ENVIRONMENTAL SIGNALS IN CORAL TISSUE AND SKELETON: EXAMPLES FROM THE CARIBBEAN AND INDO-PACIFIC

by

JEFFREY M. HEIKOOP, B.Sc. (HONOURS)

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AUTHOR: Jeffrey M. Heikoop, B. Sc. (McMaster University)

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ABSTRACT

Coral tissue and skeleton contain physical and chemical proxy signatures of the surrounding environment. The purpose of this thesis is to broaden or refine the use of corals as environmental recorders/tracers by examining the fidelity of both new and existing techniques. Corals from the Caribbean and Indo-Pacific are utilised, with primary reference to samples from Jamaica and Indonesia.

Coral skeletons from Banda Api, Indonesia, contain evidence of a short term pulse of volcanic ash and hydrothermal fluids which affected the surrounding reef during a May, 1988 eruption. Partial burial of corals by volcanic ash led to the formation of highly bioeroded death surfaces and the incorporation of ash into underlying skeletal pores. Subsequent recovery of the coral resulted in preservation of these features as death/regrowth surfaces. Hydrothermal activity is preserved in the skeleton as a distinct orange layer of iron hydroxide which has been termed the "Banda Band". X-radiography confirms that the location of these features within the coral skeletons coincides with the timing of the eruption. Preservation of an environmental pulse lasting only a few days demonstrates the fine scale resolution that can be obtained from coral skeletal records.

Coral tissues from Jamaica and Zanzibar, collected along depth/light gradients, were analysed for $\delta^{15}N$. Coral tissue $\delta^{15}N$ was found to decrease with

decreasing light availability. A conceptual steady state model has been developed to explain this relationship. In higher light conditions, corals and/or their symbiotic algae significantly deplete the internal pool of dissolved inorganic nitrogen, resulting in minimal fractionation during uptake. Under lower light conditions, however, less dissolved nitrogen is assimilated and fractionation is more fully expressed, with the light isotope of nitrogen being taken up preferentially. These results suggest corals are not conservative tracers of nitrogen. It is proposed, however, that shallow water (<5 m), autotrophic species of corals may exhibit minimal fractionation and prove to be excellent tracers of dissolved inorganic nitrogen on reefs.

$\delta^{15}N$ and $\delta^{13}C$ signals of shallow water corals collected from seven different reefs were compiled. Significant inter-reef variability in $\delta^{15}N$ was found. Both light availability and the isotopic composition of source nitrogen are thought to contribute to the variation between reefs. Other factors such as nutrient concentration may also be important. $\delta^{13}C$ variability was much less significant in the data set, with most reefs having enriched coral tissue signals, consistent with a primarily autotrophic diet.

The tissue and underlying skeleton of Jamaica and Zanzibar corals were also analysed for $\delta^{13}C$. Tissue $\delta^{13}C$ was found to decrease with decreasing light availability. Skeletal $\delta^{13}C$, however, shows no relationship with depth/light availability. It is proposed that strong kinetic isotope effects associated with the

rate of calcification mask any such relationship. A simple data transformation is applied to account for the presence of kinetic isotope effects, the result of which is a much clearer relationship between skeletal $\delta^{13}C$ and light availablity. These results suggest that it is possible to correct for the presence of kinetic isotope effects associated with the rate of skeletogenesis and to resolve meaningful environmental/metabolic information from skeletal carbon isotopic records.

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PREFACE

Chapters 2-5 are presented in publication format. The following information details the contributions of the candidate to each paper. Co-authors' contributions are identified and the status of each paper is given. The use of the pronoun "we" in the text acknowledges the contributions and shared beliefs of the co-authors.

Chapter 2: Corals as proxy recorders of volcanic activity: Evidence from Banda Api, Indonesia
Authors: J.M. Heikoop, C.J. Tsujita, M.J. Risk and T. Tomascik
Status: Published in Palaios (complete citation given at beginning of Chapter 2). Copyright has been released by SEPM. Documentation on file with McMaster Graduate Studies.

Samples were collected by M.J. Risk and T. Tomascik. All sample preparation and microscopy was performed by the candidate with help from C.J. Tsujita. Candidate was responsible for primary interpretation and writing with assistance by C.J. Tsujita. Comments and revisions were suggested by all co-authors.

Chapter 3: Relationship between light and $\delta^{15}N$ of coral tissue: Examples from Jamaica and Zanzibar
Authors: J.M. Heikoop, J.J. Dunn, M.J. Risk, I.M. Sandeman, H.P. Schwarcz and N. Waltho
Status: Submitted to Limnology and Oceanography

Jamaica coral samples were collected by N. Waltho. Light data were measured by I.M. Sandeman. Zanzibar samples were collected by M.J. Risk and J.J. Dunn. Zanzibar analyses by J.J. Dunn. All other analyses by the candidate. Primary interpretation and writing by the candidate. Comments and revisions were suggested by all co-authors.

Chapter 4: Potential influences on inter-reef variability in $\delta^{15}N$ of coral tissue
Authors: J.M. Heikoop, J.J. Dunn, M..J. Risk, T. Tomascik, H.P. Schwarcz and I.M. Sandeman
Status: subject to re-review in Estuarine, Coastal and Shelf Science

This chapter represents a compilation of data presented elsewhere in this thesis (Chapters Three and Five) and in other publications which are detailed in the text. Costa Rica and Banda data have not been presented elsewhere. Fiona van Wissen collected and analysed Costa Rica corals. Tomas Tomascik collected

Banda corals which were analysed by the candidate. Primary interpretation and writing by the candidate. Comments and revisions were suggested by all co-authors. Tomas Tomascik, in particular, contributed by improving the section on statistical analysis. Ian Sandeman provided theoretical light curves for all sites studied.

Chapter 5: Isolation of photosynthetic effects in coral skeletons from Jamaica and Zanzibar

Authors: J.M. Heikoop, J.J. Dunn, M.J. Risk, H.P. Schwarcz, I.M. Sandeman, and N. Waltho

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Chapter 1: Introduction

1.1 Basis of study

The skeleton and tissue of reef corals contain physical and chemical proxy signals of the surrounding environment. Proxy records are important in coral research, as many reefs around the world are experiencing declining health associated with environmental degradation, yet relatively few long-term reef monitoring studies have been undertaken. Tools which can be used to reconstruct changes in reef environments and resulting impacts on coral health could prove extremely beneficial to coral reef management and in assessing global change. Because many proxy records rely on a coral's metabolic or physiological response to the surrounding environment, the accuracy of any such records must be closely examined.

The purpose of this thesis is to broaden or refine the use of shallow water, hermatypic corals (reef-building corals with symbiotic algal partners) as environmental recorders/tracers by examining new applications and testing the fidelity of existing tools. The nature of several proxy records contained in both coral skeleton and tissue, primarily from Jamaica and Indonesia, will be examined. Reference to corals from other sites will also be made.

1.2 Environmental Records in Coral Skeleton

The use of corals as environmental recorders began in earnest

following the discovery, almost 25 years ago, that skeletons of hermatypic corals contain annual density bands which can be revealed by X-radiography (Knutson et al., 1972). In a typical coral, each year's growth will comprise a density couplet consisting of a high density and a low density band. The nature and causes of density banding have been shown to be extremely complex (Barnes and Lough, 1993). The formation of density bands probably reflects a forcing function with an annual cyclicity, such as light or temperature, that affects the rate of calcification within the layer of skeleton occupied by coral tissue (Barnes and Lough, 1993; Taylor et al., 1995).

The presence of density banding in coral skeletons allows for the establishment of chronologies which are in many ways analogous to those obtained from tree rings. Numerous studies have utilised this built-in coral chronometer to reconstruct the response of coral growth rate to environmental factors. A decrease in growth rate, for instance, may reflect the impact of stressful factors such as increased siltation (Cortés and Risk, 1985) or eutrophication (Tomascik and Sander, 1985).

The aragonite skeletons of corals contain physical and/or chemical proxy records of the surrounding reef environment, which can also be dated using density bands. Measurement of materials included in coral skeletons can be used to provide information on environmental variation occurring on long-term, annual or short-term scales (Taylor et al., 1995). For example, records of trapped sediment can help reconstruct siltation history on a reef over a period of several

years (Cortés and Risk, 1985). Similarly, concentrations of trace elements in the aragonite skeleton of corals can record the history of anthropogenic metal input to the ocean (e.g., Dodge and Gilbert, 1984; Shen and Boyle, 1987). Alternatively, stable isotopic measurements of coral skeletons have been utilised to reconstruct variations in temperature and insolation occurring on annual time scales (e.g., Carriquiry et al., 1988, 1994).

One of the primary goals of this thesis is to examine skeletal records of a stressful short-term environmental pulse. Any such technique could be useful in studies attempting to reconstruct factors which have affected reef health. By recognising the presence of short-term events recorded in coral skeletons, the effects of acute stresses can be taken into account, in addition to chronic stresses occurring over longer time periods.

A good example of a well studied, relatively short-term event which can be recorded in coral skeletons is climatic variation associated with the El Niño/ Southern Oscillation (ENSO) in the Pacific ocean (see Enfield, 1990, for a review of ENSO). Records of $\delta^{18}O$ in coral skeleton, for instance, can be used to reconstruct paleotemperature or rainfall patterns associated with ENSO events (e.g., Carriquiry et al., 1988, 1994; McConnaughey, 1989; Cole et al., 1993). Measurements of skeletal concentrations of nutrient analogues, such as Ba or Cd, can reflect inputs of upwelled nutrients to reefs (Shen et al., 1987; Lea et al., 1989). ENSO strongly affects the strength of coastal and equatorial upwelling in the eastern Pacific (Enfield, 1990).

It has been suggested that records of short-term pulses based on measurements of concentrations of included material (amount per weight of skeleton for instance), might be affected by geochemical "smearing" and dilution. These effects can be caused by ongoing skeletal deposition throughout the layer of skeleton occupied by coral tissue (Barnes and Lough, 1993; Taylor et al., 1995). Visible layers of included material, or layers which can be made visible (e.g. fluorescent bands), can be more accurately measured and dated (Taylor et al., 1995). Due to the complex nature of skeletal deposition, care must be taken in interpreting short-term proxy skeletal records.

While measurements of chemical or physical proxies might help reconstruct the nature of an environmental pulse, other records can be used to assess the impact on coral health. Recently, Risk and Pearce (1992) documented the occurrence of density couplets occurring with daily periodicity in hermatypic coral skeletons. These authors proposed that changes in daily growth patterns could be used to detect the effects of short-lived events. The presence of "stress bands" superimposed upon annual skeletal density variation could potentially be used to identify periods of short-term stress (e.g., Carriquiry et al., 1988; Carriquiry, 1991). In the case of extreme stress, leading to partial coral mortality, death surfaces might form (e.g., Macintyre and Smith, 1974). Skeletal $\delta^{13}C$ records can be utilised to identify the loss of symbiotic algae during coral bleaching events. This has been demonstrated in the case of coral bleaching caused by ENSO warming in the eastern Pacific (Carriquiry et al.,

1994).

Many natural short-term pulses will occur over time periods of weeks to months (e.g., ENSO, monsoonal flooding, Taylor et al., 1995). The approach taken here was to study whether coral skeletons might contain records of an event in which the initial pulse would last for only a few days. An ideal example is provided by pyroclastic volcanic eruptions. Corals were collected in the Banda Islands, Indonesia. These corals were subjected to ashfall from the eruption of Banda Api, in May 1988 (Pardyanto et al., 1991). The eruption lasted only one day, but deposited voluminous amounts of ash onto surrounding reefs. The corals in question experienced very little anthropogenic stress, so ashfall was the dominant stressing factor. Chapter 2 documents the presence of death surfaces associated with trapped ash particles and iron staining. These features are interpreted as having formed as a result of the eruption of Banda Api and accompanying hydrothermal activity.

1.3 Environmental Records in Coral Tissue

Environmental records contained in coral tissue have received much less attention than records contained in coral skeletons. Unlike skeletal records, coral tissue can only be used to reconstruct conditions immediately preceding the time of coral collection. Nonetheless, such records are potentially useful in identifying environmental gradients. The main tool for environmental analysis utilising coral tissue has been measurement of stable isotope ratios of carbon and nitrogen.

In stable isotopic analysis, the ratio of heavy to light isotope in a sample is compared to that of a standard and reported in delta notation. For example, in the case of $\delta^{15}N$ of coral tissue we have;

$\delta^{15}N = ((R_{sample}/R_{standard}) - 1) * 1000$, reported in per mille (‰) units, where $R=^{15}N/^{14}N$ (^{15}N and ^{14}N refer to the heavy and light isotopes of nitrogen, respectively).

The isotopic signatures of coral tissue may reflect the isotopic composition of dietary nitrogen and carbon (potentially with a constant isotopic offset relative to diet; cf. DeNiro and Epstein, 1978, 1981). Dietary sources include zooplankton and dissolved organic matter consumed directly by the coral (heterotrophy; e.g. Muscatine et al., 1989; Risk et al., 1994). Inorganic nutrients can also form a major portion of coral diet. Symbiotic algae in the coral (zooxanthellae) assimilate dissolved nitrogen and carbon (autotrophy) which can then be translocated to the host in the form of sugars and amino acids (e.g. Muscatine et al., 1984). Where corals behave as conservative isotopic tracers of diet they can be used to trace inputs of isotopically distinct nutrients from marine or terrestrial sources (the term conservative tracer is used here to indicate that coral tissue $\delta^{15}N$ or $\delta^{13}C$ is equal to or offset by a constant amount relative to the $\delta^{15}N$ or $\delta^{13}C$ of diet -- whether dissolved or particulate, organic or inorganic).

Identification of reef nutrients is an important factor in assessing reef health. Corals thrive in a generally nutrient poor environment due to efficient

recycling within the coral/algal symbiosis. Additional nutrient can actually be beneficial to individual corals, while causing damage to the reef as a whole through eutrophication (e.g., Tomascik and Sander, 1985; Edinger and Risk, 1995). This phenomenon has been termed the Janus effect after the two-faced god of Roman mythology (Edinger, 1991; Edinger and Risk, 1995). In some instances however, too much additional nutrient can be harmful to corals, leading to reduced growth rates (Tomascik and Sander, 1985) and reduced translocation of organic matter from the algal symbionts (Jokiel et al., 1994).

Tracing nitrogenous nutrient sources, utilising coral tissue $\delta^{15}N$, has been found to be particularly effective, especially when sources are isotopically distinct. Examples include nutrient input in the form of sewage (Allison et al. 1991; Risk et al., 1993; Dunn, 1995; Mendes et al., 1996), terrestrial organic matter, nitrogen fixation and upwelled nitrogen (Sammarco et al., manuscript in preparation). In some cases, however, even when a nutrient source is thought to be isotopically distinct, no gradient in coral $\delta^{15}N$ is present (e.g., Risk et al., 1993; Dunn, 1995). In such cases it is possible that the response of coral metabolism to some environmental factor might affect the degree of isotopic fractionation between a coral and its diet. In such a scenario, the isotopic composition of coral tissue will not serve as a conservative tracer of source carbon and nitrogen. Instead, coral $\delta^{15}N$ and $\delta^{13}C$ will reflect gradients in the magnitude of environmentally induced fractionation.

A second objective of this thesis is to study fractionation patterns in $\delta^{15}N$ of coral tissue collected over an environmental gradient. Several studies have previously looked at horizontal gradients (Risk et al., 1989; Allison et al., 1991; Risk et al., 1993; Dunn, 1995; Sammarco et al., manuscript in preparation), therefore it was decided that corals collected over a range of depths should be studied. The goal was to determine if corals acted as conservative tracers of diet or whether environmental factors affected the degree of fractionation.

Corals were collected over a depth gradient at Discovery Bay, Jamaica. It has been suggested, based on coral tissue $\delta^{13}C$, that corals living in deeper waters at Discovery Bay rely on heterotrophic feeding to a greater extent than those living in shallower, higher illumination settings (Muscatine et al., 1989). Chapter 3 documents the results of this study where it is shown that coral tissue $\delta^{15}N$ does not conservatively reflect a change in diet over a depth gradient. Instead it is demonstrated that variation in light availability affects the degree of nitrogen isotopic fractionation. Conceptual models to explain the relationship between light and coral $\delta^{15}N$ are presented.

The analysis of nitrogen isotopic fractionation patterns is extended further to examine sources of inter-reef variation (Chapter 4). Tissue $\delta^{13}C$ and $\delta^{15}N$ of corals collected from seven different reefs are compared. It is again proposed that corals do not always function as conservative tracers of nitrogen and that light may play a role in determining the magnitude of nitrogen isotopic

fractionation. Suggestions are made based on the results reported in both Chapter 3 and 4 as to which conditions might be the most conducive to corals functioning as conservative tracers of environmental $\delta^{15}N$.

1.4 Environmental Records Common to Coral Tissue and Skeleton

It is known that neither coral tissue or skeletal $\delta^{13}C$ records are conservative tracers of environmental/dietary $\delta^{13}C$. Zooxanthellae can strongly fractionate carbon during photosynthetic uptake (e.g., Muscatine et al., 1989), especially in settings with low light availability. Coral skeletons are not deposited in isotopic equilibrium with seawater. Both carbon and oxygen are depleted in the skeleton relative to equilibrium (see Fig. 13 in McConnaughey, 1989). This disequilibrium is thought to be due to a combination of kinetic and metabolic isotope effects (McConnaughey, 1989). Kinetic isotope effects cause a simultaneous depletion in oxygen and carbon, relative to equilibrium, and seem to be related to the rate of deposition of the skeleton. Metabolic effects are caused by photosynthesis and respiration modifying the isotopic composition of the carbon pool from which CO_2 for calcification is obtained (McConnaughey, 1989).

Light is a factor which affects the $\delta^{13}C$ of both coral tissue and skeleton. Coral tissue $\delta^{13}C$ decreases with increasing depth (decreasing light availability) (Muscatine et al., 1989). This is thought to be a response to increased reliance on heterotrophy as a nutritional strategy and to increased fractionation during photosynthetic uptake and assimilation of carbon (Muscatine et al., 1989).

Photosynthesis affects skeletal $\delta^{13}C$ by preferentially removing the light isotope of carbon from the internal dissolved inorganic carbon pool (e.g. Weber, 1974; Goreau, 1977a; Fairbanks and Dodge, 1979; McConnaughey, 1989; Carriquiry et al., 1994). Increased light availability/photosynthesis leads to enriched skeletal $\delta^{13}C$.

Light is a very important factor affecting coral metabolism. For example, light availability can control the amount of carbon translocated from algal symbionts to the host , which in turn may affect the degree of coral predation on exogenous food sources (Dubinsky and Jokiel, 1994). Many factors could potentially affect the amount of light received by corals on a reef. Increases in turbidity associated with phytoplankton blooms or siltation for instance, could sharply decrease light availability. Reconstructions of light availability (and its effect on carbon metabolism) based on coral skeletal $\delta^{13}C$ measurements could prove to be a powerful environmental tool.

In this thesis, the fidelity of the response of coral tissue and skeleton $\delta^{13}C$ to changing light availability is tested using the same corals from Discovery Bay as discussed in Chapter 3. Since the same process (photosynthesis) is affecting both records, the relationship between them is also compared. The results of this analysis are presented in Chapter 5. It is found that tissue $\delta^{13}C$ shows a strong relationship with light availability. In the case of skeletal $\delta^{13}C$ however, kinetic isotope disequilibrium effects occurring during calcification

(McConnaughey, 1989) mask this relationship. A simple mathematical transformation to correct for the existence of these effects is presented. This transformation allows us to evaluate metabolic disequilibrium effects associated with light/photosynthesis, in the absence of kinetic isotope effects.

Since many of the environmental records documented in this thesis reflect a coral's metabolic or physiological response to environmental conditions, any insight which might be gained regarding coral biology is also discussed.

1.5 Thesis format

This thesis is presented in a format where each chapter represents a distinct document which is either in press or in the review process. A foreword to each chapter outlines the contributions of the senior author to each study.

Chapter 2: Corals as proxy recorders of volcanic activity: Evidence from Banda Api, Indonesia

Heikoop J. M., Tsujita C. J., Risk M. J. and Tomascik T. (1996) *Palaios* **11**, 286-292.

2.1 Abstract

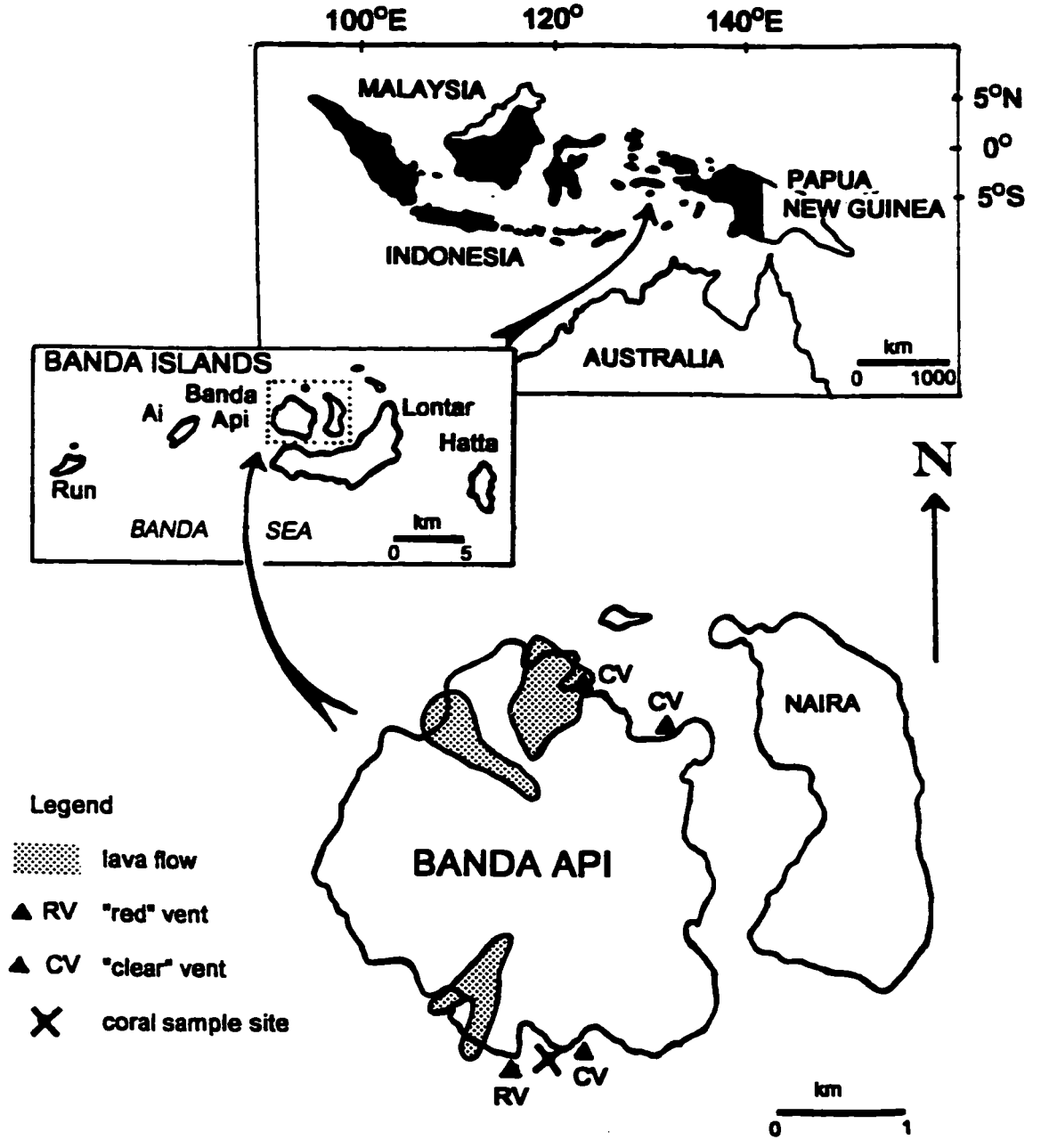
Corals growing on the flanks of Banda Api, Indonesia, contain records of volcanic activity which occurred in May, 1988. Ashfall killed portions of some massive coral colonies (Porites lobata), resulting in the formation of death surfaces. These surfaces were preferential sites for extensive bioerosion and incorporation of volcanic ash into underlying skeletal pores. Subsequent coral regeneration resulted in the preservation of death/regrowth surfaces overlying trapped volcanic ash. An orange-colored iron-rich chemical precipitate is preserved in the skeletons of corals which survived the volcanic event. These distinct orange bands are contemporaneous with the death/regrowth surfaces. The iron banding is interpreted as being a product of hydrothermal activity which accompanied volcanism. X-radiographs of coral skeletons confirm that the timing of formation of both the death surfaces and the orange banding is coincident with the 1988 eruption. The features preserved in these corals may be valuable proxy indicators of volcanic events in analogous recent and ancient environments.

