that extremely long records can be obtained. Some Pacific researchers have recently shifted their interests to another faviid, *Diploastrea,* because of its slow growth rate (e.g., Watanabe *et*

al. 2003). Very slow growing, deep water, ahermatypic corals offer similar promise of long records (e.g.Adkins *et al.* 2003).

Analysis of coral density banding provides two basic growth parameters: linear extension rate and calcification rate. Linear extension can be measured directly from banding patterns visible in X-radiographs of coral skeletons. The year is the most obvious unit of time available using this technique, particularly since gamma densitometry (see Chalker & Barnes, 1990; Barnes & Taylor, 1993) indicates that the shifts from dense to less-dense skeleton are much less abrupt than X-ray images imply. Barnes and Lough (1990) attributed square wave density banding patterns commonly described for coral skeletons to fine bands, not easily seen in X-radiographs. Calcification rate is the product of the linear extension rate and the average density of the skeleton over the time interval over which extension was measured

In early work, entire colonies were removed from the water and slices sawn from a growth axis. The slices were then X-rayed or density was measured in narrow tracks across the slices using gamma densitometry (Chalker and Barnes, 1990). This technique was destructive and could be used only with colonies less than ~0.5 m high, which contained records of, very approximately, <50 years length. Such samples were used to test records and techniques for recovery of records. Techniques for extraction of core samples from larger corals were developed to obtain records beyond the span of instrumental records (Lough and Barnes, 2000; Swart & Grottoli, 2003). The

longest records may cover environmental changes over several hundred years (e.g. Lough & Barnes, 1997; Hendy *et al.* 2003).

During the last two decades, efforts to recover proxy environmental information from corals have shifted from analyses of density banding to analyses of the composition of the skeleton. Certain materials are included in coral skeletons in trace amounts. For example, barium and strontium substitute for calcium in the aragonite matrix (see Cohen & McConnaughey 2003). Other trace elements found in coral skeletons include uranium, thorium, magnesium and caesium. Trace inclusions may be particulate materials engulfed by the coral or dissolved compounds (see Barnes *et al.* 1995) which infiltrate the skeleton. Many of the inclusions can be traced to terrestrial sources and can be used to reconstruct flood or rainfall patterns (Hendy *et al.* 2003).

It is also possible to look at trace inclusions in fossil corals that have not undergone digenesis. The shift from aragonite to calcite during digenesis changes the amount of trace inclusions in skeletons. Diagenesis also affects the stable isotope ratios in the skeleton (lowering the bias against the heavier isotopes: McGregor & Gagan, 2003). Fossil corals that have not undergone digenesis can be dated quite well. For instance, U-Th dating provides dates with errors of less than 1% for samples that are 100-200 000 years old (Gagan *et al.* 2000), with the annual density banding pattern and annual cycles in ^{18}O and ^{13}C content allowing comparisons with annual cycles in modern corals.

Initial work looked at the ratios of isotopes of oxygen (^{18}O: ^{16}O) and carbon (^{13}C: ^{12}C) in skeletal carbonate. This early research measured average isotope ratios in bulk samples representing several years' growth of the colony. Oxygen isotopic ratios are strongly affected by temperature and offer a record of seawater temperatures over the life of a colony. Latitudinal trends in these average isotope ratios correspond well with similar trends in seawater temperature. As sampling and analytical methodology and equipment improved, researchers were able to measure fluctuations in isotope ratios in samples removed from within annual growth bands. It then became possible to examine fluctuations in environmental conditions during a year. Short pulse fluctuations in isotope ratios can record up-welling events (Gagan *et al.* 2000). The most common interpretation of carbon isotope fluctuation is as a measure of the contribution of the symbiotic zooxanthellae to the nutrition of the coral (see Grottoli, 1999 for a more complete treatment).

4.1.2 Isotopic chemistry of coral skeletons

The overwhelming mass of a coral skeleton is aragonite, the metastable form of calcium carbonate. Carbon and oxygen exist in nature in several stable isotopic forms and the carbonate fraction in calcium carbonate includes these forms. Isotopes are forms of an element having identical chemical properties but different atomic weights. Atomic weight differs amongst the isotopes of an element because of different numbers of neutrons. Chemical properties of isotopes are identical since these arise from the number of electrons and the number of electrons does not differ amongst the isotopes of an element.

Different isotopes of the same element do behave slightly differently in reactions due to differences in atomic mass. Light and heavy isotopes have different momentums and inertias and, hence, have slight differences in the way they take part in chemical reactions. The most abundant isotopes of oxygen and carbon are ^{16}O and ^{18}O, and ^{12}C and ^{13}C. The products of a reaction may have slightly different ratios of ^{18}O: ^{16}O and ^{13}C: ^{12}C than were present in the reactants. The change in oxygen and carbon isotopic ratios during a reaction is called fractionation. Physical processes, such as evaporation and condensation and diffusion also result in significant fractionation. For example, the light isotope of oxygen, ^{16}O, is enriched in water evaporating from the sea as a result of the slightly lower energy required for the gas phase change in the lighter isotope relative to the heavier species. On the other hand, rain is enriched in the heavy isotope for the same reason, resulting in a further concentration of the light isotope in atmospheric water vapour. Because of differences in the processes of evaporation and condensation between equatorial regions and polar regions, with evaporation predominating at low latitudes and condensation at high latitudes, atmospheric water in the polar regions may be enriched in the light isotope by as much as 5 percent. This kinetic fractionation process underlies a great deal of the science behind stable isotope proxy records.

Coral skeletons contain more of the lighter isotopes of oxygen and carbon than calcium carbonate inorganically precipitated from seawater. Thus, coral aragonite has more ^{16}O and ^{12}C than is found in carbon and oxygen in seawater carbonates. Since fractionation of oxygen isotopes is strongly

temperature dependent, skeletal isotope ratios offer a record of seawater temperature at the time of skeletal deposition. Evaporation of seawater favours the lighter oxygen isotope more than condensation favours the heavier isotope. Hence, freshwater has relatively more ^{16}O than seawater. Consequently, skeletal deposition from seawater significantly diluted by freshwater, from land runoff or rainfall, results in lighter than normal skeleton and a skeletal record of salinity, provided allowance can be made for the temperature effect.

In practice, isotope ratios of ^{18}O: ^{16}O and ^{13}C: ^{12}C are measured relative to the ratios present in standards. The most common standard for reporting relative ^{13}C and ^{18}O measurements through the *delta* (δ) notation using calcium carbonate is the international Atomic Energy Agency (IAEA) standard, VPDB (Vienna-PeeDee Belemnite). Originally, the PDB standard was CO_2 gas created by dissolving samples taken from an internal calcite structure from a fossil belemnite (*Belemnitella Americana*) from the Cretaceous Pee Dee Formation in South Carolina in 1957. This resource, however, expired and was essentially replaced by an internationally-accepted isotope ratio scale, primarily realized through NIST (National Institute of Standards and Technology, formerly known as the National Bureau of Standards (NBS)) Reference Material 8544 (NBS 19). Today, the standard is created artificially to match the oxygen and carbon ratios of the original standards. The relative disequilibrium of biogenic carbonates is assessed against standard seawater (**VSMOW:** Vienna Standard Mean Ocean Water, now identified with the internationally-accepted isotope ratio scale reference water (NIST RM 8535)

that defines the VSMOW hydrogen and oxygen isotope ratio scales). Relative to VSMOW, VPDB has a $\delta^{18}O$ (=ratio of ^{18}O: ^{16}O) of -29.9‰ (McConnaughey, 2003).

Researchers have identified a number of factors that contribute to changes in skeletal $\delta^{13}C$ ratios. These include differences in the isotopic ratios of various carbon pools that may contribute inorganic carbon to the skeleton. Such pools include the seawater pool of dissolved inorganic carbon (DIC; Swart *et al.*, 1996), the internal carbon pools of the coral that contain organic carbon from both photosynthesis and feeding (Swart, 1983; Patzold, 1984; McConnaughey, 1989a; Klein *et al.*, 1992; Carriquiry *et al.*, 1994; Wellington and Dunbar, 1995; Swart *et al.*, 1996; Allison *et al.*, 1996; Juillet-Leclerc *et al.*, 1997). Other factors affecting the carbon isotope ratios of the internal carbon pools are metabolic demands upon the internal carbon pools (Porter *et al.*, 1989; Carriquiry *et al.*, 1994; Allison *et al.*, 1996) and changes in proportions of heterotrophic to autotrophic feeding (Carriquiry *et al.*, 1994; Swart *et al.*, 1996, Grottoli, 2002). Other factors suggested as affecting ^{12}C: ^{13}C ratios in coral skeleton include coral spawning (Gagan *et al.*, 1994; Gagan *et al.*, 1996), the topography of the colony (Cohen and Hart, 1997) and kinetic effects associated with the rate of coral growth and calcification (McConnaughey, 1989a; Aharon, 1991; DeVilliers *et al.*, 1994; Allison *et al.*, 1996; Cohen and Hart, 1997; Guzman and Tudhope, 1998).