2.2 Introduction

Many coral reefs in Indonesia are developed around volcanic islands which have experienced recent volcanic and hydrothermal activity. Records of these events, preserved in coral skeletons, could include evidence of physiological stress affecting skeletal deposition, or the incorporation of products associated with ashfalls or hydrothermal activity. Corals are useful recorders of the chronology of episodic phenomena since their skeletal density banding patterns often occur with annual periodicity (e.g., Knutson et al., 1972; Macintyre and Smith, 1974; Moore and Krishnaswami, 1974; Buddemeier et al., 1974).

Massive corals growing on the flank of a volcano, Banda Api, Indonesia (Fig. 2.1), were collected to determine if the skeletons contained a record of an eruption which occurred in May of 1988. Banda Api is a stratovolcano located in the Banda Arc, a collision zone between the Eurasian and Indo-Australian plates. Banda Api erupted May 9, 1988, ejecting ash up to 3.5 km into the troposphere (Pardyanto et al., 1991). The ash was blown from east to west, and was deposited over a total area of 4.9 km^2 on the west side of the island. The ash deposit attained an average thickness of 0.25 m and a maximum thickness of 1 m. The single day of ashfall, accompanied by three lava flows, was followed by several days of gaseous eruptions.

Fig 2.1 Map of study area showing location of hydrothermal vents and site of coral collection.


100°E
120°
140°E
5°N
0°
5°S
MALAYSIA
INDONESIA
PAPUA NEW GUINEA
AUSTRALIA
km
0 1000
BANDA ISLANDS
Banda Api
Ai
Lontar
Hatta
Run
BANDA SEA
km
0 5
N
CV
CV
NAIRA
BANDA API
RV
CV
km
0 1
Legend
lava flow
RV "red" vent
CV "clear" vent
coral sample site

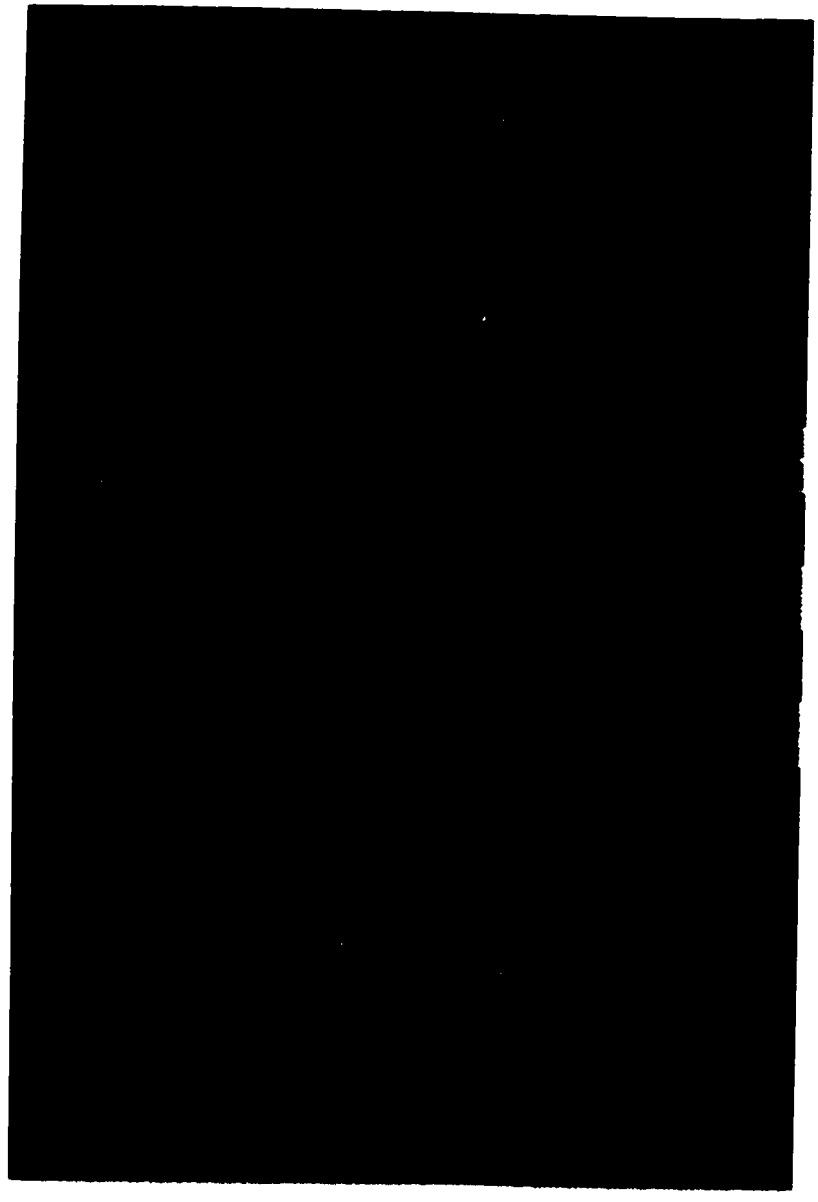
2.3 Materials and methods

Corals were collected by SCUBA in May 1992 from a site at which abundant ash was visible in the sediment. Coral mortality appears to have been extensive at the site, particularly among branching species (Fig. 2.2). Four colonies of the domal coral Porites lobata, growing at a water depth of 3 m, were selected for study. The corals were cleaved underwater to obtain samples for detailed investigation. Immediately evident in each coral corallum was a single distinct band running parallel to the growth surface (Fig. 2.3). The bands were originally black but changed to an orange color within two days of exposure to the atmosphere.

During an excursion in October, 1993, a large area affected by hydrothermal fluids was discovered approximately 300 m from the site of coral collection (Fig. 2.1). The water temperature measured in beach sand ejected by this "vent" was 60 °C at 10-20 cm below the sediment surface. Where vent water mixed with seawater, a suspended, fine-grained red precipitate was observed. This precipitate colored both seawater and interstitial water red. The precipitate was collected by forcing a 50 ml syringe into the sediment, and withdrawing an interstitial water sample. A reddish orange-colored precipitate has also been deposited on much of the surrounding rock and sediment. Waters from 3 other active vents around the island (Fig. 2.1) were clear and colorless.

Fig. 2.2 Photo of branching coral colonies killed by May, 1988 eruption of Banda Api. The small branching coral colonies on the large central head represent post-eruption recruitment of Pocillopora on dead coral. Scale bar is 25 cm.

Fig. 2.3 Photo of coral slab. Small arrow indicates "Banda Band". Large arrow indicates death surface. Increments on scale bar are 1 cm. The small drilled holes are microsampling locations for a companion study.



The corals were slabbed in the laboratory and density banding was determined by X-radiography. Thin slabs, up to 4 mm in thickness, revealed banding patterns most clearly. Bas-relief prints of X-radiographs were prepared by superimposing and slightly offsetting negative and positive exposures of X-ray images. This technique provides a pseudo-relief image, which we have found to emphasize patterns of annual density banding in corals (used for Fig. 3 in Risk et al., 1992).

Death/regrowth surfaces found within the coral skeletons (Fig. 2.3) were studied as polished sections under reflected light using Nomarski differential interference optics. The orange banding (which will subsequently be referred to as the "Banda Band") and the iron-coated sediment were sampled for petrographic thin-section investigation. The precipitate from the "red-water" vent was analyzed by X-ray Fluorescence (XRF) on a Philips PW 1480 X-ray spectrometer. A 1 mm-thick sample of coral containing the Banda Band, and a control sample from below the band, were also collected for XRF analysis. Due to the small amount of sample material available, all XRF samples were loaded as a thin layer of powder on glass fiber filters.

2.4 Results and discussion

Inspection of coral slabs and X-radiographs reveals eroded death/ regrowth surfaces which are contemporaneous with the deposition of the Banda Band (Fig. 2.3). Macintyre and Smith (1974) suggested that where hiatuses in skeletal growth and subsequent erosion can be correlated between coral

colonies, evidence is provided for catastrophic events. Death surfaces are found at the same horizon in all four of the corals studied. These death/regrowth surfaces occur both on the lower flanks and the tops of the corals studied. Nomarski images of death surfaces reveal a horizon which contains abundant algal borings (Fig. 2.4), and less commonly, shells of juvenile boring bivalves. The intensity of boring is substantially greater than that found in bands of boring produced by endolithic algae living beneath the coral surface. Abundant ash particles, trapped in pores of the coral skeleton, are visible below this bioeroded horizon (Fig. 2.4). The angular nature of the ash particles precludes extensive reworking of ash prior to its incorporation in the skeleton. It appears that local tissue necrosis rendered small portions of the coral skeleton vulnerable to bioerosion and allowed silt-sized ash particles to infiltrate skeletal pores.

The chronology of the formation of death surfaces and the contemporaneous Banda Band can be established by X-radiography. Annual density variations are well developed on the sides of the Banda Api corals (Fig. 2.5A), but are more difficult to recognise along the major growth axes. In some of the corals, annual density bands have a diffuse appearance along major growth axes (Fig. 2.5A). This may be due to rapid and relatively uniform rates of skeletal deposition or may be a function of the thickness of the slab and the orientation of

Fig. 2.4 Nomarski image of a bioeroded death surface. Small arrows indicate trapped ash particles. Large arrow indicates the bioeroded horizon. Scale bar is 100 μm.

Fig. 2.5 A) Bas-relief print of X-radiographs of slab containing the Banda Band. Dashed line shows position of the iron-rich precipitate. The Banda Band was deposited just above a high density (dark) band. The density banding pattern shows there are exactly four cycles (years) between the location of the band and the outer growth surface of the coral. Note banding is clearest on the slower growing side of the coral. Scale bar is 1 cm. B) Photo of same coral slab as in A. Arrow marks position of Banda Band. Diffuse dark banding seen above the Banda Band is of algal origin. This banding is produced by a regular cyclicity in the intensity of endolithic algal boring and is unrelated to any death surface. The thick dark band along the outer growth surface of the coral is the tissue layer extending downwards into the skeleton. Scale bar is 1 cm.


A
B

skeletal elements relative to incident X-rays (Barnes and Lough, 1989; Lough and Barnes, 1992). In other corals, fine subannual density bands are prominent along major growth axes owing to greater resolution of density variations within rapidly growing areas (e.g., Buddemeier, 1974; Barnes and Lough, 1989; Barnes and Lough, 1993). Where subannual bands occur in Banda corals, they tend to obscure patterns of annual cyclicity in X-radiographs. Slower growth rates along the sides of the corals tends to mitigate such interference, allowing better resolution of annual banding patterns. X-radiography clearly demonstrates that there are four complete annual cycles between the outer growth surface, which was deposited exactly four years after the eruption, and the Banda Band (Fig. 2.5A, B). The fact that the death/regrowth surfaces and the Banda Band are synchronous with the timing of the May 1988 eruption of Banda Api suggests that these features are linked to volcanic activity.

The deleterious effects of sedimentation stress on corals and coral reefs are well established (e.g., Dodge et al., 1974; Dodge and Vaisnys, 1977; Cortés and Risk, 1985). The 1988 ashfall on Banda Api represents a severe case of such stress. Most of the branching "table" Acropora and Pocillopora were killed by the ashfall. Massive genera such as Porites were somewhat less affected. Similar mortality patterns were observed on a reef surrounding Mount Pagan, Mariana Islands, following a 1981 eruption (Eldredge and Kropp, 1985). The greater mortality of branching species appears to be due to the presence of scoria which became trapped between corallites and closely spaced branches

(Eldredge and Kropp, 1985). Branching coral communities growing around islands a few km from our study site were unaffected.

We suggest that localized mortality of tissue on surviving Porites heads occurred as a direct result of the May 1988 ashfall, forming the death surfaces observed. Areas of tissue on the coral colonies which could not be cleared by either wave/current action or the sediment rejection mechanisms of the corals (Hubbard and Pocock, 1972; Bak and Elgershuizen, 1976; Stafford-Smith and Ormond, 1992) experienced mortality. Tissue necrosis was restricted to the sides of the coral or in local depressions on upper growth surfaces, where ash preferentially accumulated. Necrosis may have resulted from a variety of factors including anoxia and microbial action (e.g., Lasker, 1980; Hodgson, 1990).

Coral death surfaces became preferential sites for the incorporation of ash particles. Subsequently, when the ash was cleared from the corals, the exposed coral skeleton was subject to fouling and bioerosion by colonizing organisms. Coral tissues are capable of regenerating over lesions which have been occupied by fouling biota (e.g., Fishelson, 1973; Bak et al., 1977). Bak et al. (1977) have described different mechanisms of tissue and skeletal regeneration in corals which have suffered partial mortality. The skeletal growth pattern of the Banda Api corals indicates that the remaining peripheral living tissues were able to completely regenerate over the dead bioeroded skeleton (Fig. 2.3), resulting in the preservation of death/regrowth surfaces.

The orange Banda Band is present in all four colonies studied. In two

small colonies, the band is continuous, whereas in two larger colonies it is confined to the colony flanks (Fig. 2.3). In thin section, the band appears as an orange-colored mineral precipitate (identified as limonite) encapsulated within the skeletal structure. A similar precipitate coats volcanic grains collected in the vicinity of the "red-water" vent, irrespective of their lithology or mineralogy.

XRF analyses reveal that both the Banda Band and the vent-derived fine red precipitate are enriched in iron. The iron concentration in coral skeleton containing the band is 3000 ppm (by weight) whereas a control sample from the same colony shows iron concentrations of 500 ppm. It is possible that the "control" sample may have been affected by hydrothermal activity or incorporation of detrital volcanics, however, there is no visible evidence of either phenomenon. A filter blank gave an iron concentration of 450 ppm. The red precipitate collected from interstitial waters affected by the vent contains 14.6 weight percent iron

The Banda Band is clearly a chemical precipitate, derived from iron-rich fluids, which was deposited during the May 1988 eruption of Banda Api. The proximity of the corals to a site of ongoing deposition of iron-bearing precipitates from hydrothermal fluids suggests an interesting scenario. It is hypothesized that during the 1988 eruption, brief hydrothermal activity affected the site from which the coral samples were collected, depositing an iron-rich chemical precipitate on the coral skeletons. The fluids from this activity may have been similar to those presently emanating from the "red-water" vent (Fig. 2.1). Hydrothermal alteration

of the surrounding andesitic rocks would likely produce fluids rich in iron. When dissolved ferrous iron is exposed to oxygenated sea water it precipitates as an oxyhydroxide (Berner, 1980, p. 113). This mode of iron precipitation in a carbonate setting has been proposed to explain iron oxide aggregates in a dolomitized atoll (Aharon et al., 1987). Hydrothermal activity, associated with the eruption of Fernandina, has also been suggested as a potential mechanism for manganese enrichments in corals from Urvina Bay, Galapagos Islands (Shen et al., 1991). It is unlikely that hydrothermal activity was responsible for producing the death surfaces in the Banda corals, since such activity would be expected to produce extensive, uniform death surfaces, not the patchy surfaces observed in our samples.

The method of incorporation of the iron stain into the Banda coral skeletons was likely similar to that proposed by Brown et al. (1991) for corals affected by high concentrations of dissolved iron from an ore processing plant. During periods of stress coral tissues can retract, exposing mucus-covered skeletal spines to seawater. Brown et al. (1991) noted that iron precipitated on exposed skeletal spines was preserved as an orange-colored band after tissues expanded back over the colony and deposited a new layer of aragonite. In thin section, the Banda Band displays a microtopography which indicates that iron precipitation occurred on positive-relief elements of the skeletal architecture. The band is revealed to be discontinuous when viewed at high magnification, which suggests selective precipitation of iron on skeletal elements exposed by tissue

retraction. In our study area, there was ample cause for coral tissue retraction during the 1988 eruption. The corals could have been stressed by metal-laden hydrothermal fluids and the deposition of ash on the coral tissue. Iron in the hydrothermal fluids must have been present in sufficient concentration to be precipitated as a visible chemical band in the corals. The Banda Band is not well developed near the top of the larger corals, suggesting that iron precipitation was localized near the substratum or possibly in areas where coral growth was slower.

2.5 Conclusions

A coral reef adjacent to Banda Api, a volcanic island in Indonesia, was greatly affected by a volcanic eruption which occurred in 1988. Corals which survived this eruption preserve physical and chemical evidence of volcanic and hydrothermal activity. Prominent features include:

1) trapped ash within pores of the coral skeleton

2) a bioeroded death surface

3) an iron-rich precipitate forming a distinct band in the skeleton.

All of these features have a good potential of being preserved in the geological record, and could be used to detect volcanic/hydrothermal events in ancient reef/volcanic settings. Such settings are common at plate boundaries and sites of intra-plate volcanism in tropical oceans. Most significantly these proxy records provide information on short-lived, low magnitude, volcanic events which affect but do not totally devastate marine communities. The continual growth records of corals will preserve these geologically instantaneous signals

with much better resolution than the bioturbated sediment record. Moreover, large massive corals may record several such events. Accordingly, in appropriate settings, the periodicity of relatively minor volcanic events may be established in the fossil record with much greater precision than previously permitted.

Chapter 3: Relationship between light and $\delta^{15}N$ of coral tissue: Examples from Jamaica and Zanzibar

3.1 Abstract

Nitrogen isotope values from coral tissue collected over depth/light gradients are reported from Jamaica and Zanzibar. The Jamaica suite consists of multiple specimens of three coral species (Montastrea annularis, Porites astreoides and Agaricia agaricites) sampled at increasing depths. For each species, combined tissue/zooxanthellae $\delta^{15}N$ decreases significantly with decreasing availability of photosynthetically active radiation. The Zanzibar sample suite was collected from three coral colonies (all Porites lobata). Multiple samples, occupying different depths and light regimes, were collected from each coral corallum. The Zanzibar suite shows a significant decrease in $\delta^{15}N$ with increasing depth over each colony. Together, these two sample suites suggest light is an influence on the nitrogen isotopic composition in corals containing symbiotic zooxanthellae. It is proposed that in high irradiance conditions, corals strongly deplete their internal dissolved nitrogen pool, leading to reduced fractionation during uptake and assimilation by symbiotic algae and/or host. In lower light conditions, less dissolved nitrogen is assimilated and fractionation increases. Autotrophic corals living in shallow, high light settings may prove to be excellent tracers of $\delta^{15}N$ of dissolved inorganic nitrogen.

3.2 Introduction

Measurements of the natural abundance of nitrogen isotopes have been used to trace sewage (Risk et al., 1989; Risk et al., 1993; Dunn, 1995;

Mendes et al., 1996) and other terrestrial inputs to coral reefs (Risk et al., 1989; Sammarco et al., in preparation) and to aid in the identification of nutritional sources in reef settings (Schoeninger and DeNiro, 1984; Keegan and DeNiro, 1988; Yamamuro et al., 1992; Yamamuro et al., 1995; Sammarco et al., in preparation). Nitrogen isotopes are useful in such studies because different sources of dissolved and particulate nitrogen may be isotopically distinct (Sweeney et al., 1980; Owens, 1987; Michener and Schell, 1994). Nitrogen isotopes generally fractionate in a consistent manner along food chains, with enrichments of approximately 3 ‰ per trophic level (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). These fractionations have been attributed to excretion of isotopically light nitrogen (Minagawa and Wada, 1984).

Nitrogen fractionation during nutrient uptake and assimilation by primary producers (Wada and Hattori, 1978; Wada, 1980; Montoya and McCarthy, 1995) has implications for the nitrogen isotopic composition of zooxanthellate corals. Corals thrive in oligotrophic conditions, largely due to the symbiotic relationship between the coral animal and endosymbiotic zooxanthellae (Symbiodinium microadriaticum) (Muscatine and Porter, 1977). This symbiotic relationship allows corals to act, to some degree, as primary producers (photoautotrophs). Zooxanthellae fix inorganic carbon and nitrogen into organic compounds which can be translocated to the coral host (autotrophy) (Muscatine et al., 1984; Falkowski et al., 1984; Falkowski et al., 1993) to supplement available exogenous food sources (heterotrophy), including dissolved organic matter (DOM) and particulate organic matter (POM, e.g. zooplankton) (e.g. Muscatine et al., 1989; Risk et al., 1994; Dubinsky and Jokiel, 1994). The algal symbionts assimilate dissolved inorganic nitrogen (DIN) from the external

environment (e.g., Muscatine and D'Elia, 1978; D'Elia et al., 1983; Burris, 1983; Wafar et al., 1985; Wilkerson and Trench, 1986) and nitrogenous wastes excreted by the coral (Szmant-Froelich and Pilson, 1977, 1984; Muscatine and D'Elia, 1978; Rahav et al., 1989). Coral hosts may also be able to assimilate DIN directly (Miller and Yellowlees, 1990; Yellowlees et al., 1994).

Muscatine and Kaplan (1994) presented the first $\delta^{15}N$ measurements for separated coral tissue and zooxanthellae from reef corals using samples collected at Discovery Bay, Jamaica. Both the zooxanthellae and tissue were found to become generally more depleted in ^{15}N with depth. The authors argue for increasing fractionation with increasing light attenuation as a possible influence on the decrease of $\delta^{15}N$ with depth.

This study expands the work of Muscatine and Kaplan (1994) using combined coral tissue/zooxanthellae from two study areas. Replicate samples of three species of coral collected over a 30 m depth range at Discovery Bay exhibit a strong inverse relationship between mean $\delta^{15}N$ and light attenuation. In addition, we examined $\delta^{15}N$ variation along a transect from the tops to the bases of three large coral heads, at Zanzibar, Tanzania, where variability in factors such as $\delta^{15}N$ of the environment and nutrient concentrations would be minimized. Coral tissue $\delta^{15}N$ decreased from the tops to the less illuminated bases of the heads. The results in both situations suggest that light is a dominant influence on coral $\delta^{15}N$.

3.3 Materials and methods

Coral skeleton and approximately 50 cm^2 of adhering tissues per sample were chiseled from the upper surfaces of coral colonies of three species growing on the forereef at Discovery Bay, Jamaica. Samples were nearly

horizontal when in-situ, except in the case of Agaricia, for which shallower samples were somewhat inclined. Sampling was performed utilizing SCUBA in January 1993 and January 1994. Specimens of Montastrea annularis, Agaricia agaricites and Porites astreoides were collected at depths of 1m, 5m, 12m, 22m and 30m, then oven dried for 2 days at 60 °C prior to shipment to Canada (Table 3.1). Porites and Agaricia samples were collected in both 1993 and 1994, while most of the Montastrea samples were solely from 1994. Similar sampling was performed in April 1993, on large colonies of Porites lobata collected in shallow water (1.5 - 8.5 m depth) from two small fringing reefs offshore Zanzibar (Table 3.2). Coral skeleton and tissue samples were taken from the tops to the bases of three large coral heads at 25 cm depth increments and frozen for shipment.

Each sample was prepared following LeBlanc et al. (1989) and Risk et al. (1994). The sample was placed in < 1 M hydrochloric acid to dissolve the aragonite skeleton and free the tissue/zooxanthellae. Prior to dissolution, excess skeletal material was removed with a diamond circular saw. Particular attention was paid to removing the skeleton which contained the endolithic algal layer directly beneath the coral surface. Following dissolution of the skeleton, the resulting tissue mat was thoroughly rinsed in distilled water and collected on a fine nylon mesh. Samples were centrifuged, and the tissue pellet freeze-dried to ensure preservation. All data presented here represent combined tissue and zooxanthellae.

Freeze-dried samples were loaded into precombusted 6 mm diameter Pyrex tubing along with an excess of cupric oxide. Approximately 15 mg of sample was employed for nitrogen analyses and 5 mg for carbon isotopic determinations. The samples were placed under vacuum and the tubes

Table 3.1. Jamaica nitrogen isotope data

SAMPLE[1]	$\delta^{15}N$ (‰)	DEPTH (m)	Δ PAR[2] (%)	GENUS	YEAR[3]
ap1	4.3	1	9	*Porites*	1993
ap2	4.1	1	9	*Porites*	1993
ap3	3.8	1	9	*Porites*	1993
aag	2.2	1	9	*Agaricia*	1993
1p1	3.3	1	9	*Porites*	1994
1ag2	2.9	1	9	*Agaricia*	1994
1ag3	2.4	1	9	*Agaricia*	1994
1ma1	2.7	1	9	*Montastrea*	1994
1ma2	2.8	1	9	*Montastrea*	1994
bp1	3.6	5	36	*Porites*	1993
bp2	3.3	5	36	*Porites*	1993
bag1	1.7	5	36	*Agaricia*	1993
bag2	2.3	5	36	*Agaricia*	1993
5p1	3.6	5	36	*Porites*	1994
5p2	3.6	5	36	*Porites*	1994
5p3	3.6	5	36	*Porites*	1994
5p4	3.5	5	36	*Porites*	1994
5ag1	2.5	5	36	*Agaricia*	1994
5ag2	2.6	5	36	*Agaricia*	1994
5ag3	2.7	5	36	*Agaricia*	1994
5ag4	2.7	5	36	*Agaricia*	1994
5ma1	2.2	5	36	*Montastrea*	1994
5ma2	2.9	5	36	*Montastrea*	1994
5ma3	2.6	5	36	*Montastrea*	1994
5ma4	2.8	5	36	*Montastrea*	1994
cp	2.2	12	62	*Porites*	1993
cag1	2.2	12	62	*Agaricia*	1993
cag2	1.6	12	62	*Agaricia*	1993
cag3	1.5	12	62	*Agaricia*	1993
12p1	2.8	12	62	*Porites*	1994
12ag1	2.2	12	62	*Agaricia*	1994
12ag3	2.0	12	62	*Agaricia*	1994
12ag4	2.3	12	62	*Agaricia*	1994
12ma1	2.1	12	62	*Montastrea*	1994
12ma2	2.3	12	62	*Montastrea*	1994
dp	1.9	22	77	*Porites*	1993
dag1	0.1	22	77	*Agaricia*	1993
dag2	1.6	22	77	*Agaricia*	1993
dag3	1.9	22	77	*Agaricia*	1993
dag4	1.0	22	77	*Agaricia*	1993

Table 3.1. Jamaica nitrogen isotope data (continued)

SAMPLE[1]	$\delta^{15}N$ (‰)	DEPTH (m)	Δ PAR[2] (%)	GENUS	YEAR[3]
22p2	3.1	22	77	*Porites*	1994
22p3	2.6	22	77	*Porites*	1994
22ag2	2.1	22	77	*Agaricia*	1994
22ag3	2.5	22	77	*Agaricia*	1994
22ma1	2.0	22	77	*Montastrea*	1994
ep	2.0	30	82	*Porites*	1993
ema1	1.1	30	82	*Montastrea*	1993
ema2	2.1	30	82	*Montastrea*	1993
30p1	2.0	30	82	*Porites*	1994
30p2	2.3	30	82	*Porites*	1994
30ag1	0.8	30	82	*Agaricia*	1994
30ag2	1.7	30	82	*Agaricia*	1994
30ag3	1.0	30	82	*Agaricia*	1994
30ma2	2.0	30	82	*Montastrea*	1994
30ma3	2.0	30	82	*Montastrea*	1994

1 - some samples (e.g.1ag1) were not analysed due to partial bleaching or contamination with filamentous algae or bioeroding sponges

2 - decrease in PAR as a percentage of surface irradiance; values are calculated from PAR data given in Fig. 1. -- e.g. at 1 m depth % of surface PAR is 91, therefore decrease in PAR is 9 %.

3 - year of collection

Table 3.2. Zanzibar nitrogen isotope data

SAMPLE	DEPTH (M)	$\delta^{15}N$ (‰)
Head 1		
GH1105	1.75	5.4
GH1106	2.00	5.0
GH1108	2.25	4.9
GH1107	2.50	4.8
GH115	2.75	4.7
GH1116	3.00	4.7
Head 2		
GH2112	1.50	5.4
GH2111	1.75	5.2
GH2110	2.00	5.2
GH2109	2.25	4.8
GH2104	2.5	5.0
GH2103	2.75	4.8
GH2102	3.00	***
GH2101	3.25	4.8
Head 3		
Z7	7.00	5.3
Z6	7.25	5.1
Z5	7.50	5.0
Z4	7.75	4.9
Z3	8.00	4.9
Z22	8.25	5.0
Z2	8.50	4.7

***sample lost during preparation

flame-sealed. The samples were combusted at 550 °C for 2 hours, one day prior to analysis. The gases produced by combustion were cryogenically separated on line, yielding pure N_2 or CO_2 prior to admission to a VG SIRA mass spectrometer. Organic standards (gelatin and glutamic acid), as well as atmosphere standards for nitrogen, were analyzed to estimate precision (as +/- one standard deviation). Precision for these standards was 0.1 ‰ for carbon and 0.07 ‰ for nitrogen. The repeatability for four replicates of one of the corals studied was 0.2 ‰ for carbon and 0.1 ‰ for nitrogen .

$\delta^{15}N$ results are reported relative to the atmospheric standard. $\delta^{13}C$ is reported relative to the PDB standard. All results are reported in standard delta notation;

e.g. $\delta^{15}N = ((((^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{standard}) -1)*1000)$ in ‰ units.

Tests were also carried out to determine the effect of acid strength and length of exposure to acid on $\delta^{15}N$ of coral tissue. Aliquots of tissue from one coral were subjected to distilled water, 0.5, 1 and 3.5 M hydrochloric acid. The first treatment involved soaking the sample in distilled water and waterpiking to remove the tissue layer. This method was not used for all samples, because it generally yielded insufficient material for analysis in the case of material dried prior to shipment. The first three treatments yielded identical results within measurement error. The 3.5 N acid treatment resulted in a value nearly 1 ‰ depleted relative to the other tests. Aliquots of a second coral were left in 1 N hydrochloric acid for 5, 10 and 15 days. All treatments yielded identical nitrogen isotopic ratios within measurement error.

In-situ light data, used for comparison with coral tissue isotopic values, were measured by Ian Sandeman in the winter of 1986 using a LI-Cor, LT-185B

Quantum Radiometer/Photometer with a LI-192SB Cosine Collector (see Sandeman, 1996) . Data were collected for horizontal, vertical and 45 ° surfaces to depths of 40 m on the forereef (Fig. 3.1). Collection of data occurred at midday, during calm sunny periods. Simple linear regression analyses were performed in order to characterize coral isotopic variations with depth and light recorded in the two data sets.

3.4 Results

3.4.1 Light availability to Jamaica corals

Data for light, as photosynthetically active radiation (PAR), are presented in Fig. 3.1 and Table 3.1. Light availability to horizontal surfaces (corresponding to Jamaica tissue sample orientation) decreases exponentially with depth (Fig. 3.1). A strong linear relationship between PAR and ln (depth) exists (R^2=0.98). Within any given depth it can be seen that light availability decreases rapidly from the tops to the vertical sides of coral heads. This is particularly true for shaded surfaces (north facing, Fig. 3.1).

3.4.2 Jamaica coral $\delta^{15}N$

Nitrogen isotopic ratios of Jamaica corals range from 4.3 to 0.1 ‰ (the $\delta^{15}N$ values of all corals examined in this study are reported in Tables 3.1 and 3.2). A summary plot of the Jamaica data (Fig. 3.2) indicates an overall decrease in $\delta^{15}N$ with depth. The data were also plotted and regressed against light attenuation measurements from the forereef of Discovery Bay (Fig. 3.3, Table 3.3). For regression analysis, Porites and Agaricia data were not distinguished by year of collection. Regression coefficients resulting from pooling of samples from the two years (Table 3.3) are similar to those obtained if samples are separated by year. Agaricia samples from 1994, however, tend to be slightly

Fig. 3.1 Variation in light availability to horizontal and vertical coral surfaces over the depth range 0 - 30 m at Discovery Bay. Numbers adjacent to coral heads represent percentage of surface (i.e. 0 m) photosynthetically active radiation (PAR) received by the tops and sides of each coral head. Coral heads are not to scale. The depth of the top of each coral head is the depth to which data correspond. Winter surface irradiance is about 35 $Em^{-2}day^{-1}$ at Discovery Bay (summer values about 50 $Em^{-2}day^{-1}$) (Porter, 1985; Sandeman, 1996). Inset graph shows corresponding PAR data plotted versus depth. Dotted line represents light received by horizontal surfaces, solid line represents light received by south facing vertical surfaces and the dashed line represent light received by north facing vertical surfaces.

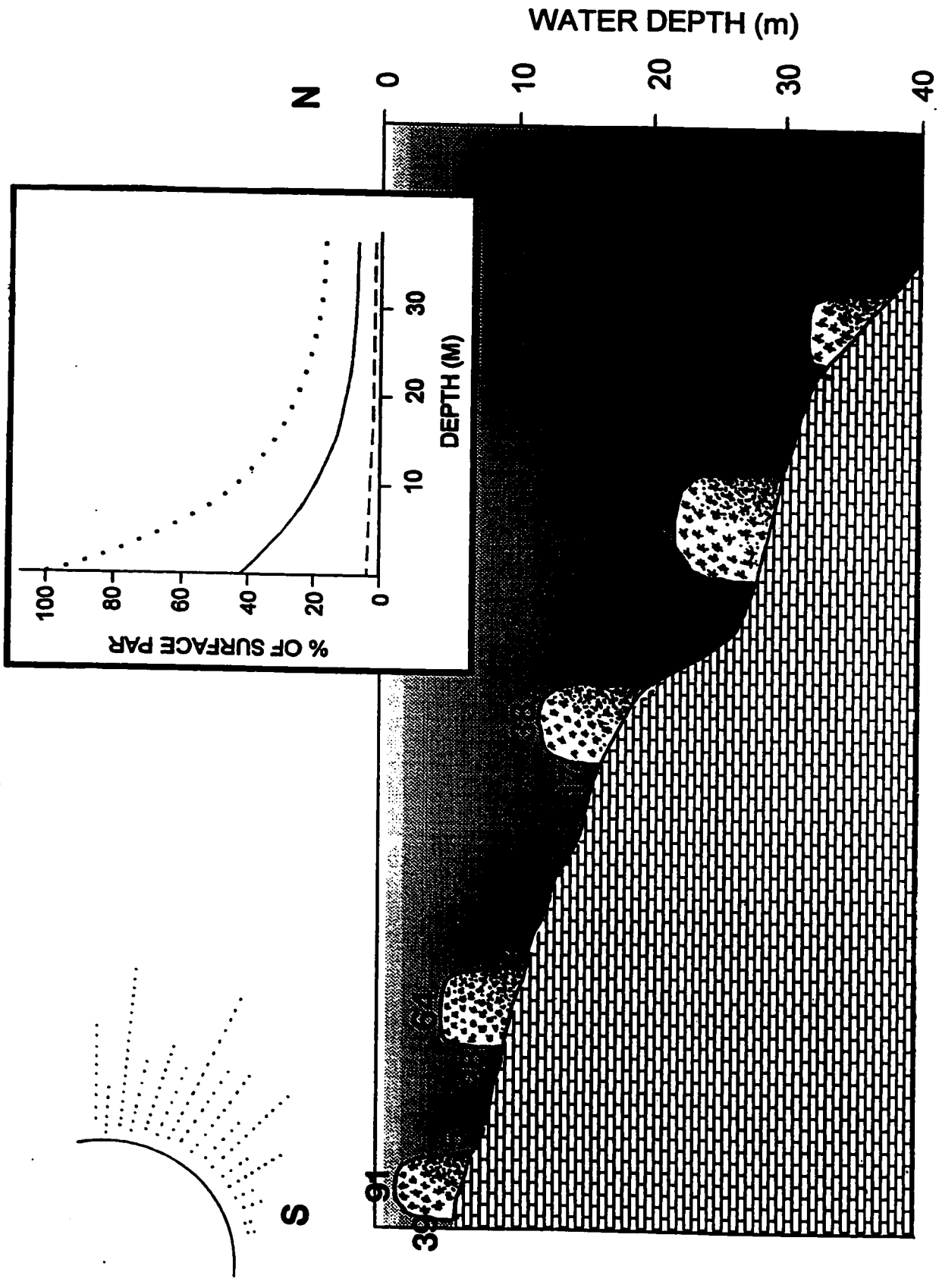

WATER DEPTH (m)
0
10
20
30
40
N
S
91
64
30
% OF SURFACE PAR
100
80
60
40
20
0
DEPTH (M)
10
20
30

Fig. 3.2. $\delta^{15}N$ versus depth for samples from Discovery Bay, Jamaica. Means are plotted as closed squares. Data ranges (minimum to maximum value) are shown. Individual graphs are shown for each species and the combined data.

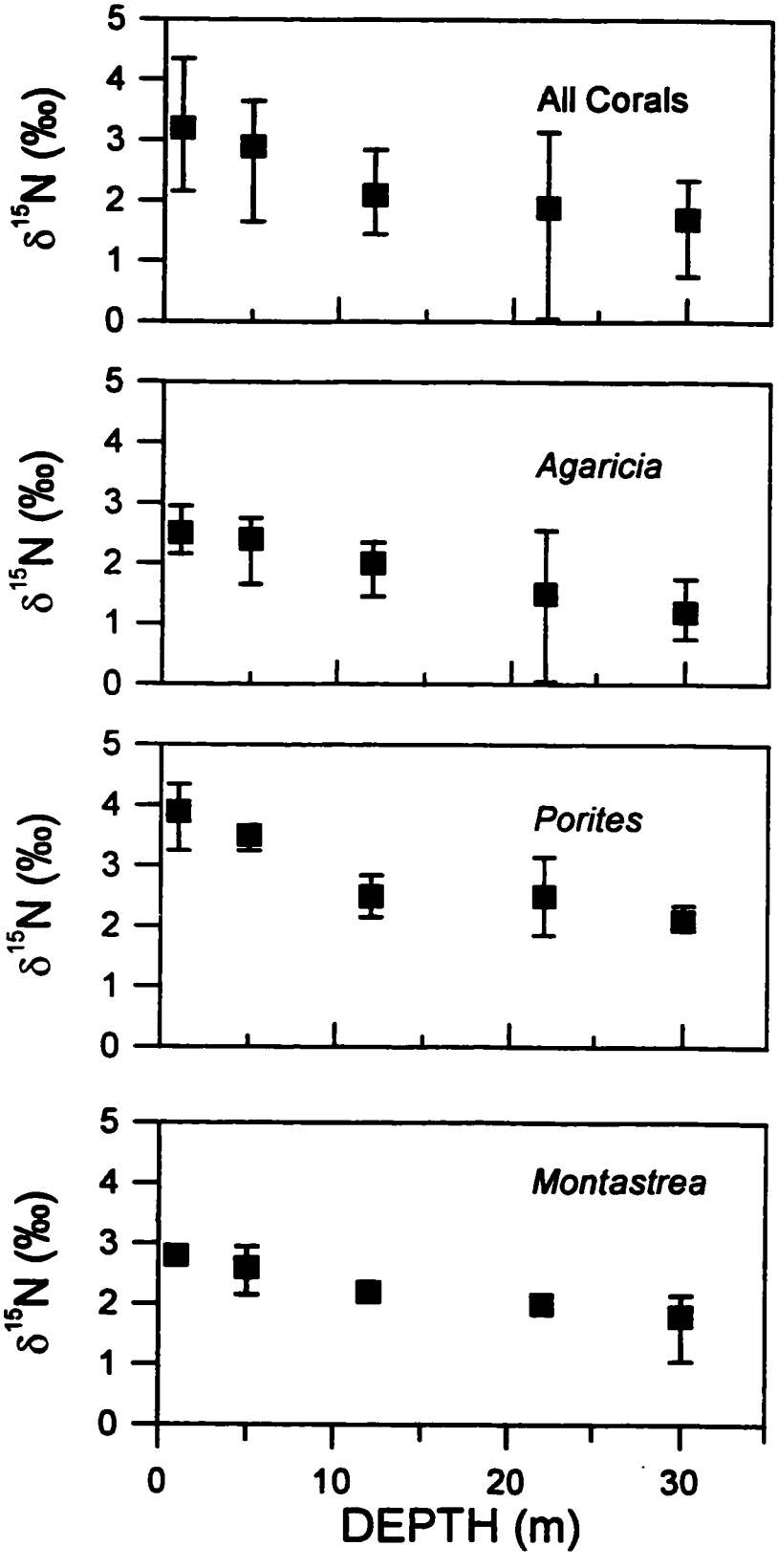

All Corals
Agaricia
Porites
Montastrea
δ15N (‰)
0
1
2
3
4
5
0
10
20
30
DEPTH (m)

Fig. 3.3. $\delta^{15}N$ versus decrease in photosynthetically active radiation (PAR) (%) for samples from Discovery Bay, Jamaica. Symbols as in Fig. 3.2. Regression lines are shown. All regressions indicate a significant decrease in $\delta^{15}N$ with decreasing light availability (see Table 3.3 for a regression summary). Individual graphs are shown for each species and for all data. PAR data is given in Table 3.1.

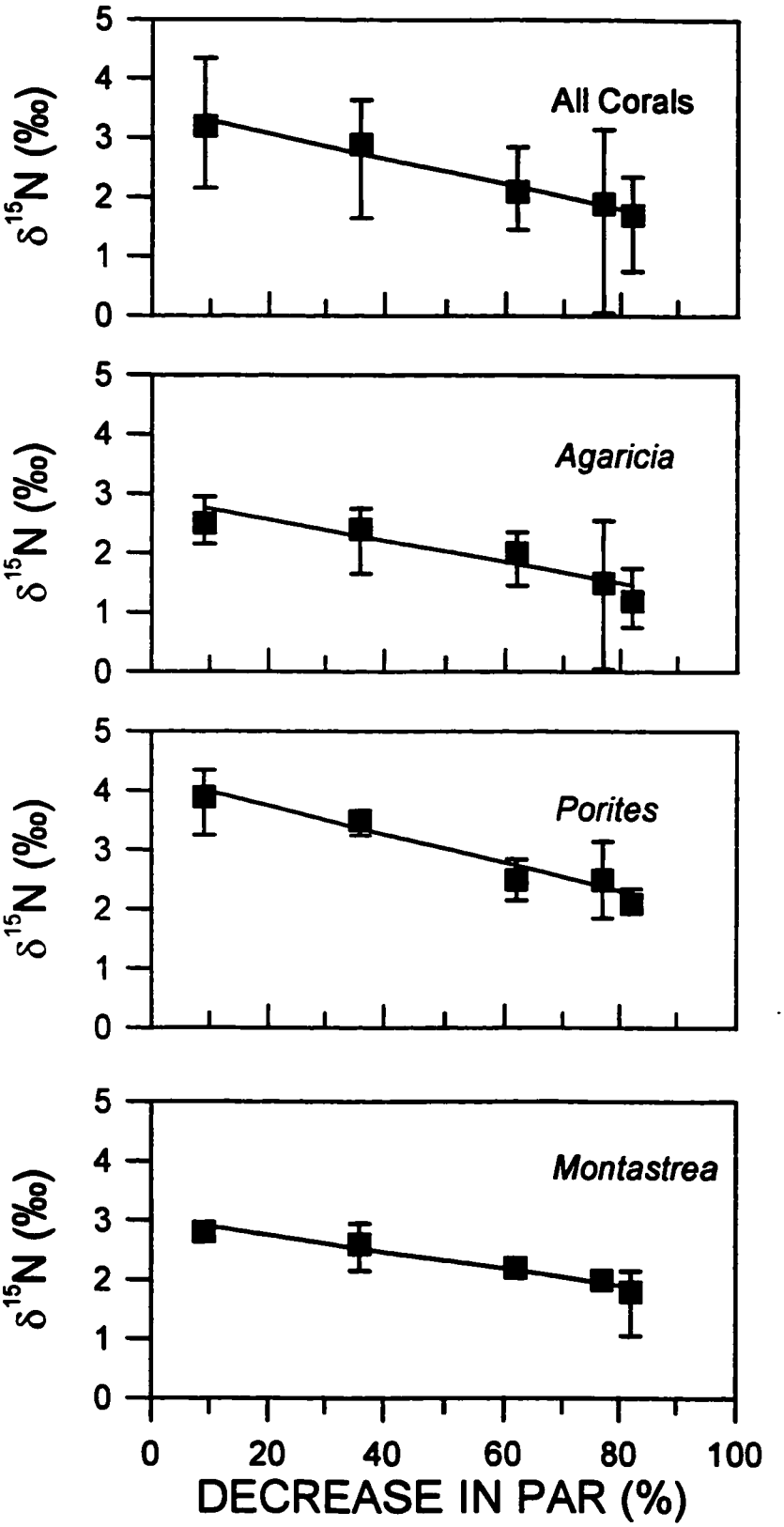

All Corals
Agaricia
Porites
Montastrea
δ15N (‰)
0
1
2
3
4
5
0
20
40
60
80
100
DECREASE IN PAR (%)

Table 3.3. Regression data[1] for Jamaica and Zanzibar corals

y ($\delta^{15}N$ of)	x	m (slope)	std dev of m	b	std dev of b	p	R^2
JAMAICA							
All Corals	% Δ PAR[2]	-0.021	0.0032	3.50	0.19	<0.001	0.45
Porites	% Δ PAR	-0.024	0.0032	4.23	0.17	<0.001	0.78
Montastrea	% Δ PAR	-0.014	0.0033	3.03	0.20	0.001	0.62
Agaricia	% Δ PAR	-0.018	0.0046	2.92	0.28	0.001	0.40
ZANZIBAR							
Head 1	LN DEPTH	-1.21	0.21	5.93	0.18	0.004	0.90
Head 2	LN DEPTH	-0.84	0.18	5.68	0.15	0.005	0.82
Head 3	LN DEPTH	-1.94	1.48	8.96	1.00	0.011	0.76

1 - for linear regression in the form y=mx +b

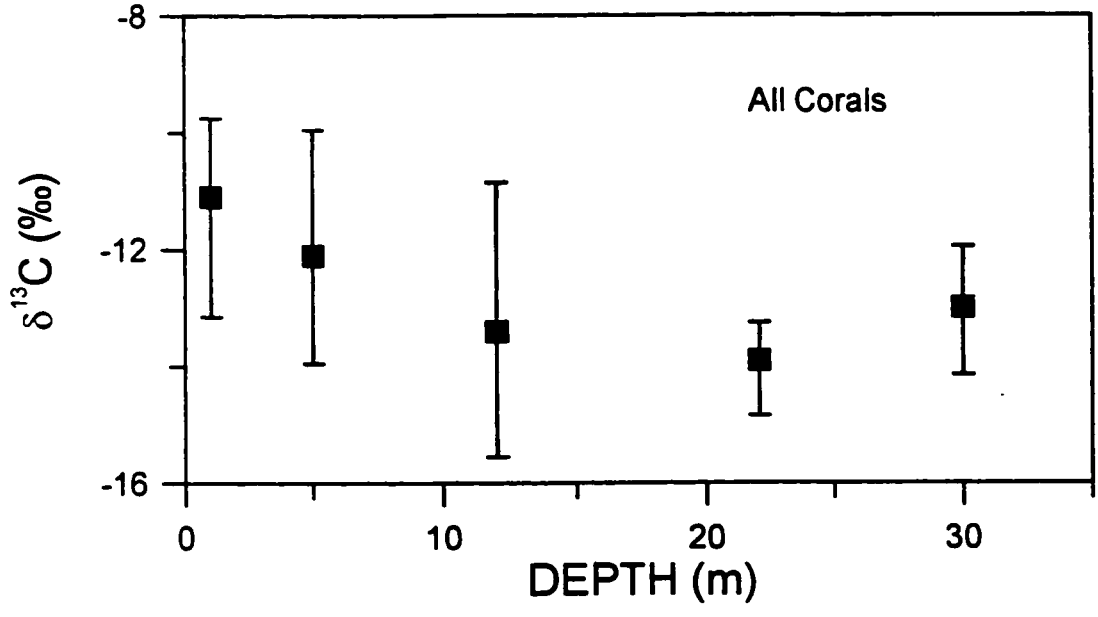
2 - % Δ PAR refers to percentage decrease in PAR relative to surface PAR as in Table 3.1.

enriched at each depth relative to samples collected in 1993. In all cases $\delta^{15}N$ decreases significantly with decreased light availability (Fig. 3.3, Table 3.3). With the exception of Agaricia, (R^2=0.47 with depth as independent variable), light availability explains a slightly greater percentage of $\delta^{15}N$ variation than depth. Quadratic regression of $\delta^{15}N$ and depth yields coefficients of determination similar to those obtained for simple linear regression with light as the independent variable.