Skeletal carbonate is considerably further from isotopic equilibrium than can easily be explained by kinetic fractionation during precipitation. This added

disequilibrium has been called a "vital" effect. McConnaughey (1989a) sought to explain the "vital effect" in terms of a combination of kinetic fractionation and metabolic effects. Kinetic fractionation is associated with hydration and hydroxylation of CO_2 and results in depletion of both ^{13}C and ^{18}O. As an example, during photosynthesis, the lighter isotope of carbon is fixed in the photosynthate at a higher rate relative to the heavier isotopes because of the kinetic effect. Photosynthetically-derived organic materials are therefore isotopically light.

The metabolic disequilibrium effect is harder to pin down. The process of calcification in corals incorporates carbonate molecules derived from metabolic sources and DIC from water which percolates through pore spaces (Cohen & McConnaughey, 2003). Skeleton which incorporates more carbon from the metabolic pool will thus be even more negatively biased from equilibrium than skeleton produced under the same conditions from carbon sources equilibrated with the environment (McConnaughey, 1989a,b). In addition, new models suggest that the internal pH of the calcification zone may be manipulated by the coral during calcification (e.g. Adkins et at. 2003, McConnaughey, 2003, Rollion-Bard *et al.*, 2003). The effects of raising pH on isotopic disequilibrium include enrichment of ^{18}O (McConnaughey, 2003, Rollion-Bard *et al.*, 2003). Intuitively, this mechanism introduces a bias in the oxygen isotope ratio. If corals raise pH to lower the precipitation threshold of $CaCO_3$, then physiological changes may make calcification processes more subject to fluctuation than previously thought. This is an additional

component to the vital effect which may change the understanding of how the process of scleractinian calcification operates.

The relative contributions of DIC and metabolic CO_2 and carbonate ions to the carbon and oxygen pools of calcifying organisms are not well known. The usefulness of isotopic analysis of skeletal material is apparently not diminished by this uncertainty, however, since the values which are measurable by standard techniques seem to track average environmental variations quite well (Risk *et al.*, 2003). The question of whether the behaviour of corals under stress can influence the process of calcification or the nature of the skeleton is open to speculation (Grottoli, 1999, 2002).

4.1.3 Factors affecting the fidelity of proxy records

Both isotope ratios and trace inclusion techniques are subject to biases and distortions due to growth processes. Initial deposition extends the skeleton at its outer edge while thickening takes place throughout the depth of the tissue layer. Linear extension is not constant. Calcification rate is likewise not constant. The initial deposition of skeleton at the outer edge of the colony is overlaid by secondary thickening within the tissue layer and the ratio of initial deposition to thickening deposition is not constant. The proxy environmental signals in initial deposition will more-or-less reflect environmental conditions at the time marked by the outer edge.

In paleo-environmental literature thus far, all dating of coral records has been carried out using the outer edge of a colony as the definitive time marker (i.e.,

the date of collection). However thickening deposition on the initial skeletal deposition can take place over a few months to nearly a year depending on the tissue thickness. Thus, a summer temperature record recorded in the initial deposition will later be moderated by thickening deposition added during autumn and winter. Environmental signals are therefore blurred across several months of growth (Taylor *et al.* 1995). The amount of thickening is further complicated because the thickness of the tissue layer is not constant (Chapter 2). Summer tissue thickness is greater than winter tissue thickness; therefore the averaging effect of secondary thickening will be greater in summer than winter.