The decrease in light availability explains between 40 to 80% of Discovery Bay coral $\delta^{15}N$ variation (Table 3.3). If mean $\delta^{15}N$ values at each depth are regressed against light, approximately 95% of the variance is explained for Porites, Montastrea and all corals combined. In the case of Agaricia, 99% of the variance in the means is explained by regression with depth. The most obvious inter-species difference is the enrichment in ^{15}N for Porites tissue at each depth, relative to that of the other two species. The paucity of data at some depths makes interpretations of changes in the variance of the data with decrease in light availability problematic. Some of the apparent increase in variability with decrease in light availability (particularly at 22 m in the case of Porites and Agaricia) (Fig. 3.3) is a function of samples having been collected from different years.

A summary diagram of $\delta^{13}C$ values of Jamaica corals (Fig. 3.4) shows an overall trend similar in form to that of $\delta^{15}N$ (Fig. 3.2). Individual species studied follow trends similar to that of the combined data, with corals showing decreasing $\delta^{13}C$ over the upper half of the depth range (closest to the surface), then more constant values at greater depths. Carbon data from these corals will be presented in greater detail in Chapter 5.

Fig. 3.4. $\delta^{13}C$ versus depth for all corals from Discovery Bay, Jamaica. Symbols as in Fig. 3.2. Note the similarity between the C and N trends.


All Corals
-8
-12
-16
δ13C (‰)
0
10
20
30
DEPTH (m)

3.4.3 Zanzibar coral $\delta^{15}N$

Samples from the three Zanzibar Porites lobata coral heads display an overall decrease in $\delta^{15}N$ with ln (depth) (Fig. 3.5) . The regressions are highly significant and explain between 75 - 90% of the variation in $\delta^{15}N$ (see Table 3.3). Light data were unavailable for this site. Heads 1 and 2 were collected from the same fringing reef, while Head 3 was collected from a fringing reef approximately 10 km distant.

Regression coefficients for Head 1 and Head 2 are similar (Table 3.3). Given the large standard error for Head 3, it is not possible to state that the slope of the $\delta^{15}N$ versus ln (depth) regression is greater than that of Heads 1 and 2. Coral tissue $\delta^{15}N$ is similar for the tops of all three heads (Table 3.2). $\delta^{13}C$ data show a decrease with depth (see Chapter 5). When $\delta^{13}C$ data are regressed against depth, the simple linear regression model explains 25 - 96 % of the observed $\delta^{13}C$ variance. The decrease in $\delta^{13}C$ with depth, however, is only significant at the 10% significance level for Head 3 and is not significant for Head 1.

3.5 Discussion

The decrease in $\delta^{15}N$ of Jamaica coral with increasing depth (Fig. 3.2) is consistent with trends found for separated tissue and zooxanthellae from Discovery Bay corals (Fig. 3.6; Table 1 in Muscatine and Kaplan, 1994). The isotopic shifts in this study, therefore, reflect simultaneous isotopic depletions in animal and algal components of the symbiosis. Several factors could explain the observed trends, including variations in $\delta^{15}N$ of the external seawater DIN pool, changes in concentration of DIN with depth, trophic level shifts and changes in nitrogen fractionation related to light attenuation (Muscatine and Kaplan, 1994).

Fig. 3.5. $\delta^{15}N$ versus the ln (depth) (m) for samples from three Porites lobata colonies from Zanzibar. Regression lines are as indicated (refer to Table 3.3 for regression summary).

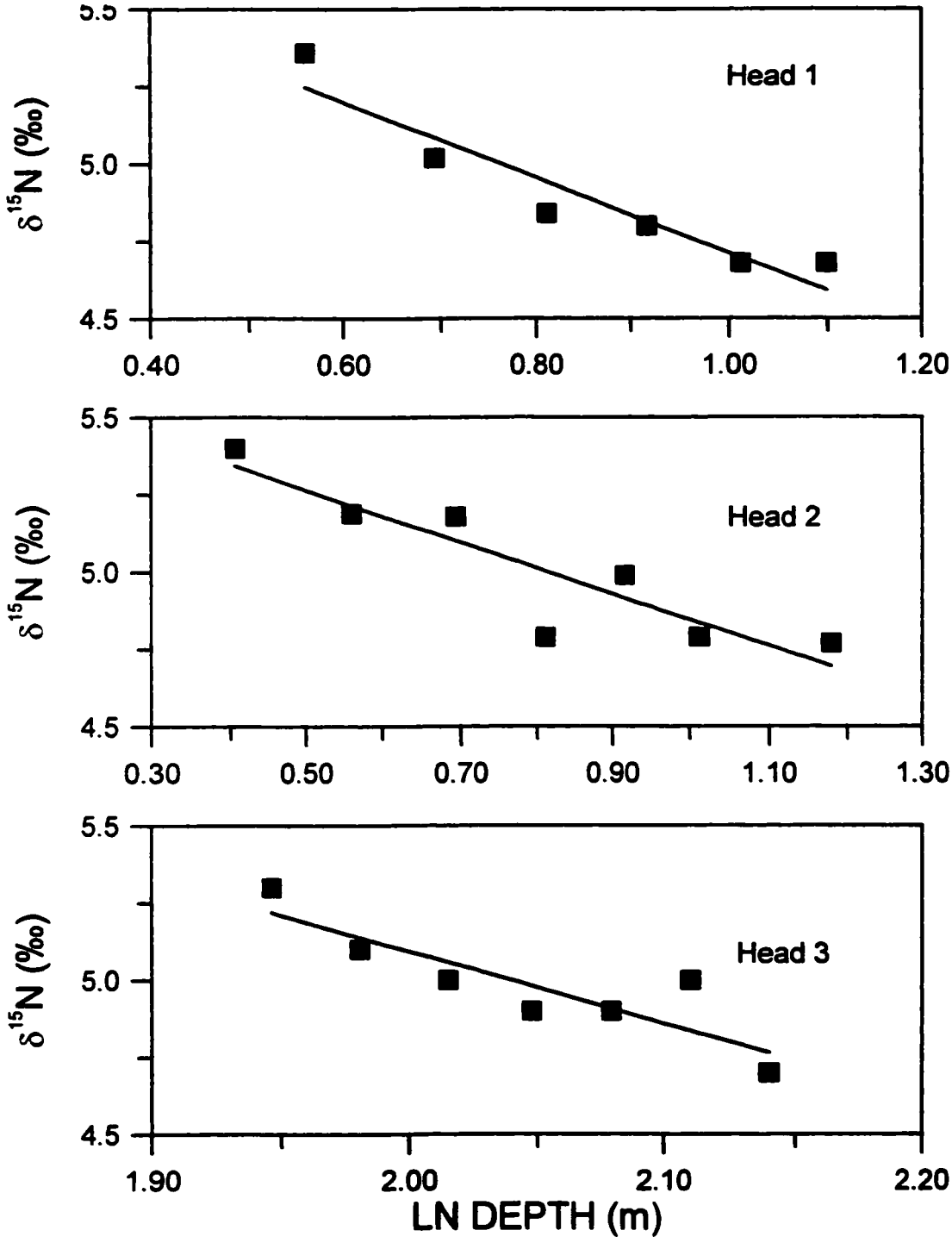
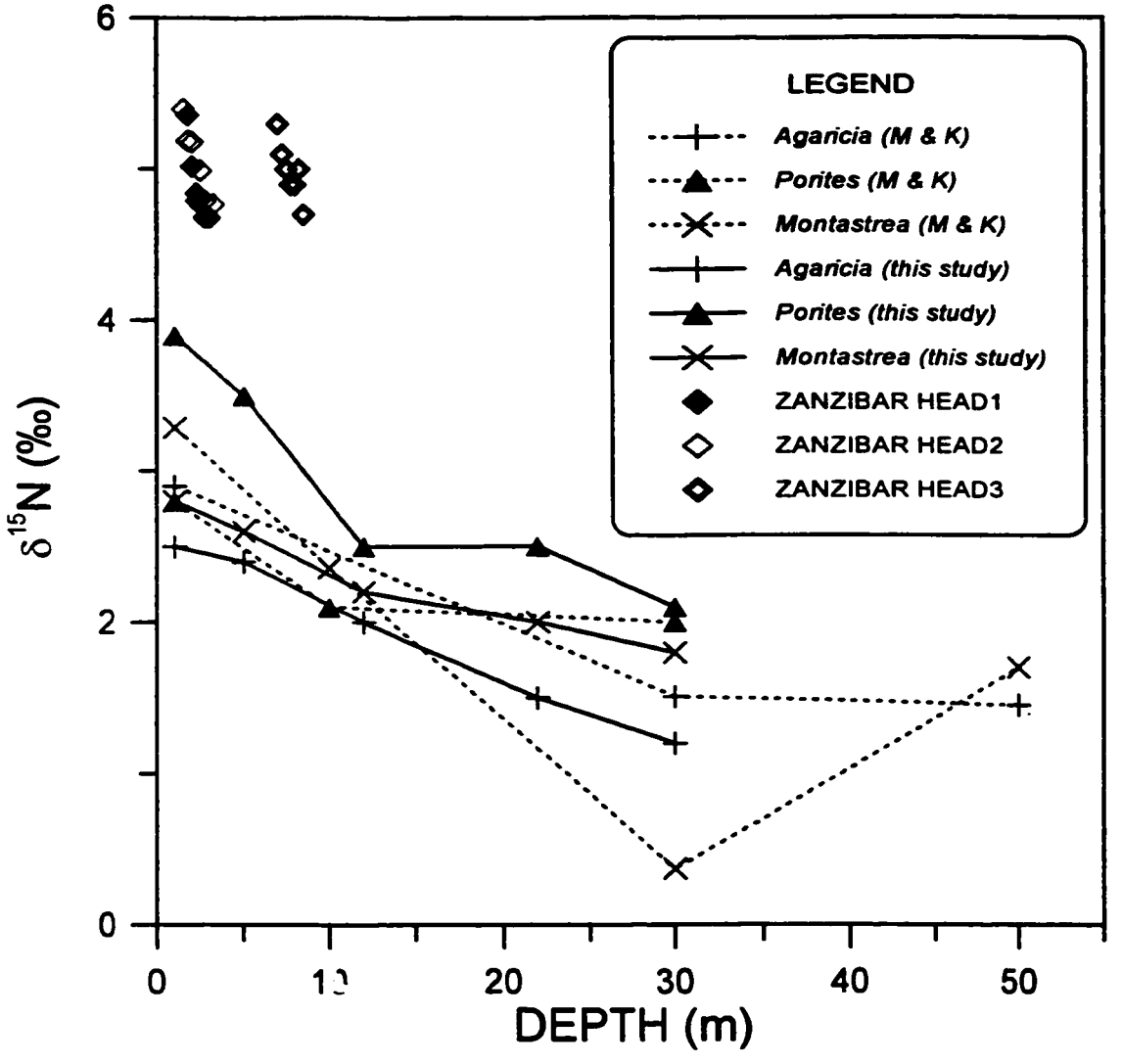

Head 1
Head 2
Head 3
δ15N (‰)
5.5
5.0
4.5
0.40
0.60
0.80
1.00
1.20
0.30
0.50
0.70
0.90
1.10
1.30
1.90
2.00
2.10
2.20
LN DEPTH (m)

Fig. 3.6. Compilation of $\delta^{15}N$ data versus depth; this study and Muscatine and Kaplan (1994). Note the sharp trend in $\delta^{15}N$ over a very small depth range corresponding to the distance between the tops and bases of Zanzibar coral heads. These trends display a significant proportion of the variation seen over 30 m at Discovery Bay, Jamaica. Muscatine and Kaplan (1994) data were originally presented for separated tissue and zooxanthellae. We have combined the data assuming the zooxanthellar nitrogen content was 7% of the nitrogen contained in the symbiosis. This arbitrary figure is based on nitrogen or protein ratios of zooxanthellae versus hosts in several coral species (e.g. Muscatine et al., 1984; Szmant et al., 1990; Falkowski et al., 1993).


LEGEND
Agaricia (M & K)
Porites (M & K)
Montastrea (M & K)
Agaricia (this study)
Porites (this study)
Montastrea (this study)
ZANZIBAR HEAD1
ZANZIBAR HEAD2
ZANZIBAR HEAD3
δ15N (‰)
DEPTH (m)
0
2
4
6
0
20
30
40
50

Variation in $\delta^{15}N$ of PON or DON with depth could also occur.

3.5.1 Relationship Between Jamaica Coral $\delta^{15}N$ and Light

The strong relationship between $\delta^{15}N$ of Jamaica corals and light availability between 0 - 30 m depth (Fig. 3.3, Table 3.3) supports the hypothesis that light has an important influence. The availability of downward irradiance to horizontal surfaces (Jamaica samples) will tend to decrease exponentially with depth according to the Bouguer-Lambert-Beer law ($I=I_o e^{-kz}$, where I=irradiance, z=depth (m) and k= extinction coefficient (m^{-1})). This exponential decrease in light availability might explain the strong quadratic relationship between Jamaica coral tissue $\delta^{15}N$ and depth.

The amount of photosynthetically active radiation available to reef corals is extremely variable. Changes in extinction coefficients with depth will affect the shape of a light/depth curve. For instance, yearly means for extinction coefficients at Discovery Bay are 0.127 m^{-1} for 0-5 m depth , 0.067 for 5-10 m^{-1} and ~0.05 m^{-1} for greater depths (Sandeman, 1996). Variation in turbidity, related to storms or runoff for example, will also affect scattering and reflectance of light. Time of day will affect the path light takes through the water, and hence light availability to surfaces of different orientations. The flux of light received by corals is also affected by shading by surrounding fauna and reef topography as well as the reflectance of different substrates (Brakel, 1979). Coral orientation will greatly influence the amount of light received (Fig. 3.1). This will be particularly important for Zanzibar samples which include a wide range of orientations.

In order to determine how light might affect the nitrogen isotopic composition of reef corals we must consider the sources of "new" dietary nitrogen

(Cook and D'Elia, 1988; Rahav et al., 1989) and the pathways by which this nitrogen might be assimilated, cycled and eliminated from the symbiosis. The effect of variation in light levels on coral diet and the isotopic composition of that diet must be taken into account. It is also important to consider under which conditions nitrogen might be limiting to the host or symbiont in order to assess whether potential fractionations might occur.

3.5.1.1 Possible influences of light on coral diet and its isotopic composition

Sources of new nitrogen to reef corals include seawater DIN, PON (particulate organic nitrogen, e.g. zooplankton) and DON (dissolved organic nitrogen). It has often been assumed that one of the advantages the zooxanthellae receive from the symbiotic association with corals is abundant nitrogen generated by host catabolism of exogenous protein sources such as zooplankton (see discussion in Szmant et al., 1990). If zooplankton were the dominant source of new nitrogen to corals and their zooxanthellae, then a trend in the isotopic composition of zooplankton with depth could explain the observed coral isotopic trends.

Trends towards lighter $\delta^{15}N$ values of PON do occur within the euphotic zone of the open ocean (e.g. Altabet and McCarthy, 1986; Altabet 1989), particularly during periods when these waters are poorly mixed. Increased phytoplankton fractionation associated with the influx of nutrient rich waters from below the nutricline may account for this trend (Altabet and McCarthy, 1986). Light may play a role by affecting the magnitude of phytoplankton fractionation relative to DIN (Montoya and McCarthy, 1995). Phytoplankton isotopic signatures in turn affect $\delta^{15}N$ of total PON via the planktonic food web.

The external influence of gradients in PON (or DON) on $\delta^{15}N$ of coral

tissue is unlikely in the setting described here. The Discovery Bay forereef waters are well mixed, displaying nearly constant temperature over depth (Land et al., 1975b). Moreover, the benthic corals feed largely on demersal zooplankton as they rise towards or return from the surface where they feed each night. The isotopic signatures of demersal zooplankton from various depths will be biased towards that of phytoplankton from shallower water.

While zooxanthellate corals may be able to exist as total heterotrophs, there is generally insufficient zooplankton on reefs for them to do so (Muscatine and Porter, 1977; Olhorst, 1985). Corals growing in high light settings exhibit abundant translocation of carbon from the symbiont to the host (Muscatine et al., 1984; Falkowski et al., 1984). This translocation may act as a negative feedback, reducing coral predation (Dubinsky and Jokiel, 1994). In the case of shallow water poritid corals, predation on zooplankton may not be a significant source of nutrition based on analyses of gut contents and observation of feeding behavior (Johannes and Tepley, 1974; Edmunds and Davies, 1986). Similar conclusions have been reached based on comparison of coral and zooplankton $\delta^{15}N$ (Eustice et al., 1995; Yamamuro et al., 1995).

The above arguments suggest that seawater DIN could be a quantitatively important alternative source of new nitrogen, particularly to shallow water reef corals. Nutrient budgets for Acropora palmata and Stylophora pistillata for instance, suggest significant proportions of coral nitrogen demand are obtained from DIN (Bythell, 1987; Fig. 5 in Falkowski et al., 1993). It has been suggested that host cytoplasmic levels of DIN are actually very low (D'Elia and Cook, 1988). This effect could be caused by conservation of nitrogen by the host (manifested as low rates of amino acid catabolism; Szmant et al., 1990) or

uptake of DIN from host tissues by the zooxanthellae (D'Elia and Cook, 1988) or by the host itself (Miller and Yellowlees, 1989). Low cytoplasmic levels could create a nutrient gradient leading to net uptake of DIN from seawater into the coral (Cook and D'Elia, 1988).

Many studies have demonstrated the uptake of seawater DIN, both nitrate and ammonium, by zooxanthellate corals (e.g. Franzisket, 1974; D'Elia and Webb, 1977; Webb and Wiebe, 1978; Muscatine and D'Elia, 1978; Propp, 1982; Burris, 1983; Wafar et al., 1985; Wilkerson and Trench, 1986; Bythell, 1990). Ammonium appears to be the most readily assimilated dissolved nitrogen species and can be taken up at micromolar concentrations typical of reef waters (Muscatine and D'Elia; 1978, Burris, 1983; Bythell, 1990). Uptake across host membranes may be diffusive or may involve enzyme-mediated transport, particularly of charged species (Propp, 1982; D'Elia and Cook, 1988; Miller and Yellowlees, 1989). Uptake is light dependent in many cases (Muscatine and D'Elia, 1978; Wilkerson and Trench, 1986) and appears to require the presence of zooxanthellae (azooxanthellate or aposymbiotic corals do not exhibit uptake behavior, Muscatine et al., 1979; Burris, 1983).

The possibility of variation in $\delta^{15}N$ of external DIN as a factor contributing to the $\delta^{15}N$ trend with depth at Discover Bay must be considered (Muscatine and Kaplan, 1994). Decreases in $\delta^{15}N$ of nitrate with depth do occur over the upper 200 m of the open ocean (Liu and Kaplan, 1989). This is likely due to preferential uptake of $^{14}NO_3^-$ by phytoplankton living in well illuminated surface waters (Altabet et al., 1986; Liu and Kaplan, 1989). Such variation with depth will be small over the well mixed upper 30 m of Discovery Bay, where phytoplankton activity is concentrated.

To evaluate the relationship between $\delta^{15}N$ and light availability, the effect of light levels on the proportion of seawater DIN in coral diet should also be considered. Reef corals may exhibit a shift in trophic status with changing light levels. In deeper water, translocation of carbon from the zooxanthellae to the host is often insufficient to meet host needs (e.g. Muscatine et al., 1984; Falkowski et al., 1984). Predation on zooplankton most likely makes up for this shortage (Dubinsky and Jokiel, 1994). Further evidence for increased reliance on heterotrophy comes from the $\delta^{13}C$ of separated tissue and zooxanthellae from Discovery Bay corals (Muscatine et al., 1989). Both the zooxanthellae and tissue become more depleted in ^{13}C with depth but the tissue component becomes depleted at a faster rate. This may result from increased reliance on isotopically light heterotrophic food sources such as zooplankton (Muscatine et al., 1989). Our $\delta^{13}C$ data (Fig. 3.4) suggest an overall decrease with depth which is similar to that found by Muscatine et al. (1989) for separated coral tissue and zooxanthellae.

A trophic shift of this nature would generally be expected to raise coral $\delta^{15}N$ (e.g. Minagawa and Wada, 1984). It is possible, however, that increased reliance on a heterotrophic food source with lower $\delta^{15}N$ than seawater DIN, could explain the trends in $\delta^{15}N$ observed for Discovery Bay corals. This assumes little or no fractionation of seawater DIN by autotrophic corals. Any such heterotrophic source would have to have $\delta^{15}N$ less than 1 ‰, in order to account for the nitrogen data (Table 3.1, Fig. 3.2) Zooplankton are enriched in ^{15}N relative to phytoplankton forming base of the food chain (Minagawa and Wada, 1984). For zooplankton to be isotopically lighter than the DIN source utilized by corals, the phytoplankton upon which the zooplankton feed would have to exhibit strong

fractionations during DIN uptake or utilize an isotopically lighter source of nitrogen than corals. Large discriminations against ^{15}N can occur however, during phytoplankton assimilation, particularly under nutrient sufficient conditions (e.g. Wada and Hattori, 1978).

To examine this possibility, we measured $\delta^{15}N$ of two samples of POM collected from the Discovery Bay forereef at 10 m depth in March, 1993. The samples were collected at 7:30 a.m. utilizing a plankton net with an effective mesh size of approximately 100 µm. The POM consisted largely of zooplankton dominated by planktic copepods and chaetognaths . Both plankton samples yielded $\delta^{15}N$ values of 3.5 ‰. This value is lighter than that reported for many other zooplankton samples (e.g. Wada et al., 1975). This low value may reflect an isotopically light source of DIN (e.g. fixation of N_2 by cyanobacteria or excretory ammonium, Wada and Hattori, 1976; see review in Michener and Schell, 1994) or large phytoplankton fractionation at the base of the plankton food chain. It is unknown whether $\delta^{15}N$ of these samples is representative of $\delta^{15}N$ of the zooplankton being consumed by corals at depth on the Discovery Bay forereef. Different size classes of zooplankton, for instance, have been shown to have different isotopic signatures (Minagawa and Wada, 1984). The isotopic signature of DON at Discovery Bay is also unknown.

Isotopically heavier tissue values were recorded by Muscatine and Kaplan (1994) at 50 m depth for Montastrea annularis and Montastrea cavernosa (i.e. the trend towards lighter values with depth is reversed). These values could reflect dominance of a heterotrophic dietary signal under very low irradiance (Muscatine and Kaplan, 1994). In addition, an azooxanthellate coral from Discovery Bay was enriched in ^{15}N relative to all zooxanthellate samples

(Muscatine and Kaplan, 1994). These data suggest that tissue trends are not being driven by increased contributions of a heterotrophic diet source with low $\delta^{15}N$.