Multi-proxy records (i.e. those obtained by using several techniques to extract proxy environmental data from the same core) are now finding currency amongst researchers (e.g. Fallon *et al.* 2003; Quinn & Sampson, 2002). An example of this is the use of the Sr/Ca ratio, which is vulnerable to temperature and salinity variations, to cancel out the effects of salinity on the $\delta^{18}O$ thermometer (Gagan *et al.* 2000). High resolution techniques, based on laser or particle beam ablation of skeleton coupled with absorption spectroscopy allow detailed information to be extracted from small skeletal elements (e.g. Fallon *et al.* 1999).

4.1.4 Rationale

The skeletons of *Porites* extend by the formation and extension of the vertical skeletal elements. Trabeculae are formed by groups of sclerodermites. Sclerodermites are three-dimensional fans composed of fine aragonite crystal

fibres growing around a calcification centre in spherulites (which are themselves grouped as fishscale-shaped bundles called fasciculi). The linear extension phase of growth appears to occur exclusively at night (Vago, 1997, Cohen & McConnaughey, 2003). The trabeculae terminate in dentations at the growing tips of the septa. Lateral spikes from these dentations, which are in the form of splayed fans, may link periodically to form synapticulae. The fans form anew at night and are subject to secondary thickening and cross-linking during the day (Cohen & McConnaughey, 2003). Environmental signals are thus captured by the vertical elements of the skeleton. That is, the conditions prevailing at the time of formation of the vertical elements define the nature of the skeleton at that point. This signal is subsequently blurred by secondary thickening within the tissue layer.

Some dissention exists about the amount of secondary thickening within the tissue layer: Wellington *et al.* (1996) for instance, suggest that ~90% of skeletogenesis occurs within the first 1mm of skeleton. Barnes & Lough (1993, 1996) and Taylor *et al.* (1993, 1995) prefer that substantial thickening occurs throughout the tissue layer. The second theory therefore predicates that environmental signals will represent less of a snapshot than a running mean of environmental conditions for the entire time the skeleton was within the tissue layer.

The study in this chapter was undertaken to examine whether isotope-based techniques used to reconstruct past marine environments have the ability to detect sublethal stress events in the life of a coral. These techniques have

been developed in geochemical labs over the last several decades to a high level of sophistication, yet they have never been tested against histories of known biological variation – they are essentially correlative, and in need of biological ground-truthing at several levels.

Stable isotopes were chosen as the test proxy because of their increasing use in the field of paleo-climate reconstruction and the relevance of this investigation in respect of recently-published investigations. The degree of precision of isotopic analysis (sampling up to 50 times within an annual cycle of skeleton formation) potentially allowed near weekly resolution of the effects of environmental impacts and the corals' responses to them. Depending on the degree of blurring by the process of secondary thickening, therefore, these techniques should have the ability to detect the onset of the stress events with a high degree of precision. This study represents the first time that a long term record of biological variation has been compared to a skeletal proxy record. It is also the first time that manipulative field experiments have been performed which may affect the skeletal record and (quite importantly) can be directly compared with a natural stress event.

4.2 Materials and Methods

4.2.1 Source of study cores

The corals used in this study were selected from the groups used in previous studies (Chapters 2, 3) in Pioneer Bay, Orpheus Island. The thickness of the tissue layer of all colonies had been monitored at regular intervals between 1994 and 1996, and again from 1998 to 2000. Corals that had been part of the experiment to explore the effects of shading stress (Ch. 3) were affected by the shade treatment from October 1994 to July 1996. All of the colonies in the treatment group experienced sustained tissue loss during the experiment, losing an average of approximately 50% of pre-treatment tissue thickness. This tissue loss was reversed after the removal of the shading stress. Seasonal variation in tissue thickness, observed in the control colonies, was much less pronounced in the shaded colonies. Early in 1998, 20 months after the shade was removed, all colonies (both shaded and control) were affected by a mass bleaching event (see Chapter 3). Colonies used in the current study bleached during this event, but appeared to recover fully in the months afterwards.

Corals used in the study described in this chapter were randomly chosen from amongst the colonies used in the shading experiment. Seven colonies were cored as described below (four shaded – obtained in August 1999 – and three unshaded – obtained in April 2000) and the cores that most closely followed the central growth axis (identified from X-radiographs taken of core slices) were used in the current analysis. This procedure resulted in a mixture of species: one shaded and the unshaded coral were identified as *P. australiensis*

and the second shaded coral was *P. lobata.* Since the bulk of bio-geochemical studies published to date do not identify massive *Porites* to species level, it was considered of no consequence at the time.

4.2.2 Collection of cores and preparation of samples

In August 1999, 50mm diameter cores were taken from the uppermost surface of two colonies using a 30cm long stainless steel barrel fitted with carbon-steel cutting bit. A core from a third colony, which had not been shaded, was obtained in April 2000 in the same way. The core barrel was cleaned with 0.5M HCl and rinsed in fresh water before use to remove any traces of hydrocarbon. Colonies were drilled normal to the surface so that a vertical growth axes was sampled. Cores removed were 150 mm long. Such cores potentially sampled 10 years of growth before shading and 5 years of growth after shading, as well as the shading period. Cores were taken to nearby Orpheus Island Research Station, and thoroughly washed with fresh water. They were then allowed to air dry in sunlight for 6 hours before being wrapped in plastic film to protect them during transportation.

Cores were taken to the Research School of Earth Sciences at the Australian National University in Canberra. Three 7mm-thick slices were cut longitudinally from close to the centre of each core using a 120 mm diameter water-lubricated diamond saw. The slices were dried for 2 days at 35°C. Each slice was X-radiographed using medical radiology equipment.

The sampling device used in this study was adapted from a milling machine and sampled at precise intervals in a straight line. Growth of *Porites* is not strictly radial. Because the surface area of the corallum increases with linear extension, new corallites are formed at intervals in the centre of a fan as space becomes available. Darke and Barnes (1993) showed that the tracks of individual calices deviate from the axis of growth because new calices are constantly formed at the centre of the growth axis. Examination of a coral skeleton reveals fans of corallites centred on the axis of growth (Figure 4.1). Visual inspection of X-radiographs confirms that the centre of growth "wanders" as new calices are budded off. The coral slice was therefore shaped so that all sampling would occur within the centre of growth. This was to ensure that the proxy record represented a linear time progression of growth along the slice. The slices were shaped along the reverse edge using a carbon-steel band saw to provide suitable attachment surfaces when mounting the slice in the sampling device.

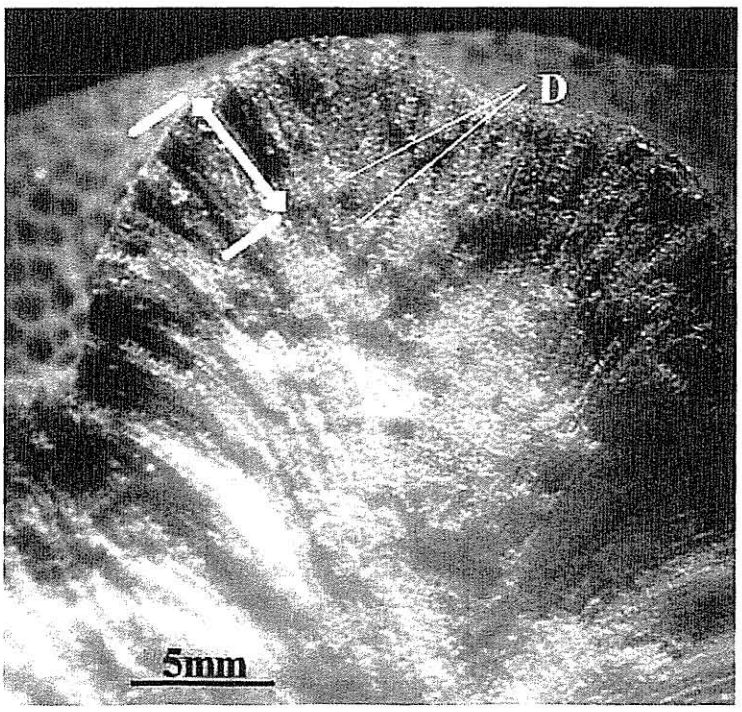

Figure 4.1

Fractured core from *Porites lutea* specimen showing the corallite tracks radiating out from the central growth axis. Arrows = extent of the tissue layer, D=dissepiments

The slices were then placed in the milling machine fitted with rigorously cleaned tungsten milling bits, controlled by software developed at ANU. Skeletal material above and below the growth axis was removed to leave a ledge, precisely 2mm thick and 5mm deep to expose the centre of the growth axis (Figure 4.2). The slices were then cleaned in Millipore-filtered water using an ultrasonic probe to remove any small particles and contaminants (including the tissue at the outer margin of slice). Slices were placed into the 35°C oven for a further two days to dry.