To summarize, gradients in DON or PON are unlikely to explain the observed trends of $\delta^{15}N$ with depth for both Jamaica and Zanzibar corals. Increasing reliance on an isotopically light heterotrophic food source is possible at both sites, but unlikely. When discussing the relative proportions of DIN versus organic sources, we assumed no fractionation relative to seawater DIN during uptake by zooxanthellae. We will now consider models that could potentially explain coral isotopic variation at both Zanzibar and Jamaica in terms of light-mediated endogenous fractionation associated with assimilation of DIN. The effect on both zooxanthellae and tissue $\delta^{15}N$ will be examined.

3.5.1.2 Light mediated isotopic fractionation during nitrogen uptake and assimilation

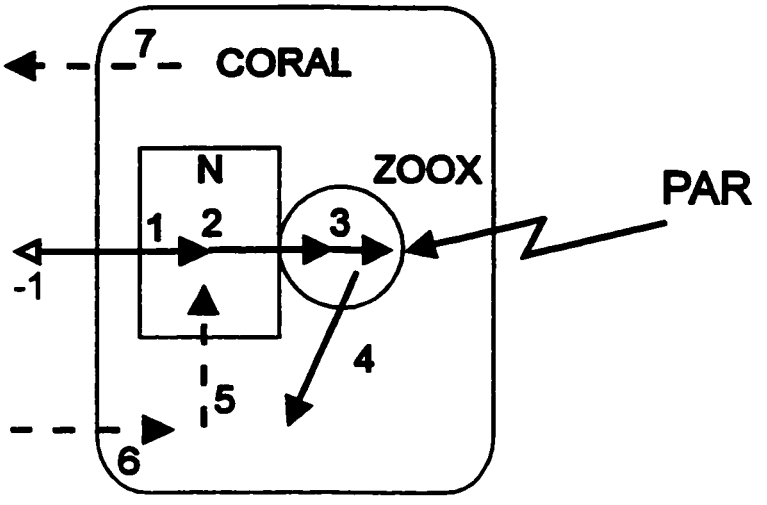
Muscatine and Kaplan (1994) first explored the issue of light-mediated fractionation using $\delta^{15}N$ values of separated zooxanthellae and host tissue. They assumed that zooxanthellae behave similarly to free-living phytoplankton in terms of isotopic discrimination. It was suggested that under lower light conditions, zooxanthellar growth rates would be reduced and nitrogen fractionation during zooxanthellar assimilation of DIN could potentially increase (c.f. Wada and Hattori, 1978). In view of the strong relationship between combined coral tissue $\delta^{15}N$ and light (Fig. 3.3, Table 3.3) we have re-examined the effect that light might have as an influence on coral $\delta^{15}N$.

Two models have been proposed to explain the uptake of DIN by reef corals and other marine invertebrate symbioses (Fig. 3.7): the diffusion/depletion

Fig. 3.7. Schematic diagram detailing fluxes of inorganic nitrogen into and out of the coral symbiosis. (a) corresponds to the diffusion/depletion model (D'Elia and Cook, 1988). (b) corresponds to the "host-assimilation" model (Miller and Yellowlees, 1989). PAR is photosynthetically active radiation. ZOOX refers to zooxanthellae while CORAL refers to the coral host. N is the coral internal (cytoplasmic) dissolved inorganic nitrogen pool. Solid arrows refer to pathways by which DIN can be acquired and assimilated into organic compounds by the zooxanthellae and coral host. Fluxes are as follows; Flux 1: uptake of DIN into host cytoplasm (passive or active), Flux -1: release of DIN from host cytoplasm (passive or active), Flux 2: uptake of DIN by zooxanthellae from host cytoplasm, Flux 3: assimilation of DIN into organic nitrogen (amino acids) by zooxanthellae, Flux 4: translocation of organic nitrogen to the host from the zooxanthellae, Flux 5: host catabolic nitrogen released into the host cytoplasmic DIN pool, Flux 6: host feeding on particulate organic nitrogen (e.g. zooplankton) or dissolved organic matter, Flux 7: release of organic nitrogen in the form of mucoprotein, planulae, zooxanthellae or dissolved organic matter, Flux 8: host assimilation of DIN from the cytoplasmic nitrogen pool via the action of glutamine dehydrogenase or glutamine synthetase.

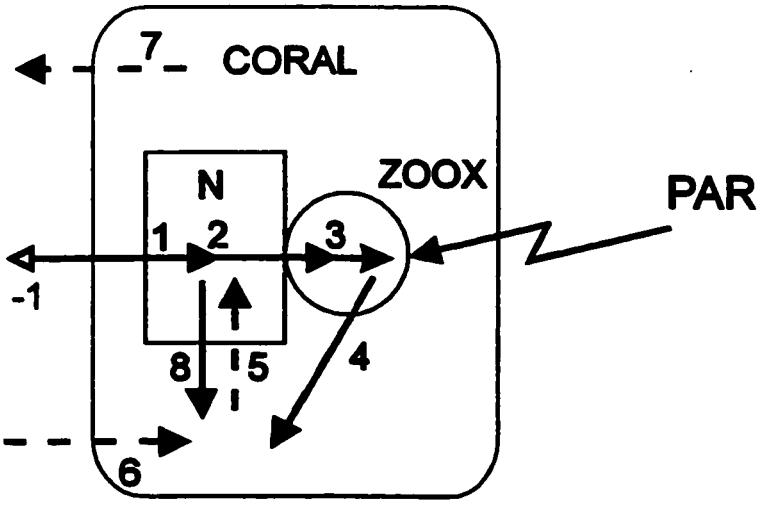
DIFFUSION-DEPLETION MODEL

(a)



HOST ASSIMILATION MODEL

(b)



model (Cook and D'Elia, 1988) and the host assimilation model (Miller andYellowlees, 1989). In the diffusion/depletion model the zooxanthellae are thought to deplete actively the internal coral DIN pool leading to passive diffusion (or possibly enzyme facilitated transport) of seawater DIN into the coral tissue (Cook and D'Elia, 1988). Light could be an important factor by providing an energy source for active algal uptake or by providing carbon skeletons upon which to fix nitrogen molecules (Muscatine and D'Elia, 1978; Cook and D'Elia, 1988). It has been demonstrated experimentally and in field studies that uptake of DIN by phytoplankton involves discrimination against the heavy isotope of nitrogen leading to a fractionation relative to source, with fractionations up to 12 ‰ possible (e.g. Wada and Hattori, 1978; Wada, 1980; Cifuentes et al., 1989; Montoya et al., 1991; Goericke et al., 1994; Montoya and McCarthy, 1995).

Montoya and McCarthy (1995) have postulated that light may directly affect the fractionation factor, α (ratio of kinetic rate constants, $^{14}k/^{15}k$), for phytoplankton DIN uptake by affecting the amount of energy available for active uptake. These authors believe that most of the fractionation occurs during uptake of DIN (see also Goericke et al., 1994) as opposed to during assimilation within the algal cell (Wada and Hattori, 1978). They contend that under low light conditions less of the nitrogen immediately adjacent to the cell will be taken up, leading to increased values of α . This same study found no clear relationship between α and growth rate of algal cells, contrary to the results of Wada and Hattori (1978). Montoya and McCarthy (1995) argue that since light controlled growth rate in the study of Wada and Hattori (1978), the principal control on variation in α could be light level.

Here we utilize the observations and conceptual model of Montoya and

McCarthy (1995), developed for phytoplankton, to explain potential zooxanthellar fractionations within a diffusion-depletion scenario. Since zooxanthellae are not directly in contact with a large external DIN pool, the effect of nutrient limitation upon the degree of expression of the fractionation factor must also be considered. A simple model is presented below, (modified for DIN uptake and assimilation in corals from an algal carbon uptake model presented in Goericke et al., 1994). The simplest case of steady state isotope kinetics with the assumption of no zooxanthellar growth is:

(1) $DIN_{external} \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} DIN_{coral\text{-}int} \xrightarrow{k_2} DIN_{zoox\text{-}int} \xrightarrow{k_3} OBN \xrightarrow{k_4} (TRANSLOCATE)$

(numerical subscripts correspond to numbered fluxes in Fig. 3.7)

The subscript *external* refers to the external seawater DIN pool, *coral-int* is the internal coral DIN pool and *zoox-int* is the internal zooxanthellae DIN pool. OBN refers to organic-bound nitrogen formed by assimilation of DIN within the zooxanthellae. k_1 is the uptake of DIN into the coral internal DIN pool, k_{-1} is the release of DIN from this pool (back diffusion or possibly active excretion from host). k_2 is uptake of DIN by the zooxanthellae from the internal coral DIN pool, k_3 is the assimilation of DIN into organic-bound nitrogen within the zooxanthellae and k_4 is the translocation of OBN from the zooxanthellae to the coral host.

In isotopic delta notation the model becomes:

(2) $\delta_{external} \underset{\varepsilon_{-1}}{\overset{\varepsilon_1}{\leftrightarrow}} \delta_{coral\text{-}int} \xrightarrow{\varepsilon_2} \delta_{zoox\text{-}int} \xrightarrow{\varepsilon_3} \delta_{OBN} \xrightarrow{\varepsilon_4} host$

Per mil isotopic enrichment factors ($\varepsilon = 1000\ (\alpha - 1)$) are presented using the convention that positive values represent isotope effects in which the lighter species reacts more quickly (i.e. product is enriched in the light isotope). We will

assume that $\varepsilon_4 = 0$.

(3) $\delta_{OBN} = \delta_{zoox\text{-}int} - \varepsilon_3 + \varepsilon_4$

(4) $\delta_{zoox\text{-}int} = \delta_{coral\text{-}int} - \varepsilon_2 + \varepsilon_3$

(5) $\delta_{coral\text{-}int} = \delta_{external} - \varepsilon_1 + f(\varepsilon_{-1}) + (1-f)\varepsilon_2$

and where $f = \frac{k_{-1}}{k_1}$ (i.e. outputs from the internal coral DIN pool must be weighted).

Substituting (4) and (5) into (3) gives the isotopic composition of the zooxanthellae OBN pool;

(6) $\delta_{OBN} = \delta_{external} - \varepsilon_1 + f(\varepsilon_{-1} - \varepsilon_2)$

For simplicity only inputs of new seawater to the internal coral DIN pool and fractionations occurring relative to this source are considered in (6) but the influence of catabolic nitrogen (flux 5 in Fig. 3.7) as an additional source will be considered below. We have assumed that all of the nitrogen entering the zooxanthellae is assimilated (Goericke et al., 1994), such that ε_3 disappears in equation (6). This model is greatly simplified in that we only show uptake across one host membrane. We have ignored the presence of additional membranes such as the perialgal membrane surrounding each zooxanthellae.

Light can have two possible effects on nitrogen fractionation within this model. With decreasing light levels, ε_2 may increase as suggested for free-living algae (Montoya et al., 1995). More importantly, because the zooxanthellae are not free-living but contained within coral tissue (a diffusion barrier or barrier requiring energy for active uptake), light levels could affect the value of f in (6). f is the "leakiness" of the cell membrane to DIN (c.f. Goericke et al., 1994). The

value of *f* is unknown for DIN in coral tissues, but can be evaluated using the relationship:

(7) $f = 1 - (k_2/k_1)$

(Goericke et al., 1994). With high levels of irradiation, zooxanthellar uptake of dissolved nitrogen (flux 2) should increase (as a result of energy availability for DIN uptake, D'Elia and Cook, 1988). With greater zooxanthellar usage of DIN from the internal coral DIN pool, *f* will approach zero (all of the nitrogen entering the internal coral DIN pool will be taken up, including any catabolic nitrogen). Under these conditions the only fractionation observed will be the fractionation, ε_1, associated with the initial diffusion into the animal tissue (see equation (6)).

Under lower light conditions, zooxanthellar uptake of DIN will be reduced and larger fractionations relative to new seawater nitrogen could potentially occur. Corals from Discovery Bay appear to rely more heavily on exogenous food sources at greater depths (Muscatine et al., 1989) but seawater nitrogen could still provide an important source of DIN. In low light, there should be correspondingly more DIN in the host cytoplasm derived from host catabolism (flux 5) due to increased heterotrophic feeding (D'Elia and Cook, 1988).

With both new seawater nitrogen and catabolic nitrogen (fluxes 1 and 5 in Fig. 3.7) contributing to the internal coral DIN pool, excess nitrogen could be present relative to zooxanthellar demand under low light levels. Under these conditions zooxanthellae will be able to fractionate relative to these sources and unused heavier DIN can periodically leak (or be transported) from the coral into the external environment (k_{-1} in (1)). Essentially, equilibrium will nearly be established between internal and external DIN concentrations, with resultant minimal net fractionation across the host membrane. With reference to equation

(6), as k_{-1} becomes larger (k_2 becomes smaller) *f* will approach unity (or its maximal value dependent upon membrane characteristics). Under these conditions fractionation associated with zooxanthellar uptake, ε_2 , will be more fully expressed. In other words, since all of the seawater nitrogen entering the coral will not be utilized, zooxanthellar uptake fractionation can occur. The model and the isotopic offset in coral tissue $\delta^{15}N$ with depth suggest ε_2 is greater than ε_1.

Light is likely a determining factor in the nutrient limitation status of corals (Dubinsky and Jokiel, 1994). It has been suggested that while corals under high light are almost certainly nitrogen limited, corals under low light may not be nitrogen limited at all (e.g. Muscatine and Kaplan, 1994; Dubinsky and Jokiel, 1994). This state will tend to promote larger fractionation with decreasing light availability. In addition, it is possible that different strains of Symbiodinium microadriaticum (e.g. Trench, 1987; Trench and Blank, 1987; Rowan and Powers, 1991; Sandeman, 1996; Eustice personal communication), like different species of free-living algae, might have different nitrogen fractionation factors. Light related gradients in zooxanthellae assemblages or physiologies could potentially lead to different values of ε_2 under different light conditions.

The above model is most appropriate for diffusive fluxes but also provides a useful starting point for considering active uptake and release of DIN (cf. Goericke et al., 1994). A similar model has been employed to discuss fractionation of ammonium by a marine bacterium (Hoch et al., 1992). Until more is known about the nature of processes involved in nitrogen uptake and release, as well as the species involved, it will not be possible to utilize this model in anything but a conceptual fashion.

In the host assimilation model, the host can take up and assimilate

ammonium from the internal coral DIN pool (flux 8, Fig. 3.7b) using the enzymes glutamate dehydrogenase (GDH) or glutamine synthetase (GS). GDH and GS activities have been found to be higher in coral hosts than in zooxanthellae (e.g. Yellowlees et al., 1994). Light and zooxanthellae also play a role in nutrient acquisition in this model. Miller and Yellowlees (1989) have proposed that carbon-rich translocated sugars from the zooxanthellae (flux 4, Fig. 3.7b) drive host DIN uptake by providing an energy-rich source to fuel enzymatic uptake processes. We will consider organic nitrogen fixed in this matter to be photoautotrophic since uptake is dependent upon the presence of zooxanthellae and energy from light. Since light is driving nitrogen uptake and leading to depletion of the internal coral DIN pool, arguments used to explain zooxanthellar fractionation in the diffusion-depletion model apply equally well for host assimilation here. Equation (6) would still apply, with ε_2 replaced by ε_8 (fractionation during host uptake). GDH and GS mediated uptake of ammonium has been demonstrated to involve fractionation against the heavy isotope with fractionations of about 8 ‰ at pH 7.1 (relative to NH4+; Weiss et al., 1988; Hoch et al, 1992).

In shallow water, high light settings, both the zooxanthellae and host will have a large energy source for abundant nitrogen uptake from the internal coral DIN pool (fluxes 2 and 8, Fig. 3.7b). Only initial diffusion fractionation, ε_1, will tend to be expressed. In deeper water with reduced light availability, both algal and tissue demand for DIN will be reduced and greater fractionation (ε_2 (zooxanthellae) and ε_8 (host)) can occur. In this model algal and tissue fractionation occur separately from the same source pool.

3.5.1.3 Influence of light on coral tissue $\delta^{15}N$

Fractionation associated with both the depletion/diffusion and host assimilation models are adequate to explain the decrease in $\delta^{15}N$ of zooxanthellae with depth at Discovery Bay (Muscatine and Kaplan, 1994). With increasing reliance on an exogenous diet at greater depths, the tissue component of the corals would tend to be driven towards food (probably zooplankton) $\delta^{15}N$ values . We now consider how decreased light availability, might lead to simultaneous depletions in the tissue component (see Muscatine and Kaplan, 1994) without requiring an isotopically depleted heterotrophic food source (Flux 6 in Fig. 3.7).

Under low light conditions, if the component of host nitrogen derived from DIN (taken up directly by the host and/or translocated by way of the zooxanthellae) is sufficiently isotopically depleted, its isotopic signature could overwhelm that of the heterotrophic diet. The net effect could be to produce tissues with lower $\delta^{15}N$ under reduced light conditions. The large isotopic depletions observed for zooxanthellae $\delta^{15}N$ with depth (see Table 1 in Muscatine and Kaplan, 1994) and predicted by equation (6), for both zooxanthellae and host assimilated nitrogen, suggest this situation can occur. If the zooxanthellae are depleted it can be expected that photosynthate translocated to the host (Flux 4, Fig. 3.7) would tend to be similarly depleted. In order to affect host tissue $\delta^{15}N$, the autotrophic contribution to the diet of the coral animal must be both sufficiently isotopically depleted and abundant to result in lower $\delta^{15}N$ tissue values under decreased light conditions. Light, by affecting the autotrophic contribution to the coral nitrogen diet, could also affect tissue $\delta^{15}N$.

The heavy $\delta^{15}N$ values of separated tissue from corals at 50 m at Discovery Bay (Muscatine and Kaplan, 1994) suggest that autotrophic

contributions can only overwhelm heterotrophic signatures to a certain level of low illumination. That is, in very low light conditions, translocation and direct host uptake of DIN may be so reduced that corals are predominantly heterotrophic and so become isotopically heavy. We can only assert that there is a strong relationship between coral ^{15}N and light over the range of irradiance studied (about 6 - 35 $Em^{-2}day^{-1}$ at Discovery Bay; Fig. 3.1).

The preceding discussion has assumed an increase in heterotrophy with depth. It is possible, however, that ratios of heterotrophy to autotrophy do not increase over the Discovery Bay forereef, but rather the metabolic activity of both host and symbiont decrease with decreasing light availability. This situation may well occur, as benthic zooplankton availability actually decreases over depth at Discovery Bay, thereby making increased heterotrophy more difficult (Ohlhorst, 1985). Isotopically depleted translocated material could more easily affect tissue $\delta^{15}N$ if there was no corresponding increase in heterotrophy with depth.

In invoking translocated organic nitrogen to explain lighter tissue values with decreasing light, we have assumed abundant translocation of nitrogen-rich metabolites (e.g. amino acids such as alanine). Translocated organic matter, however, is generally carbon-rich and has been viewed as "junk food" providing an energy source to corals (Falkowski et al., 1984). For translocation to explain the observed isotopic trends, the translocate would have to provide a higher quality "health food" in terms of nitrogen content or be of sufficient flux to affect tissue isotopic signatures. It has been suggested that most of the DIN assimilated by the zooxanthellae is translocated to the host (Muscatine et al., 1984; Falkowski et al., 1984; Falkowski et al., 1993). It is likely that translocated organic nitrogen is an important nitrogen source, especially to shallow water

corals with reduced rates of predation. This source of nitrogen may, for example, provide some of the essential amino acids that the coral host can not generate (Falkowski et al., 1993).

3.5.2 Sources of variability in Jamaica coral $\delta^{15}N$

Causes of variability between species at Jamaica should be considered. In general, the regression coefficients are similar for all three species suggesting that there is not a lot of variability attributable to species effects. One exception however, is the higher surface value (y- intercept) for Porites (Table 3.3). $\delta^{15}N$ of Porites at each depth tends to be higher than that of the other two species. This may imply that this species is the most autotrophic with respect to nitrogen at any given depth. As such, it will more strongly deplete its internal DIN pool, leading to reduced fractionation relative to the other two species. This is consistent with the small polyps, feeding behavior and energy budgets of this genus (cf. Porter, 1976; Edmunds and Davies, 1986). It is interesting to note that the tissue and zooxanthellae $\delta^{15}N$ tend to be closer for Porites astreoides than Montastrea annularis (see Table 1 in Muscatine and Kaplan, 1994), suggesting Porites does rely more on autotrophy as a nutritional strategy.

Light could explain some of the variation about the mean at each depth for Jamaica corals (Fig. 3.3). Small variations in sample orientation, shading by surrounding fauna and reflectance of surrounding substrate (Brakel, 1979) could influence the amount of light received by individual corals. Since we believe $\delta^{15}N$ is affected by the amount of photosynthesis occurring (as influenced by light), variations in individual coral metabolic response to light availability could also play a role.

3.5.3 Other possible influences on Jamaica coral $\delta^{15}N$

The models presented above have dealt with fractionation associated with uptake and assimilation of new nitrogen and recycling of catabolic nitrogen. Processes by which nitrogen is eliminated from corals must also be considered. Nitrogen is eliminated as DIN, DON, mucoproteins, expelled zooxanthellae and planulae (Fluxes -1, 7, Fig. 3.7) (Muscatine and Porter, 1977). Fractionation associated with these processes could affect coral tissue $\delta^{15}N$. Until more is known about the relative importance of these processes and their associated fractionations, however, their effect on coral isotopic nitrogen balance will remain unclear. The efficiency of recycling of DIN via the zooxanthellae may be affected by light availability. Light could therefore affect the excretory balance and hence the $\delta^{15}N$ of coral tissue.

Concentration of DIN is another potential factor that could cause isotopic shifts. If increased external concentrations of DIN were present (perhaps as a result of eutrophication or upwelling) greater nitrogen fractionation could occur during host or zooxanthellar assimilation (Muscatine and Kaplan, 1994). With greater external concentrations, internal concentrations might tend to increase. Given the same demand for nitrogen under a particular illumination, f in equation (6) will be correspondingly closer to unity and ε_2 will be more fully expressed. It is also possible however that under conditions of nutrient enrichment, translocation from zooxanthellae to host is reduced and the host obtains more of its food from heterotrophic sources (Dubinsky and Jokiel, 1994; Jokiel et al., 1994). It will be difficult to evaluate the effect of concentration on $\delta^{15}N$ of corals until controlled experimental work is performed.

3.5.4 Influence of light on Zanzibar coral $\delta^{15}N$

Notwithstanding the strong correlation between $\delta^{15}N$ of Jamaica coral tissue and light attenuation, we cannot be certain that light is the dominant factor responsible for the observed variations (Fig. 3.3, Table 3.3). The Zanzibar data set, however, provides an opportunity to evaluate the degree to which light alone could be responsible for the observed trend in $\delta^{15}N$ at Discovery Bay. Over the scale of a single large coral head, $\delta^{15}N$ and concentration of DIN in seawater can be assumed constant, as can $\delta^{15}N$ of PON or DON. Light levels, however, can vary greatly. Although we lack light data from the reefs studied in Zanzibar, we can use appropriate data from Discovery Bay (Fig. 3.1) to illustrate the potential variation in light over a coral head. At Discovery Bay the light received by a vertical surface at 1 m depth, which is exposed to the maximum amount of incoming insolation, is about 60% less than that received by a horizontal surface (i.e. the top of a coral head). At the same depth, a vertical shaded side of a coral head would receive about 95% less light than the top of a coral head. The decrease in light received by the top of a coral head at 30 m relative to the top of a coral head growing near the surface is about 80 % (Fig. 3.1). Therefore, based on Discovery Bay data, we can say that the variation in light over a single shallow water coral head can be a very large proportion of the variation in light received by the tops of coral heads over the depth range of a reef.

The decrease in $\delta^{15}N$ with depth, from the top to the sides of the large Zanzibar coral heads, is a substantial fraction (approximately 50 %) of the decrease seen over 30 m at Discovery Bay. This decrease occurs over a depth range of only one to two meters (Fig. 3.6). In each case, the samples ranged from the horizontal tops of the head to vertical sides near the base of the coral.