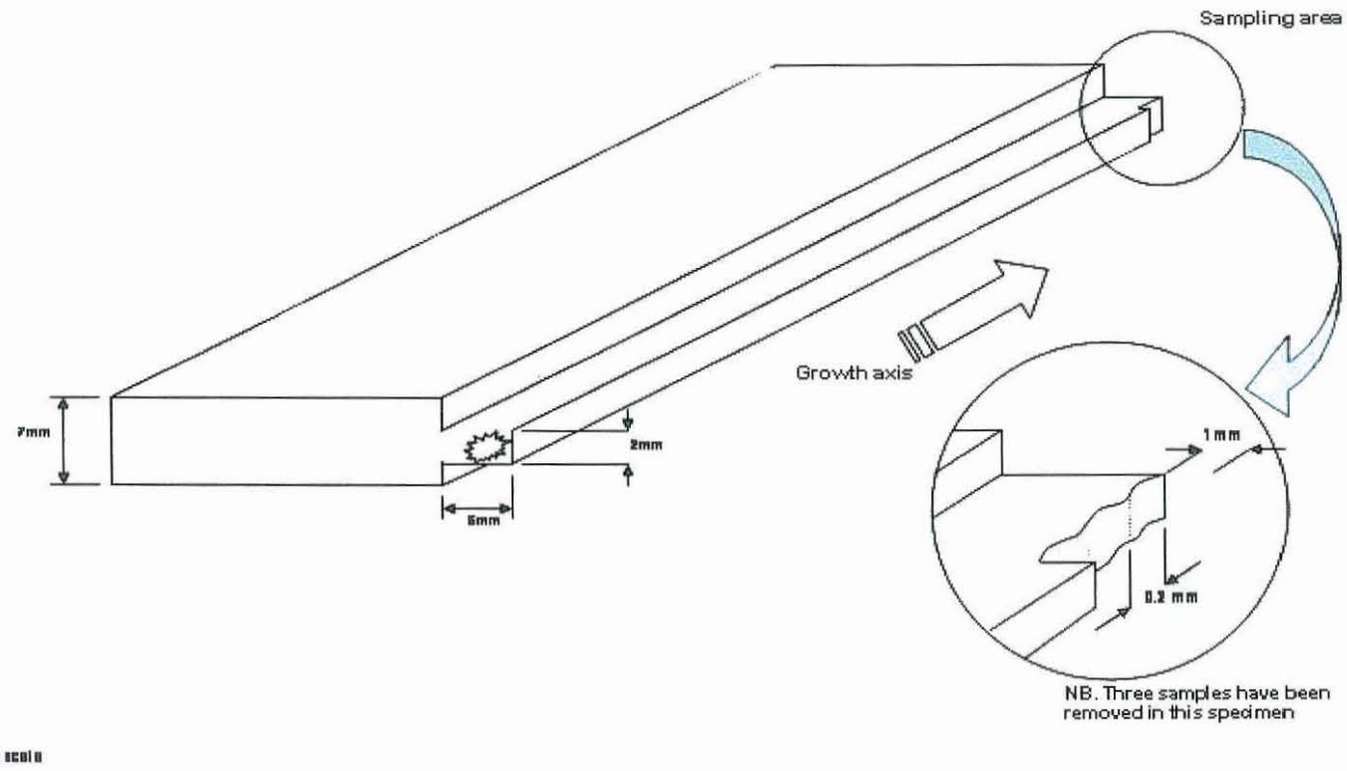

Figure 4.2 Detail of sampling of a slice from a skeletal core used for fine-scale isotopic analysis.

The prepared skeleton was mounted in the milling machine and a series of samples were removed from one end of the ledge using a 0.7mm diameter tungsten milling bit. During each sampling iteration, the bit travelled 1mm inwards from the point of contact with the ledge and 0.2mm along the axis of the slice (Figure 4.2). Each 0.2mm increment yielded approximately 400-450μg of fine aragonite powder. The powder generated by milling one increment was allowed to fall onto a clean piece of plasticised paper. The powder was then transferred to a clean, labelled plastic vial, which was then sealed to prevent possible contamination. The milling face of the coral slice and the tool were cleaned using blasts of clean compressed air between milling each increment. Sampling continued for 105mm along the length of the slice, yielding slightly over 500 samples.

4.2.3 Measurement of stable isotopes

200μg sub-samples were portioned from the material obtained from each sampling increment. The remainder of the material was reserved in case duplicate measurements were required (or in the case that some measurement failure led to the initial sub-sample being discarded). Sub-samples were placed into cleaned quartz vials. The samples were analysed with a Finnigan MAT 251 mass spectrometer using a Kiel Microcarbonate device. The Kiel device added 105% orthophosphoric acid at 90°C to the powder to produce CO_2 gas. Gas evolved in this reaction was pumped into the MAT 251 for analysis. The reaction chamber and mass spectrometer were purged with reference gas (KAZZA: δ^{18}OV-PDB = - 1.88; δ^{13}CV-PDB = +2.39) between

samples to ensure that levels detected in each measurement were not contaminated by traces from previous samples.

Batches of skeletal samples were interspersed at defined intervals with calibration samples of the NBS 19 calcite standard to allow corrections to be made for "machine drift". Machine drift is a characteristic of mass spectrometers. It may arise from differences in environmental conditions in the building where the machine is housed, in electrical power supply, in age of componentry or in some cases, quirks developed by the machine over years of operation can influence results obtained during analysis. Other contributors to machine drift include: the laser becoming slightly unfocussed, the detector less efficient and changes in the working gas. Overall, there are many factors that can change or influence results obtained with a mass spectrometer. Internal standards, usually emulating something like VPDB, are used to keep track of the machine's performance, allowing correction of sample readings or intervention in machine operation if a trend away from normal is identified. In a typical pattern at ANU, 5 out of 22 samples in a carousel will be standards, spaced at defined intervals (i.e. the first two samples and the final sample, plus two samples spaced evenly in the carousel).

These analyses provide data equivalent to data provided using the McCrea (1950) technique, in which calcium carbonate is dissolved in 105% H_3PO_4at 25°C for more than 8 hrs. McCrea (1950) provided much of the baseline isotopic disequilibrium data used since in most geochemical analyses of carbonates. Subsequent techniques usually attempt to corroborate the results against baseline analyses using the McCrea process.

Isotope ratios initially recorded by the MAT251 were adjusted in two stages. Data reduction was performed by the software that controls the MAT 251. During this stage, data were corrected for ^{17}O interference using the method of Santrock *et al.* (1985).

Data were also corrected for deviations from the McCrea process:

1. The reaction temperature inside the Kiel device being 90°C;
2. No equilibration time of the reaction products CO_2 and H_2O (the McCrea reaction occurs over an 8 hour period, allowing some isotopic equilibration of reaction products. In contrast, the Kiel device evacuates reaction products within 15 minutes, therefore introducing a small, but correctable bias in the isotope ratios of the reaction products relative to products derived from the McCrea process *sensu strictu*).

In the second stage, data provided by the MAT 251 were scaled to the PDB scale.

Temperatures in the environment around the coral colonies were obtained from a Great Barrier Reef Marine Park Authority GBRMPA online sea temperature monitoring database:
(http://www.grbmpa.gov.au/corp_site/info_services/science/seatemp/).

Temperatures were recorded at 3m depth (relative to MLWS) in Pioneer Bay, Orpheus Island (Berkelmans, 1998, pers.com.), within 50m of the corals used in this study. All corals used in this study were located in <5m of water

(MLWS); within this narrow depth range, thorough mixing is assumed, so that conditions recorded by instrumental data apply to all of these corals.

Rainfall at the nearest Australian Bureau of Meteorology recording site (Lucinda Jetty, 12 km WNW of Orpheus Island) and river flow data for the Burdekin River (which is the outflow which most influences the region (see McCulloch *et al.* 2003)) were obtained from the Australian Bureau of Meteorology.