The different $\delta^{15}N$ responses of the three Zanzibar coral heads (Fig. 3.5) likely reflect variations in coral head morphologies. Due to the different shapes of the coral heads, no single function can adequately describe the change in $\delta^{15}N$ with depth. The common linear relationship between $\delta^{15}N$ and ln (depth), however, emphasizes similarity in trends. The logarithmic transformation is appropriate, because in general these coral heads steepened quickly from the top to the sides, with much of the depth range occupied by near vertical coral flanks. This would be consistent with a rapid decrease in light received by coral surfaces, from the top to the side of each head, followed by a more gradual decline in light availability down the side of the coral colony.

Based upon the decrease in light availability from the tops to the bases of the large corals, we can infer that there is a corresponding decrease in zooxanthellar activity. The carbon data for the Zanzibar corals show a slight decrease in $\delta^{13}C$ with depth. Both Zanzibar and Discovery Bay corals exhibit decreasing $\delta^{15}N$ with decreasing light availability. In the case of Zanzibar corals, however, light is the only apparent factor that seems capable of explaining this decrease. Although we cannot use Zanzibar data to imply that light is the only factor leading to the decrease in $\delta^{15}N$ with depth at Discovery Bay, the results suggest that light could be the main control.

3.5.5 Significance of light mediated fractionation models

Neither this study nor Muscatine and Kaplan (1994) can discern whether diffusion/depletion with algal translocation or host assimilation is the route by which coral tissues may be affected by light. It is possible that both processes are involved. If the heterotrophic food source does not have low $\delta^{15}N$ (see above), then both fractionation models (i.e. models based on diffusion/

depletion and host assimilation scenarios) strongly support the importance of seawater nitrogen even under the relatively low light conditions prevalent at 30 m depth. If all of the DIN being assimilated by the host or zooxanthellae was derived from host catabolism of an exogenous diet, then isotopic mass balance would require that the combined tissue/zooxanthellae $\delta^{15}N$ closely resemble that of the heterotrophic food source. The trend with decreased light would be expected to be one of enrichment rather than depletion. The fact that there is an additional dissolved source against which nitrogen fractionation can occur during uptake by the host and/or zooxanthellae helps account for the observed trends. The models also support the importance of photoautotrophy with respect to nitrogen even under relatively low light conditions. Nitrogen fractionation at depth also suggests that the host is not totally controlling the amount of nitrogen available to the zooxanthellae (see review in Miller and Yellowlees, 1989).

Without knowing $\delta^{15}N$ of the various forms of DIN available to reef corals at Discovery Bay we cannot say what form is being utilized. We can however suggest that if ammonium is the predominant form being initially taken up (k_1) then it is not only as diffusion of the uncharged form (ammonia). Membrane diffusion of ammonia involves fractionation on the order of 39 ‰ (relative to ammonium; see discussions of the causes of this fractionation in Hermes et al., 1985; Hoch et al., 1992). In the high light case where f is close to zero and the only fractionation expressed is ε_1 , if ammonia diffusion was the only process involved we would expect much lower coral tissue $\delta^{15}N$ than is observed. This may suggest a role for active uptake across host membranes. It is also possible that more than one species of nitrogen is utilized, particularly in shallow water where demand is greater. If an additional DIN species was enriched (e.g.

nitrate relative to ammonia) this factor could also affect observed trends.

The results of this study allow us to suggest that shallow water autotrophic corals could prove to be ideal tracers of DIN in reef environments. Porites is an excellent example of such a coral. In shallow water this coral seems to rely largely on autotrophy as a nutritional strategy. If nitrogen is limiting, the only fractionation of concern is that which occurs during initial uptake across the host membrane. If this fractionation can be experimentally determined, we could calculate $\delta^{15}N$ of the DIN source(s) being utilized. If we assume ε_1 is negligible then the minimum DIN $\delta^{15}N$ value would be about 3-4 ‰ at Discovery Bay and about 5.5 ‰ at Zanzibar. These values are at the lower end of the range of typical seawater DIN values (see Fig. 5 in Owens, 1987). This assumes that corals are not enriched relative to diet due to excretion of light nitrogen (cf. Minagawa and Wada, 1984). If all excretory nitrogen (as DIN) is recycled by shallow water autotrophic corals, loss of nitrogen from the system will occur primarily as substances such as mucus or DON. If these substances have $\delta^{15}N$ values close to the bulk tissue, no fractionation associated with loss of nitrogen will occur.

There is no evidence in the Discovery Bay data set that even surface water corals are nitrogen limited. There is no definite plateau in $\delta^{15}N$ (Fig. 3.3) over a range of shallow depths that suggests all corals within this range are nutrient limited and therefore exhibiting minimal fractionation. The very strong relationship between mean $\delta^{15}N$ and light supports this contention. It is possible this is a function of the resolution of sampling (i.e. we have not sampled every meter for instance in shallow depths) or may reflect the particular light and nutrient regime prevalent at Discovery Bay at the time of collection. A single

sample of Porites furcata sampled at 5m had $\delta^{15}N$ of 5.4 ‰. This branching, small -polyp form may be more autotrophic than the other species considered (c.f. Porter, 1976), and as such may be less fractionated relative to source. If so, then all other Jamaica corals in this study may be exhibiting fractionation relative to source DIN.

3.6 Conclusions

Symbiosis, conservation of DIN and multiple nitrogen sources to both host and symbionts make it difficult to apply the empirical relationships of simple food chains to corals. This study suggests light is a further complicating factor. Data from Jamaica and Zanzibar show decreasing $\delta^{15}N$ over gradients corresponding to decreasing light availability. By analyzing replicates of three species of coral over a 30 m depth range, we have demonstrated strong correlations with the light attenuation profile at Discovery Bay, Jamaica. Light may not be the sole factor leading to the observed trends, but the strong correlation suggests it is certainly a variable which must be considered in future coral $\delta^{15}N$ studies. Data from Zanzibar coral, collected over single heads, show variation in $\delta^{15}N$ of the same sense and appropriate magnitude relative to Jamaica corals. The absence of other factors strongly suggests that light is an important control on $\delta^{15}N$. We believe light acts as an endogenous control on the degree of fractionation expressed during nitrogen uptake and assimilation, affecting both the zooxanthellae and tissue components of the symbiosis. Variation in light affecting $\delta^{15}N$ could be due to the overall light attenuation profile on a reef or to smaller scale factors such as coral orientation.

Under conditions of internal nutrient limitation (as affected by light and nutrient concentration) corals may be excellent tracers of $\delta^{15}N$ of reef seawater

DIN and could prove useful in determining the relative proportions of multiple nitrogen sources being utilized by reef corals. Because they are contained within host tissues, zooxanthellae will have less opportunity for DIN fractionation than other primary producers on the reef, perhaps making corals better tracers of the $\delta^{15}N$ of DIN. To test this hypothesis detailed isotopic studies of inorganic and organic nitrogen sources on reefs will have to be undertaken.

Chapter 4: Potential influences on inter-reef variability in $\delta^{15}N$ of coral tissue

4.1 Abstract

A comparison of $\delta^{15}N$ and $\delta^{13}C$ values of zooxanthellate coral tissue from eight different reef sites is presented. One-way ANOVA analysis indicates that highly significant inter-reef differences exist in both $\delta^{13}C$ and $\delta^{15}N$. Corals from five of the studied reefs have average $\delta^{13}C$ values ranging from approximately -10 to -12 ‰, suggesting a predominantly marine diet with strong reliance on photoautotrophy as a nutritional strategy. Corals from the reef at Jepara (Indonesia) and Australian reefs have lighter values suggesting that heterotrophic feeding on terrestrial carbon may be important. Average $\delta^{15}N$ values for corals from the eight reefs range from approximately 3 to 7 ‰ with an average for all corals of 4.8 ‰. Possible reasons for inter-reef differences in $\delta^{15}N$ are discussed. Potential factors include trophic status, $\delta^{15}N$ of source nitrogen, and light and/or concentration mediated fractionation of dissolved inorganic nitrogen. It is suggested that these factors act synergistically to produce the observed inter-reef differences.

4.2 Introduction

Several studies have demonstrated that significant variation in $\delta^{15}N$ of coral tissue exists along environmental gradients within individual reefs or

between reefs in a regional reef system. This variation has frequently been related to localised input of an isotopically distinct nutrient source, often of anthropogenic origin (Risk et al., 1989; Allison et al., 1991; Risk et al., 1993; Dunn, 1995; Sammarco et al., in preparation; Mendes et al., in press). Variation within reefs can also be caused by gradients in environmental factors such as light (Muscatine and Kaplan, 1994; Chapter 3). Variation in $\delta^{15}N$ between major reef systems, on the other hand, has received little attention.

An extensive analysis of $\delta^{15}N$ (and $\delta^{13}C$) of reef coral tissue has been performed in the Stable Isotope Laboratory in the Department of Geology at McMaster University. We present here the results from 123 coral tissue samples collected from reefs located in the Indo-Pacific and the Caribbean Sea (Figs. 4.1, 4.2). Variation between reefs is shown to be significant and possible factors leading to this variability are discussed. The focus of this paper is on $\delta^{15}N$ of coral tissue (as opposed to $\delta^{13}C$), as this variable shows the greatest inter-reef variation and in general is poorly understood. In particular, we examine whether corals might be acting as conservative tracers of $\delta^{15}N$ of DIN on each reef, or whether fractionation related to different environmental conditions could be responsible for the observed variation.

Fig. 4.1 Location map for Indo-Pacific reefs. Month or season of collection for each site are provided below to illustrate seasonal distribution of samples in addition to latitudinal distribution shown in the figure (refer to discussion of the influence of light on coral $\delta^{15}N$): Banda -- May and October; Australia -- July; Maldives -- May; Zanzibar -- April and July; Jepara -- July.

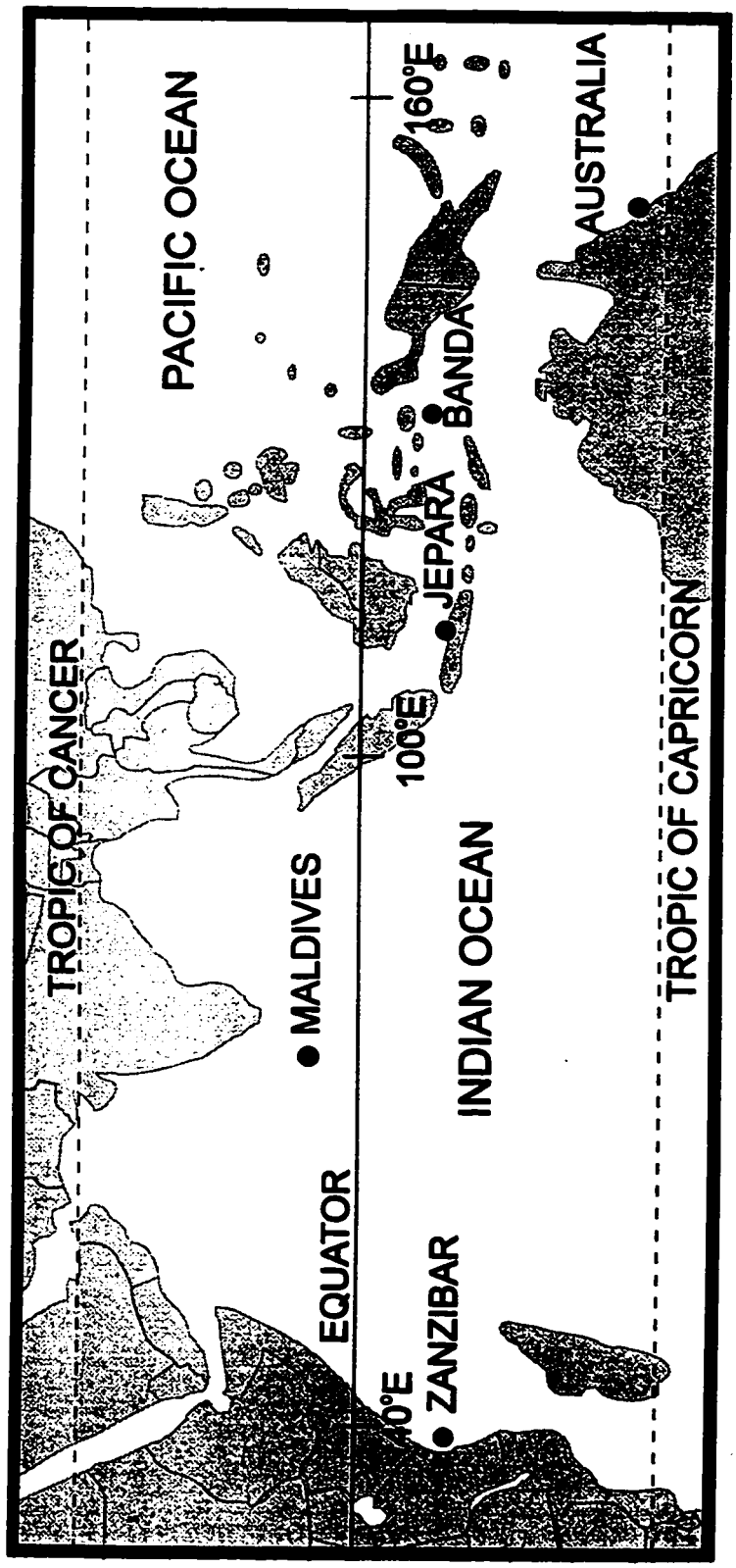
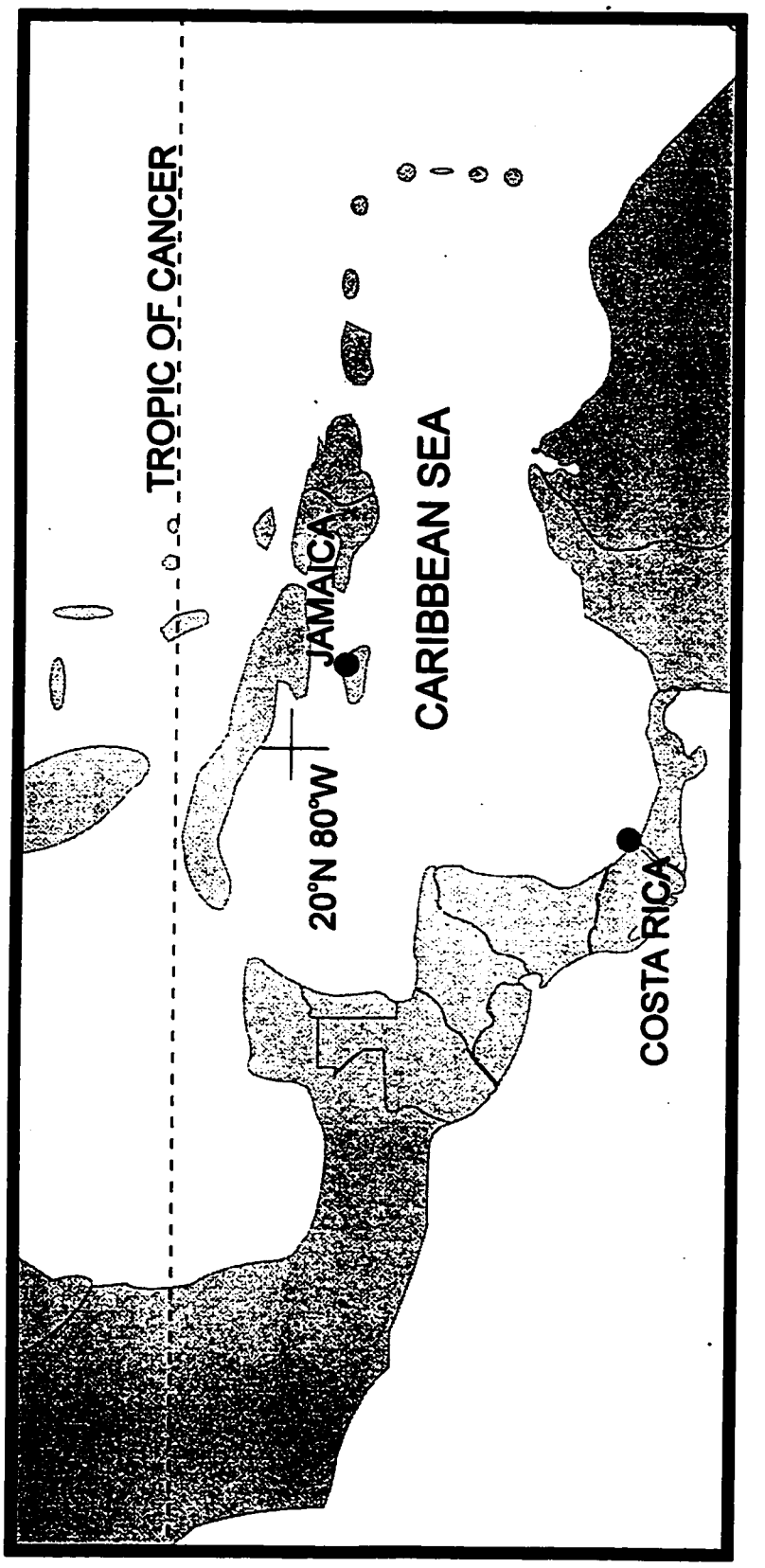

TROPIC OF CANCER
PACIFIC OCEAN
160°E
AUSTRALIA
MALDIVES
JEPARA
BANDA
100°E
INDIAN OCEAN
EQUATOR
ZANZIBAR
TROPIC OF CAPRICORN

Fig. 4.2 Location map for Caribbean reefs. Months of collection: Jamaica -- January; Costa Rica -- April and February;


TROPIC OF CANCER
JAMAICA
CARIBBEAN SEA
20°N 80°W
COSTA RICA

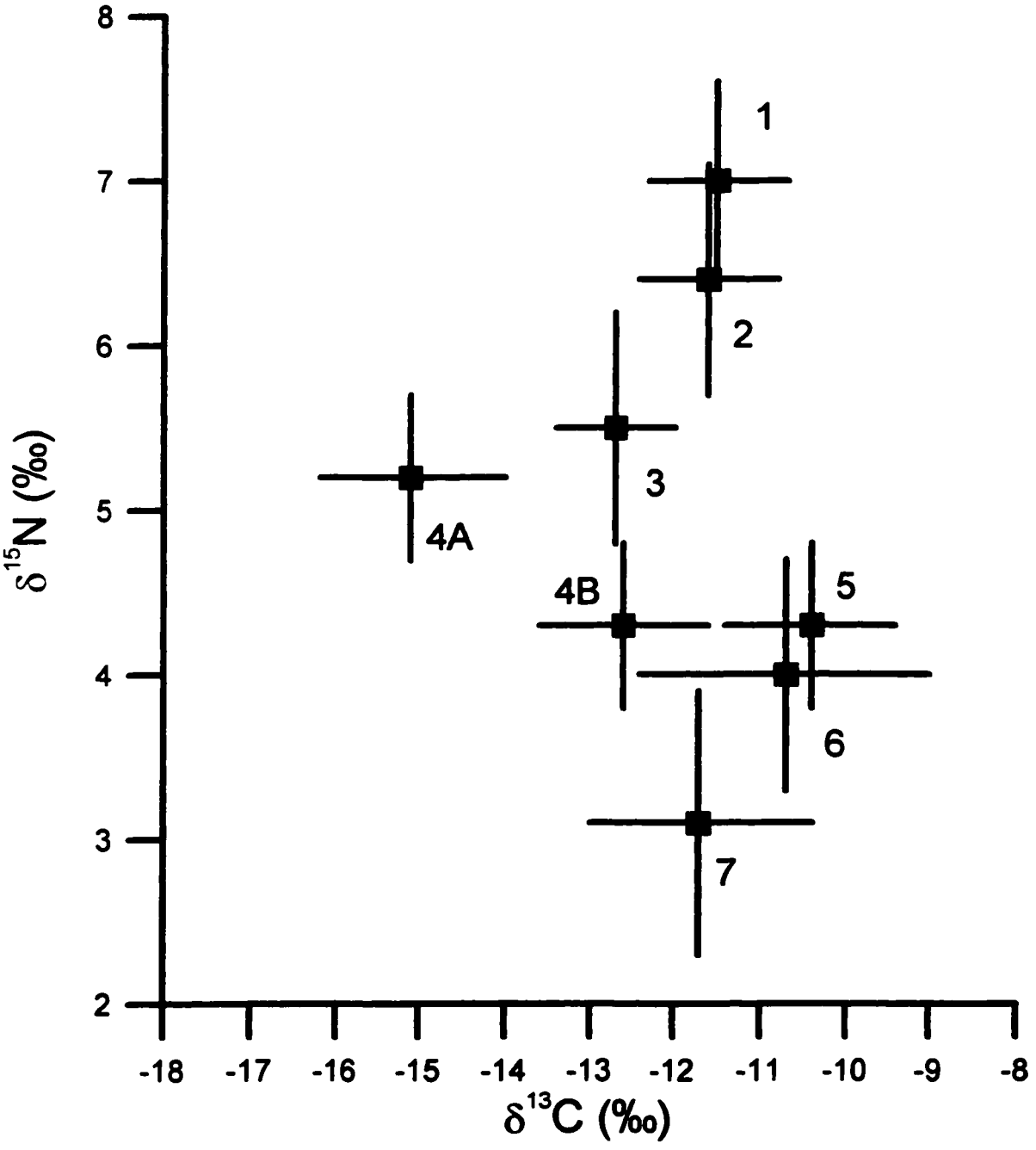
4.3 Materials and methods

Sample preparation has been described in detail in Chapter 3 and in individual references from which data have been derived (see Fig. 4.3). Approximately 15 mg of coral tissue was analysed for $\delta^{15}N$ using a VG SIRA mass spectrometer. 3-5 mg of tissue was used for $\delta^{13}C$ analysis. Repeatability (1 σ) on all analyses was generally 0.1 ‰ or better. $\delta^{15}N$ and $\delta^{13}C$ values are reported relative to atmospheric nitrogen and PDB standards respectively.

The locations of sampled reefs are shown in Figs. 4.1 and 4.2. The reefs studied are all fringing reefs, with the exception of samples from the barrier reef of Australia. Values for individual Australian corals represent averages of one to three replicates from each colony. Australian samples were collected as part of a cross-shelf survey and include reefs from inshore to shelf edge (Risk et al., 1994). These corals were divided into inshore, mid-shelf and outer shelf groupings based on their distance from shore. For the purpose of data analysis, mid-shelf and outer-shelf reefs were further grouped based on the lack of statistically discernible differences in average $\delta^{13}C$ and $\delta^{15}N$. As such, data from eight sites, representing seven different geographic locations are presented.

All samples included in this compilation were collected from less than 5 m depth and consist of tissue from the horizontal tops of coral heads. Corals from such shallow depths were chosen because they are potentially fully

Fig. 4.3 $\delta^{15}N$ and $\delta^{13}C$ of coral tissue from reefs compiled in this study. Squares represent mean values. Range bars give one standard deviation on either side of the mean. 1. Banda (this study); 2. Maldives (Allison et al., 1991; Risk et al., 1993); 3. Jepara (Dunn, 1995); 4A. Australia inshore reefs (Risk et al., 1989; Risk et al., 1994, Sammarco et al, in preparation) ; 4B. Australia mid-shelf and outer-shelf reefs (Risk et al., 1989, Risk et al., 1994, Sammarco et al., in preparation); 5. Zanzibar (Dunn, 1995); 6. Costa Rica (this study); 7. Jamaica (Chapter 3).


8
7
6
5
4
3
2
δ15N (‰)
1
2
3
4A
4B
5
6
7
-18
-17
-16
-15
-14
-13
-12
-11
-10
-9
-8
δ13C (‰)

photoautotrophic with respect to carbon metabolism (e.g., Porter, 1985; Edmunds and Davies, 1986; Muscatine et al., 1989). As such, these corals may also be deriving much of their nitrogen requirements through zooxanthellar uptake of dissolved inorganic nitrogen (DIN) as opposed to predation on zooplankton (see model of Dubinsky and Jokiel, 1994). The results of Chapter 3 suggest that under these conditions corals may prove to be useful tracers of $\delta^{15}N$ of reef DIN. All of the Indo-Pacific corals are Porites lobata. Caribbean corals include Porites astreoides, Montastrea annularis, Siderastrea radians, Diploria sp. and Porites furcata.

Maldives and Zanzibar corals were originally collected to facilitate comparison of eutrophied and unaffected reefs. Except where pertinent, data from these reefs are not identified according to position on a nutrient gradient (either intra-reef or between reference and affected reefs). Differences between sites in this compilation are generally larger than intra-site variation related to nutrient gradients. Jepara (Indonesia) samples were collected along an onshore to offshore nutrient gradient. In general, samples closest to shore (and the anthropogenic nutrient source under study) at Jepara have the highest $\delta^{15}N$ and lowest $\delta^{13}C$ (Dunn, 1995). The reader is referred to individual studies for more in-depth discussion of intra-reef variability.

4.3.1 Statistical analysis

Prior to statistical analyses, all original $\delta^{13}C$ and $\delta^{15}N$ were tested for violations of normality (using normal probability plots; Wilkinson, 1989) and homogeneity of variance (using F_{max} and Bartlett's tests; Zar, 1984) assumptions inherent in all linear model statistics. Outliers were identified using the box and stem plot procedures (Wilkinson, 1989) and as a result of ANOVA analyses. While the $\delta^{13}C$ data conformed to the normality assumption, violation of the homogeneity of variance assumption was detected. The homogeneity of variance violation occurred primarily due to the presence of three outliers. Removal of the three outliers from the data set (i.e., they were replaced with the corresponding cell means; Sokal and Rohlf, 1981) corrected the homogeneity of variance violation, and as a result data transformation was not necessary. The $\delta^{15}N$ data showed no violations of either normality or homogeneity of variance assumptions. To test the null hypothesis that there were no statistically discernible differences in the average $\delta^{13}C$ and $\delta^{15}N$ values among the eight different sites in the study, a one-way ANOVA was employed (Zar, 1984). Differences between specific sites were tested using the Bonferroni's pairwise comparison test (Wilkinson, 1989).

4.4 Results

Data from the Banda Islands and Costa Rica have not been previously reported and are presented in Table 4.1. A comparison of coral isotopic signatures from the eight reefs is presented in Figure 4.3 (see Table 4.2 for

Table 4.1. Banda and Costa Rica Coral Tissue $\delta^{15}N$ and $\delta^{13}C$

Sample	Location	Genus[1]	Date[2]	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Costa Rica					
sid7	Manzanillo	*Siderastrea*	April 1990	3.5	-13.1
sid8	Manzanillo	*Siderastrea*	April 1990	3.2	-11.3
dip	Manzanillo	*Diploria*	April 1990	4.8	-9.4
sid4	Pto. Viejo	*Siderastrea*	April 1990	4.6	-9.2
CP	Cahuita	*Porites*	Feb. 1992	3.7	-8.2
CS	Cahuita	*Siderastrea*	Feb. 1992	4.8	-11.3
CD	Cahuita	*Diploria*	Feb. 1992	4.3	-12.2
CAP	Cahuita	*Acropora*	Feb. 1992	3.2	-11.1
Banda					
Run1	Run	*Porites*	Oct. 1993	6.6	-11.2
Run2	Run	*Porites*	Oct. 1993	7.6	-12.1
Run3	Run	*Porites*	Oct. 1993	7.3	-12.7
Run4	Run	*Porites*	Oct. 1993	8.3	-10.5
Run5	Run	*Porites*	Oct. 1993	7.2	-10.0
Run6	Run	*Porites*	Oct. 1993	7.3	-12.2
LF1	Banda Api	*Porites*	May 1993	6.7	-12.4
LF2	Banda Api	*Porites*	May 1993	6.8	-11.8
LF3	Banda Api	*Porites*	May 1993	6.4	-12.1
LF4	Banda Api	*Porites*	May 1993	5.9	-10.7
LF5	Banda Api	*Porites*	May 1993	7.0	-11.3
LF6	Banda Api	*Porites*	May 1993	6.8	-11.2
LF7	Banda Api	*Porites*	May 1993	7.5	-12.1
LF8	Banda Api	*Porites*	May 1993	6.6	-12.2
LF9	Banda Api	*Porites*	May 1993	7.4	-10.5

[1] full genus and species names

Siderastrea radians
Diploria sp.
Acropora palmata
Porites astreoides – Cahuita
Porites lobata -- Banda

[2]date of collection

Table 4.2. Site Summary Statistics: $\delta^{15}N$ and $\delta^{13}C$

site	mean	std. deviation	min.	max.	N
$\delta^{15}N$					
Banda	7.0	0.6	5.9	8.3	15
Maldives	6.4	0.7	5.6	7.5	18
Jepara	5.5	0.7	4.8	7.0	11
Australia-in[1]	5.2	0.5	4.0	5.8	9
Australia-mid/out[2]	4.3	0.5	3.5	5.2	15
Zanzibar	4.3	0.5	3.2	5.4	21
Jamaica	3.1	0.8	1.7	5.4	26
Costa Rica	4.0	0.7	3.2	4.8	8
$\delta^{13}C$					
Banda	-11.5	0.8	-12.7	-10.0	15
Maldives	-11.6	0.8	-13.1	-10.4	18
Jepara	-12.7	0.7	-13.8	-11.8	11
Australia-in[1]	-15.1	1.1	-16.9	-13.0	9
Australia-mid/out[2]	-12.6	1.0	-14.1	-10.1	15
Zanzibar	-10.4	1.0	-12.7	-8.7	21
Jamaica	-11.7	1.3	-13.9	-9.8	26
Costa Rica	-10.7	1.7	-13.1	-8.2	8

[1] inner shelf reefs
[2] middle and outer shelf reefs

Note: No outliers have been removed for this statistical summary

statistical summaries). The average $\delta^{15}N$ of all corals studied is 4.8 ‰ while the average $\delta^{13}C$ is -11.8 ‰. $\delta^{15}N$ of all corals ranges from 1.7 ‰ to 8.3 ‰ while $\delta^{13}C$ ranges from -16.9 to -8.2 ‰. There is essentially no correlation between carbon and nitrogen isotopic values of coral tissue in this study (r= -0.056).

One-way ANOVA suggests that there are significant differences in the mean values of the reefs studied for both $\delta^{13}C$ and $\delta^{15}N$ ($p<<0.0001$ in both cases; Tables 4.3, 4.4). Statistically significant differences between individual reefs are identified in Tables 4.5 and 4.6. Inter-reef differences in $\delta^{13}C$ mean values are dominated by the lower values for Australia and Jepara corals and by the slightly heavier values of Zanzibar corals. Twenty-three of twenty-eight possible pairwise comparisons show significantly different mean $\delta^{15}N$ values. Corals collected from reefs in the Maldives and Banda have the highest $\delta^{15}N$ signatures. Corals from Jamaica are the most depleted in the heavy isotope of nitrogen. The remaining reefs tend to have an average $\delta^{15}N$ of approximately 4 to 5 ‰ (Fig. 4.3, Table 4.2). The observed variation in $\delta^{15}N$ is a significant proportion of total variation observed in the marine realm, excluding areas of intense denitrification or vent seeps (See Figure 5 in Owens, 1987). Corals from most of the reefs have similar mean $\delta^{13}C$ values (-10 to -12 ‰) while samples from Australia and Jepara show greater isotopic depletion. While mid- and outer shelf Australian corals are statistically indistinguishable there are trends within this grouping. Outer shelf corals tend to be enriched in the heavy isotope of both

Table 4.3. One-way ANOVA of coral tissue $\delta^{13}C$

Source	Sum-of-squares	DF	Mean-square	F-ratio	P
Reef	179.342	7	25.620	26.742	<<0.0001
Error	110.176	115	0.958		

Table 4.4. One-way ANOVA of coral tissue $\delta^{15}N$

Source	Sum-of-squares	DF	Mean-square	F-ratio	P
Reef	226.298	7	32.328	93.817	<<0.0001
Error	39.628	115	0.345		

Table 4.5. Results of the Bonferroni's pairwise comparison test for inter-reef differences in $\delta^{13}C$. NS indicates no significant differences; * indicates significant differences at least at the $P < 0.05$ level.

	Costa Rica	Jepara	Zanzibar	Maldives	Jamaica	Banda	Australian shelf inner	Australian shelf mid & outer
Costa Rica	----							
Jepara	*	----						
Zanzibar	NS	*	----					
Maldives	NS	NS	*	----				
Jamaica	NS	NS	*	NS	----			
Banda	NS	NS	*	NS	NS	----		
Aust.-inner	*	*	*	*	*	*	----	
Aust.-mid&out	*	NS	*	*	NS	*	*	----

Table 4.6 Results of the Bonferroni's pairwise comparison test for inter-reef differences in $\delta^{15}N$. NS indicates no significant differences; * indicates significant differences at least at the $P < 0.05$ level.

	Costa Rica	Jepara	Zanzibar	Maldives	Jamaica	Banda	Australian shelf inner	Australian shelf mid & outer
Costa Rica	----							
Jepara	*	----						
Zanzibar	NS	*	----					
Maldives	*	*	*	----				
Jamaica	*	*	*	*	----			
Banda	*	*	*	NS	*	----		
Aust.-inner	*	NS	*	*	*	*	----	
Aust.-mid&out	NS	*	NS	*	*	*	*	----

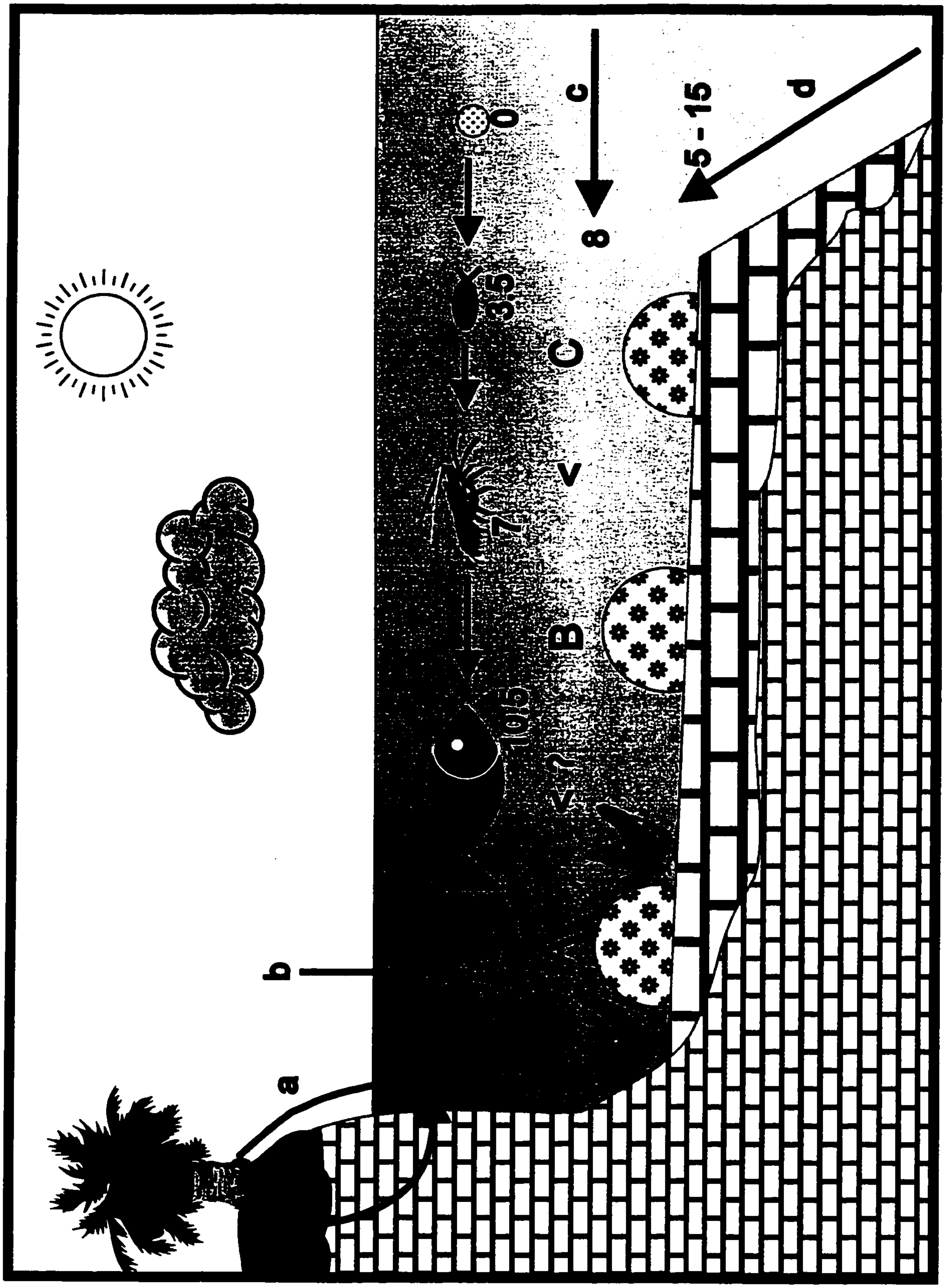
nitrogen and carbon relative to mid-shelf corals (average values of 4.7 ‰ versus 3.9 ‰ for nitrogen and -12 ‰ versus -13 ‰ for carbon).

It is difficult to address taxonomic effects in this data set since most of the corals are from a single species (Porites lobata) or genus (Porites). Inter-species differences in $\delta^{15}N$ of Jamaica corals are discussed in Chapter 3. These differences were found to be small compared to the inter-reef variation shown in Fig. 4.3. This is also true of Costa Rica corals (Table 4.1).

4.5 Discussion

The observed variation in $\delta^{15}N$ could reflect 1) differences in trophic status of corals on individual reefs, 2) differences in $\delta^{15}N$ of reef DIN or 3) factors such as light levels or concentrations of DIN which might affect the degree of fractionation during uptake or release of nitrogen. When all of the various reefs were sampled, it was believed that simple food chain nitrogen isotopic relationships would apply to corals and that $\delta^{15}N$ of source nitrogen would be the main influence on coral $\delta^{15}N$. The data presented in Muscatine and Kaplan (1994) and Chapter 3, however, suggest that corals, due to their symbiotic nature, exhibit complex nitrogen isotopic behaviour and strong fractionations. Although we lack sufficient data for many environmental factors (light, concentration of DIN or $\delta^{15}N$ of DIN), we can at least qualitatively address the potential magnitude and direction these influences might have on inter-reef coral $\delta^{15}N$ variability (Fig. 4.4).

Fig. 4.4 Diagram of selected aspects of the nitrogen cycle on reefs. A simple food chain is illustrated (diatom consumed by micro-zooplankton consumed by macro-zooplankton consumed by a fish -- organisms not to scale) illustrating stepwise enrichment up the food chain. Major inputs (a-d) of nitrogen are shown with arrows indicating direction of flux. Values adjacent to flux arrows give possible $\delta^{15}N$ of input in ‰. a) groundwater or fluvial inputs -- (value used is an average value for terrestrial/freshwater nitrogen -- see Table 2 and Fig. 5 in Owens, 1987) b) nitrogen fixation; c) advection -- a possible surface nitrate isotopic composition for the euphotic zone is given (Liu and Kaplan, 1989); d) upwelling -- lower isotopic values represent "normal" upwelled nitrate while higher values represent residual nitrate resulting from denitrification (Liu and Kaplan, 1989). Coral A is shown in a nutrient enriched, low light setting near to shore. The effect on nutrient fractionation is unknown but would likely result in coral tissue strongly depleted in the heavy isotope of nitrogen. Coral B is shown in a low light setting (no nutrient enrichment) and Coral C in a higher light setting (no nutrient enrichment). The <> signs indicate the expected relative nitrogen isotopic compositions of these three corals.


a
b
c
d
0
3.5
7
10.5
B
C
8
5 - 15

4.5.1 Trophic level

One of the most striking features of nitrogen isotope systematics in both marine and terrestrial food webs is a step-wise enrichment in $\delta^{15}N$ of organisms at higher trophic levels within a food web. This enrichment is on the order of 3.5‰ per step in the food chain, and is due to excretion of nitrogenous wastes, isotopically depleted relative to diet (DeNiro and Epstein, 1981; Minegawa and Wada, 1984). Given the fact that corals are mixotrophs, capable of occupying different trophic levels, it is tempting to suggest that the observed variability represents a gradient from autotrophy (Jamaica corals) to heterotrophy (Banda and Maldives corals). The range in $\delta^{15}N$ is indeed similar to that observed between primary producers (benthic algae) and high-level carnivores (e.g., barracuda) on reefs in the Bahamas and Turks and Caicos (Keegan and DeNiro, 1988).

The inter-reef variability in $\delta^{13}C$ is useful in addressing this possibility. Five of the eight reefs in this compilation have enriched mean $\delta^{13}C$ values (~ -10 to -12 ‰). Isotopically enriched carbon signatures in coral tissue have been interpreted as representing a more autotrophic diet (Land et al., 1975b; Muscatine et al., 1989; Risk et al., 1994) of isotopically heavy dissolved inorganic carbon (HCO_3^- ~ 1 ‰, e.g., McConnaughey, 1989; fractionation occurs relative to this source during zooxanthellar photosynthesis; Muscatine et al., 1989). In shallow high light conditions, ratios of photosynthesis to respiration in zooxanthellate

corals have been used to demonstrate that autotrophy could provide all of the organic carbon necessary for coral metabolism (e.g., Porter, 1985; Edmunds and Davies, 1986; Muscatine et al., 1989). Corals relying largely on a heterotrophic diet of zooplankton, should have much lighter $\delta^{13}C$ values, typical of zooplankton (~ -17 - -20 ‰; e.g. Land et al., 1975b; Yamamuro et al., 1995). The zooxanthellae of shallow water Jamaica corals have been shown to have similar $\delta^{13}C$ to the coral tissue in which they were contained and this has been interpreted as strong evidence for photoautotrophy (Muscatine et al., 1989). Among this group of enriched corals, the reason for significantly higher Zanzibar coral $\delta^{13}C$ is unclear.

Similarly, a significant proportion of these corals' nitrogen requirements could be derived from inorganic sources, via zooxanthellar uptake, assimilation and translocation. This has been demonstrated in the limited number of coral nitrogen budgets available (Bythell, 1988; Falkowski et al., 1993). In the case of Porites porites, at Discovery Bay, Jamaica, analysis of gut contents shows a general lack of zooplankton. Edmunds and Davies (1986) have suggested that this reliance on autotrophy may be a general strategy for this genus. In general, shallow reef corals have been found to be depleted in ^{15}N relative to zooplankton and azooxanthellate corals on the same reef (Yamamuro et al., 1992; Muscatine et al., 1994; Yamamuro et al., 1995; Eustace et al., 1995; Chapter 3), suggesting that DIN always provides a significant portion of a coral's nitrogen requirements.

Jepara and Australia corals are significantly depleted in ^{13}C relative to the other sites. This depletion is thought to be due to the importance of heterotrophic feeding on terrestrial carbon (Risk et al., 1994; Dunn, 1995). While these corals do not have the highest $\delta^{15}N$ signatures expected of marine heterotrophs, their nitrogen isotopic compositions are likely influenced by heterotrophic feeding on dissolved and particulate terrestrial organic matter. The influence of terrestrial organic matter across the Great Barrier Reef, Australia is thought to extend to the mid-shelf (Risk et al., 1994). Outer shelf corals may have a fully autotrophic diet (Risk et al., 1994; Sammarco et al., in preparation).

At the time of collection, the Banda and Maldives corals were experiencing very high irradiance in waters with very low turbidity. In these high light conditions it is unlikely that these isotopically enriched Porites lobata colonies were deriving more of their nitrogen from heterotrophy than the most depleted corals from Jamaica (which were collected in much lower light conditions -- see below). It is more likely that corals from the majority of the studied reefs relied on autotrophy for a substantial proportion of their nutrient requirements. Therefore, for these corals, no trophic level effect is indicated.

4.5.2 Concentration of DIN

Concentration of DIN is an environmental factor which could potentially affect coral nitrogen fractionation (Fig. 4.4). It has been suggested that with higher external concentrations of nitrogen, fractionation potential during zooxanthellar uptake might be increased, leading to lighter coral $\delta^{15}N$ (Muscatine

and Kaplan, 1994; Chapter 3). This assumes that corals are not actively controlling their internal DIN concentrations, independent of external concentrations. Conversely, if corals experience net excretion, rather than conservation (e.g., Muscatine and D'Elia, 1978), of isotopically depleted ammonia under nutrient enrichment conditions, tissues might be enriched in ^{15}N relative to diet (cf. DeNiro and Epstein, 1981; Minegawa and Wada, 1984). In severe cases of nutrient enrichment, it appears that the coral/zooxanthellae relationship is disrupted, such that translocation to the host is reduced (Jokiel et al., 1994). Under these conditions it is possible that the coral may have to supplement its diet through heterotrophy, which again could affect the nitrogen isotopic balance. Given this uncertainty, since the effect of nutrient enrichment on coral tissue $\delta^{15}N$ has not been tested experimentally, it is difficult to evaluate this factor.

Nutrient data were not collected at the time of coral sampling. It is difficult therefore, to appraise the possible magnitude or even the direction of fractionation that might be associated with varying nitrogen levels. It is interesting to note however, that several reefs (Banda, Maldives, Zanzibar, Costa Rica (Cahuita)) and outer shelf Australia Reefs (Tables 4.1, 4.2, 4.7) include sites which are not eutrophied. None of the seas surrounding these reefs has mean annual surface nitrate concentrations greater than 2 μM (Conkright et al., 1994) yet their coral $\delta^{15}N$ signatures differ significantly.

Table 4.7. Maldives and Zanzibar: Affected Versus Reference Sites

Site	$\delta^{15}N$		$\delta^{13}C$		
	mean	std. deviation	mean	std. deviation	N
Maldives					
affected	7.2	0.3	-11.9	0.9	7
reference	5.8	0.5	-11.4	0.7	9
Zanzibar					
affected	4.2	0.3	-9.9	0.8	10
reference	4.2	0.5	-10.7	1.0	9

Note: This comparison does not include all values used for Figure 4.2 and Table 4.2.

Similarly, the degraded sites, showing evidence of nutrient enrichment and eutrophication (Zanzibar, Maldives, Costa Rica (Manzanillo) and Jepara) cover a significant proportion of the entire range of $\delta^{15}N$ observed in this study (Fig. 4.3, Tables 4.1, 4.2, 4.7). Another way to assess the magnitude of $\delta^{15}N$ variability caused by nutrient concentration would be to examine the differences between reference and affected sites in some of the individual reefs studied (e.g., Risk et al., 1993; Dunn, 1995). The results of these studies are not consistent. In the Maldives, a site receiving anthropogenic nutrient has somewhat enriched coral $\delta^{15}N$, while in Zanzibar no significant differences were found (Table 4.7). In both the Maldives and Zanzibar nutrient enrichment situations, it is difficult to assess the effect nutrient enrichment may have on $\delta^{15}N$, because of opposing isotopic effects. The presence of an isotopically heavy nutrient source may mask increased fractionation due to higher nutrient concentrations or lower light levels resulting from shading by benthic algae or increased turbidity due to higher productivity in the water column (Dunn, 1995)

Water turbulence has been demonstrated to affect the rate of ammonium uptake in corals (Atkinson et al., 1994). By affecting internal DIN concentrations water turbulence could affect coral $\delta^{15}N$. In this study however, Banda and Jamaica corals are both collected from the most turbulent fore-reef environments and have the extreme high and low nitrogen isotopic compositions respectively.

No consistent patterns emerge which suggest that nutrient concentration is a dominant factor controlling inter-reef variability in $\delta^{15}N$. Measurement of reef water DIN concentrations at the time of coral collection, as well as controlled experimental work would be useful to address this problem. It would be important to measure both ammonium and nitrate concentrations, as the actual species of nitrogen used by corals still remains contentious (e.g., Muscatine and D'Elia, 1978; Wilkerson and Trench 1985).

4.5.3 $\delta^{15}N$ of reef DIN

Differences in the isotopic composition of reef DIN is one way to account for the observed differences in coral $\delta^{15}N$. Unfortunately, few measurements of $\delta^{15}N$ of DIN exist for the oceans in general (Liu and Kaplan, 1989) and reef measurements are rare. None of the sampled reefs have been studied for $\delta^{15}N$ determinations of DIN. As in the case of nitrogen concentration, it is even uncertain as to what DIN species might show the strongest relationship to coral $\delta^{15}N$.

$\delta^{15}N$ of corals in general, fall within or below the typical lower range for marine nitrate (see compilations in Wada et al, 1975; Owens, 1987) and within the known range for marine ammonium (Wada et al, 1975). Despite the fact that the corals were all collected from shallow water, there is the potential for fractionation during DIN uptake and assimilation into the zooxanthellae or host (beyond that associated with initial diffusion or transportation across the

plasmallema; Muscatine and Kaplan, 1994; Chapter 3). In addition, some part of the nitrogen requirements for all of these corals is likely met by predation on zooplankton (or assimilation of DOM) which could be isotopically distinct relative to DIN.

Despite these difficulties it is possible to consider factors which might cause enrichment or depletion of ^{15}N in reef waters (Fig. 4.4), which could in turn affect coral $\delta^{15}N$. In particular, it is instructive to look at the $\delta^{15}N$ signatures of various processes which supply nitrogen to reefs and processes by which nitrogen can be lost from a reef system. The main sources of new DIN (usually as nitrate, cf. Dugdale and Goering, 1967) to a reef include advection, upwelling, groundwater/fluvial inputs, nitrogen fixation and endo-upwelling (D'Elia, 1988). Nitrogen can be lost from the reef by advection, as PON sedimented away from the reef or buried in reef sediments, and by denitrification in anaerobic reef environments (see reviews in Wiebe, 1985; D'Elia, 1988).

Some of these phenomena could result in distinct isotopic signatures for reef DIN pools. Nitrogen fixation, which can be important in coral skeletons (Shashar et al., 1995) and on reefs as a whole (e.g., Wilkinson et al., 1994), involves little fractionation, producing organic matter with $\delta^{15}N$ close to that of atmospheric nitrogen (0 ‰). Upwelling or endo-upwelling (Rougerie and Wauthy, 1993) has the potential to deliver nitrogen to a reef with isotopic values distinct from that of surface waters. Normally, nitrate from deep waters (>200 m) is

depleted in $\delta^{15}N$ relative to surface waters (Liu and Kaplan, 1989). In areas where deep waters are suboxic, however, denitrification can occur. Denitrification involves very large fractionations, up to 40‰ (Cline and Kaplan, 1975; Liu et al, 1987). Residual nitrate will therefore be strongly enriched , and has values up to 19 ‰ (see compilation in Liu and Kaplan, 1989). Groundwater/fluvial DIN has a wide range of $\delta^{15}N$ values which may be isotopically distinct relative to marine sources (see Table 2 and Fig. 5 in Owens, 1987). The effect of advection will depend on the background isotopic composition of surrounding water masses.

DIN losses from reefs are more difficult to characterise isotopically. Some important processes, such as denitrification (e.g., Wiebe, 1985), may involve large fractionations. The partitioning of nitrogen between organic and inorganic sources and between different dissolved nitrogen species will also be important.

It is interesting that in general, Indo-Pacific reefs seem to be enriched relative to Caribbean reefs (Fig. 4.3). Liu and Kaplan, 1989, proposed that the $\delta^{15}N$ of nitrate in the Indo-Pacific would be enriched relative to the Atlantic because denitrification is much more intense in the Indo-Pacific oceans (Deuser, 1975). Measurements of subsurface nitrate confirmed this for the central and western Pacific and the northern Atlantic (Liu and Kaplan, 1989). The general spread in the data (Fig. 4.3) may therefore reflect the background $\delta^{15}N$ of marine DIN in surrounding oceans. Analyses from other studies tend to confirm the

above observation. Eustace et al. (1995) found an average $\delta^{15}N$ value of 2.4 ‰ for Discovery Bay (Jamaica) corals and a Porites porites from Turks and Caicos in the Caribbean yielded a value of 3.7‰ (Keegan and DeNiro, 1988). Corals from Palau and Ishigaki Island in the Western Pacific have higher values of about 4 - 6 ‰ (see Fig. 2 in Yamamuro et al., 1995). There is still considerable overlap, however, in reefs from both areas (e.g., Costa Rica and Zanzibar corals, Fig. 4.3).

The Maldives site is closest to an area of large scale denitrification (Arabian Sea. Deuser, 1975). While it is over 1000 km away from an actual source of suboxic waters (<0.1 ml/l, Liu and Kaplan, 1989), enriched nitrate from centres of denitrification can be transported such distances (Liu and Kaplan, 1989). Upwelling, or endo-upwelling through Maldivian atolls, could be a source of any such isotopically enriched nutrient.

Banda reef corals (most enriched in ^{15}N), however, are near no large source of denitrification and occur in well ventilated waters (Sukarno et al., 1984; Levitus et al., 1994). The nearest potential source of nitrate affected by denitrification is Kau Bay (Halmahera, Indonesia), approximately 700 km north of Banda. Deeper waters of this fjord-like bay are suboxic or periodically anoxic and are probably flushed at least annually (Van der Weijden et al., 1989; Van Aken and Verbeek, 1989). Banda corals collected in October were in a water column containing abundant marine snow. Denitrification may occur in anoxic zones associated with organic flocculations of marine snow (Alldredge and

Cohen, 1987), however, under these conditions all of the nitrate in the microcosm might experience denitrification leading to no net fractionation (Wada et al., 1975).

It has been suggested that outer shelf Australian reefs may receive inputs of enriched nitrogen upwelled from an oxygen minimum zone located just off the shelf (Wyrtki, 1962; Andrews and Gentien, 1982; Sammarco et al., in preparation). If localised denitrification from this zone is affecting shelf edge reef corals it is not producing overly enriched tissue signals compared to other sites (Fig. 4.3). It is possible that inputs from nitrogen fixation on these reefs (Willkinson et al, 1984) could have an opposing effect.

Little is known about the importance of nitrogen fixation at Discovery Bay, although predominance of this process might explain the low nitrogen isotopic compositions at this site. It has been suggested that groundwater inputs are likely a more significant source of nutrients to this reef and that in general nitrogen fixation will be more significant on low islands without significant terrestrial input (D'Elia et al., 1981). Maldivian corals would be the closest to this situation, yet they have amongst the most enriched nitrogen isotopic signatures. Nitrogen fixation is thought to be a dominant source of nitrogen for mid-shelf Australian reefs (Wilkinson et al., 1984; Sammarco et al., in preparation). These reefs do have amongst the lowest $\delta^{15}N$ of all the sites included in this compilation (Fig. 4.3, Table 4.2).

Perhaps the ground water inputs at Discovery Bay may ultimately prove to be a depleted source of nitrogen, helping contribute to the observed depletion in coral ^{15}N. It is interesting to note, however, that Discovery Bay waters may not be strongly depleted in $\delta^{15}N$ relative to other sites. A single sample of Porites furcata is enriched relative to other Jamaica samples ($\delta^{15}N$ = 5.4 ‰). Based upon polyp size and surface area (Porter, 1976) this coral might be expected to be the most autotrophic of all the species examined, and hence may be the least fractionated with respect to $\delta^{15}N$ of DIN.

Corals collected from Banda Api in the Banda islands (Table 4.1) were collected from a lava flow deposited in the sea in 1988. These corals are near sites of active hydrothermal vents, that have been proposed as a nutrient source to nearby corals (Tomascik et al., 1996). This source of terrestrial nitrogen might also be isotopically distinct, leading to the enriched nitrogen signatures of Banda corals. Corals from Run Island however, are similarly enriched and experience no DIN input from hydrothermal vents (Table 4.1). Corals from inshore reefs of Australia, on the other hand, do appear to utilise significant amounts of isotopically distinct terrestrial nitrogen and carbon (Fig. 4.3, Table 4.2; Risk et al., 1994; Sammarco et al., in preparation), some of which will be dissolved inorganic species. The isotopic composition of anthropogenic dissolved nutrients affecting contaminated reefs (e.g., Jepara; Dunn, 1995) will almost certainly influence coral $\delta^{15}N$.

It is impossible at this point to determine how much of the variation observed in Fig. 4.3 is due to variation in the $\delta^{15}N$ of average oceanic DIN surrounding the reef or from inputs or losses of isotopically distinct sources of nitrogen. Analysis of $\delta^{15}N$ of reef DIN and a better understanding of coral nitrogen metabolism and fractionation is required before we can determine how much of the variation in coral $\delta^{15}N$ is due to $\delta^{15}N$ of reef nitrogen.

4.5.4 Light

Light availability has been suggested as a strong influence on the degree of nitrogen fractionation during uptake and assimilation of DIN by zooxanthellate corals (Muscatine and Kaplan, 1994; Chapter 3). It has been proposed that under conditions of high irradiance/photosynthesis, corals exhibit minimal fractionation because all nitrogen in the internal DIN pool is utilised. Under lower light conditions, fractionation by host and/or zooxanthellae during nitrogen DIN assimilation can be more fully expressed (Fig. 4.4; Chapter 3). No in-situ light data are available for the time periods up to and including collection of corals on each of the reefs studied. We can nevertheless look at factors such as seasonal insolation and water clarity to assess whether light might have had an influence on the spread in isotopic values in Fig. 4.3.

The pattern of variation in $\delta^{15}N$ demonstrated in Fig. 4.3 has some aspects which might correlate with light availability. The first factor to examine is light availability as affected by season and latitude of each site. Banda and

Maldives corals were collected in spring and fall months, during periods of low cloud and rainfall (Mike Risk and Tomas Tomascik, personal observation). These near equatorial sites therefore receive high insolation during these periods (theoretical irradiance of ~58 $Em^{-2}day^{-1}$, ignoring cloud cover and atmospheric haze).

The higher latitude Jamaica corals were collected during the winter months (~ 40 $Em^{-2}day^{-1}$), which experience significant afternoon cloudiness which can drastically decrease insolation to the reef (see Porter, 1985 and Sandeman, 1996 for actual surface irradiances). Australian corals were also collected during the austral winter, but under relatively clear skies (Mike Risk, personal observation). Based on latitude and season, Zanzibar and Costa Rica corals theoretically should have experienced relatively high irradiance. Cloud cover, however, may have been a factor for these sites as well (WeatherDisc Associates, 1990 and personal observations). At Jepara, water clarity is extremely low and is likely the more important factor.

Water clarity varies significantly between the various sites. An increase in attenuation from $0.03m^{-1}$ (e.g. Sargasso Sea; Kirk, 1983) to $0.1m^{-1}$ (e.g., Discovery Bay, Sandeman, 1996) at 5 m depth, will result in an extra 25% reduction in light availability relative to surface irradiance (Bougeur-Lambert-Beer Law $I_z=I_oe^{-kz}$, I=irradiance ($Em^{-2}day^{-1}$), z=depth (m), k= attenuation coefficient (m^{-1})). This reduction in light availability is similar in magnitude to that found in the surface irradiance difference between Banda and Jamaica (given the

seasons of collection, see above). We do not have attenuation coefficients for each of the reefs, but visibility has been qualitatively rated (Mike Risk). For example, Banda and Maldives have the greatest visibility (Banda visibility is somewhat reduced in October by the presence of marine snow). This, in concert with high surface irradiance, should produce the highest light availability for these two reefs. Australian shelf edge reefs also have high visibility, while the Discovery Bay reef is among the lowest. The high value for a single Porites furcata sample, known to be highly autotrophic (Edmunds and Davies, 1986), may reflect high $\delta^{15}N$ values for Jamaica reef waters, and might imply that most of the corals on this reef are actually fractionating nitrogen quite strongly. Jepara had by far the lowest visibility. It has been suggested that visibility is so low that the corals were likely feeding dominantly as heterotrophs, possibly accounting for their enriched tissue $\delta^{15}N$ (Dunn, 1995).

Thus, it is possible to discount the Jepara case and to say that the relative $\delta^{15}N$ values of the various reefs are consistent with the amount of insolation individual corals might have been receiving. Assuming that light is a factor, how much of the observed inter-reef $\delta^{15}N$ variation could be due to light-dependent fractionation? Comparing results for Zanzibar and Jamaica corals from Chapter 3, it is possible to estimate that an approximate 1.5-2‰ decrease in $\delta^{15}N$ accompanies a decrease in insolation of approximately 30 $Em^{-2}day^{-1}$. Assuming the relationship between light and coral $\delta^{15}N$ can be linearly

extrapolated to higher light levels, we might expect a comparable isotopic effect in the shallow water corals from these various reefs (assuming a 20 $Em^{-2}day^{-1}$ difference in theoretical surface irradiance is exaggerated by factors such as water clarity and cloud cover). This would represent a significant proportion of the observed variation (Fig. 4.3). It would be interesting to see if corals from these various reefs all contain the same strain of zooxanthellae. Changes in zooxanthellar composition could affect light dependent fractionation factors (Chapter 3; Eustice, personal communication).

All else being equal (e.g. nutrient concentration and $\delta^{15}N$ of source DIN), increased productivity on a reef could lead to isotopic enrichment of the total biomass and DIN (assuming the system is partially closed; cf. Altabet and Francois, 1994). Light could be a factor which causes variation in total productivity on reefs (assuming light is limiting to productivity) and hence, may have a small effect on the nitrogen isotopic composition of the DIN pool and primary producers on the reef (including corals). It is even possible that when nitrogen demand is high, under high light conditions, corals may use additional enriched species of DIN (e.g. nitrate in addition to ammonium; in some corals at least, ammonium appears to be the preferred species (e.g., Muscatine and D'Elia, 1978).

There is no evidence in the data set suggesting that light influences inter-reef variation in $\delta^{13}C$. It is quite likely, however, that at the higher irradiances experienced by fully autotrophic corals, nitrogen isotopic fractionation

is more sensitive to light availability/photosynthesis than carbon isotopic fractionation. Given the large flux of carbon, relative to nitrogen, fixed by the zooxanthellae and translocated to the host (e.g., Falkowski et al., 1984), it is probable that dissolved carbon will tend to be a more limiting substrate (within the coral tissue pool) in terms of fractionation potential.

The influence of light on coral fractionation of nitrogen must be assessed with caution. This effect has only been studied for the limited range of light variability occurring from the surface to 30 m depth at Discovery Bay, Jamaica. during winter months, and over three single coral heads (samples were analysed from tops to bases) from Zanzibar (Chapter 3). It is not known how coral $\delta^{15}N$ will respond to higher levels of light availability, within or between reefs. Nevertheless, the pattern of variability in Fig. 4.3 suggests that light is potentially an important factor in inter-reef $\delta^{15}N$ variation. If so, in-situ measurements will be necessary to understand the relationship, as factors such as cloudiness and turbidity will be very important. This hypothesis also implies that some of the variation shown in Fig. 4.3 could be seasonal in origin. It is important to note that since it is the effect of light on photosynthesis which determines the degree of fractionation (Chapter 3), any factor which affects photosynthesis could potentially affect coral $\delta^{15}N$.

4.6 Conclusions

This compilation of $\delta^{15}N$ data from reefs throughout the tropics suggests that significant inter-reef variability does exist. Coral tissue $\delta^{15}N$ would therefore appear to merit further study. It is unknown at this time if the approximately 6‰ spread in $\delta^{15}N$ reflects a 6‰ spread in $\delta^{15}N$ of dissolved nitrogen on the reefs or whether much of the variation is due to fractionation (of a source with limited range of $\delta^{15}N$) associated with factors such as light and/or concentration of DIN. If fractionation was more important it would suggest a minimum $\delta^{15}N$ value of the DIN source of about 7-8 ‰, which is within the range for marine nitrate in surface waters (Liu and Kaplan, 1989). It would also suggest an average fractionation of 3-4 ‰ based on an average coral tissue value of approximately 4-5 ‰ (Fig. 4.3). It is more likely, however, that all of the factors mentioned in the discussion act synergistically in a complex manner to produce the observed variation. $\delta^{15}N$ of waters surrounding reefs will be modified by localised inputs of nitrogen to reefs. Corals will in turn fractionate the available nitrogen to some degree, depending on the prevailing environmental conditions.

The magnitude of variability caused by each of these factors will remain unknown until all of the possible factors are assessed both in field and controlled laboratory studies. The results do suggest, however, that corals may not be conservative tracers of nitrogen under all conditions and care must be taken in interpretation of tissue $\delta^{15}N$ measurements. When shallow water,

autotrophic corals are collected under high irradiance conditions, along environmental gradients in which light and concentration of DIN are relatively constant, corals will likely prove to be excellent tracers of reef DIN. In cases where nutrient input changes the light and concentration regime, the various isotopic effects described above may tend to counteract one another (Dunn, 1995). If we can learn how to account for the affect of fractionation on coral tissue $\delta^{15}N$, important information might be obtained regarding both the $\delta^{15}N$ of source nitrogen and coral nitrogen metabolism.

Chapter 5: Isolation of photosynthetic effects in coral skeletons from Jamaica and Zanzibar

5.1 Abstract

$\delta^{13}C$ signals of light availability/photosynthesis in coral skeletons from Jamaica and Zanzibar are masked by strong kinetic isotope effects. A simple data transformation has been developed to correct for the presence of these disequilibrium kinetic influences. The resulting transformed isotopic data show significant correlations with associated coral tissue $\delta^{13}C$ and with depth/light availability. These relationships demonstrate the value of the transformation and suggest that transformed skeletal data faithfully record the carbon isotope effect associated with photosynthesis. Possible applications of these transformations are discussed.

5.2 Introduction

Measurement of stable isotopic ratios of carbon and oxygen in coral skeletons has been frequently employed to reconstruct environmental conditions on modern and ancient tropical reefs. $\delta^{18}O$ of coral skeletons can provide information on sea surface temperature (SST) and the oxygen isotopic composition of seawater (Weber and Woodhead, 1972). $\delta^{13}C$ of coral skeleton reflects patterns of light availability associated with the seasonal cycle of insolation, depth and cloudiness (Weber and Woodhead, 1970; Weber, 1974; Weber et al., 1976; Goreau, 1977a,b; Fairbanks and Dodge, 1979; Swart and

Coleman; Weil et al, 1981; Swart, 1983; Patzold, 1984; McConnaughey, 1989a; Carriquiry, 1991; Cole et al., 1993; Carriquiry et al., 1994) and can be used to evaluate the status of the symbiotic relationship between corals and their endosymbiotic algae (zooxanthellae) (Carriquiry et al., 1994). Carbon isotopic variation is also often used alone or in conjunction with coral density banding to establish chronology in coral skeletons (e.g., Cole and Fairbanks, 1990; Shen et al.. 1992; Carriquiry et al., 1994).

Reconstruction of important climatic phenomena such as the El Niño/Southern Oscillation (ENSO) provides an excellent example of the usefulness of stable isotopic measurements of coral skeletons. $\delta^{18}O$ of coral skeleton can provide information on the often catastrophic temperature increases associated with ENSO events (e.g., Druffel et al., 1985; Carriquiry et al., 1988, 1994; McConnaughey, 1989a; Wellington and Dunbar, 1995). In areas where temperature does not change significantly during ENSO events (e.g., mid Pacific), but where rainfall patterns are strongly altered, $\delta^{18}O$ of coral skeleton can be used to reconstruct changes in the isotopic composition of the surrounding water mass (Cole and Fairbanks, 1990; Cole et al., 1993). Carriquiry et al. (1994) have used carbon isotope measurements in coral skeletons from Isla del Caño, Costa Rica, to reconstruct patterns of coral bleaching (loss of zooxanthellae) associated with SST rise at the time of the largest El Niño this century (1982-1983 ENSO).

The use of stable isotopes of carbon and oxygen in coral skeletons can be confounded by various disequilibrium effects. Corals do not precipitate their skeleton in isotopic equilibrium with surrounding seawater. Coral skeletal $\delta^{18}O$ and $\delta^{13}C$ are consistently depleted in the heavy isotopes of oxygen and carbon relative to equilibrium (Weber and Woodhead, 1970, 1972; Weber, 1974; McConnaughey, 1989a). In the case of oxygen isotopes, it has been suggested that this offset from equilibrium is constant within a given coral genus (Weber and Woodhead, 1972). This means that while paleotemperature equations developed for molluscs (which precipitate their skeleton in equilibrium with seawater; Epstein et al., 1953) cannot be applied to corals, similar relationships, nearly parallel to the mollusc paleotemperature curve, can be employed. This offset from equilibrium has been termed an isotopic "vital effect" (Weber and Woodhead, 1972).

McConnaughey (1989a,b) has recently argued that vital effects are manifestations of kinetic isotope effects associated with the hydration and hydroxylation of carbon dioxide during coral skeletogenesis. These kinetic isotope effects result in simultaneous isotopic depletion of carbon and oxygen (relative to isotopic equilibrium), with carbon being approximately three times more depleted in its heavy isotope than oxygen. In general, more rapidly growing/calcifying parts of coral skeletons seem to show greater depletions (i.e. stronger kinetic isotope effects; see Fig. 13 in McConnaughey, 1989a). If the most rapidly extending primary growth axis of the coral is analysed, variation in

kinetic isotope effects will be minimised, and coral paleotemperature equations can be applied (McConnaughey, 1989a; see also Land et al., 1975a).

Superimposed on kinetic isotope effects is a carbon isotope effect associated with photosynthesis (i.e. metabolic effects; McConnaughey, 1989a). The prevailing hypothesis is that photosynthesis preferentially removes the light isotope of carbon from the internal pool of dissolved inorganic carbon (DIC) while respiration enriches the pool in the light isotope (Weber and Woodhead, 1970; Weber, 1974; Weber et al., 1976; Goreau, 1977a,b; Swart, 1983). In corals in which zooxanthellae are photosynthesising rapidly, the skeleton will tend to be enriched (heavier) compared to those in which photosynthesis is less important to coral diet. Evidence for this comes from several studies showing trends towards depletion in ^{13}C with depth or during periods of low insolation (e.g., Weber et al., 1976; Land et al., 1975a; Swart and Coleman, 1980; Carriquiry, 1991; Carriquiry et al. 1994). Studies contradictory to this prevailing hypothesis exist (Erez, 1977, 1978; Swart et al. 1996), but potential explanations for these discrepancies have been presented (McConnaughey, 1989a; Swart et al., 1996). $\delta^{18}O$ of coral skeleton is apparently not directly affected by photosynthesis (McConnaughey, 1989a).

In this paper, we analyse isotopic data from coral skeletons collected in Jamaica and Zanzibar to see if the presence of the photosynthetic effects can be isolated from the skeletal kinetic isotope effects. We attempt to transform the data to correct for the presence of kinetic isotope effects then

compare the transformed data to $\delta^{13}C$ of associated coral tissue and light availability.

5.3 Materials and Methods

Collection of samples has been described in detail in Chapter 3. Discovery Bay, Jamaica samples consisted of near-horizontal chips of skeleton and adhering tissue from three species: Montastrea annularis, Agaricia agaricites and Porites astreoides. Samples were collected at depths of 1m, 5m, 12m, 22m and 30 m (Table 5.1). Zanzibar samples consisted of similar chips collected from the tops to the bases of three large Porites lobata colonies (Table 5.2).

Each sample was divided into two aliquots to facilitate isotopic measurements of tissue and skeleton. In the case of Zanzibar corals, this meant two adjacent chips from each depth were utilised. For Jamaica corals, the centre of each chip was used for skeletal analyses while surrounding material was used for tissue measurements.

Each tissue sample was prepared following LeBlanc et al. (1989) and Risk et al. (1994). Excess skeletal material was removed with a diamond circular saw, with particular attention paid to removing the endolithic algae layer beneath the surface of the skeleton. Samples were dissolved in < 1 M HCl to free the tissue/zooxanthellae. The resulting tissue mat was thoroughly rinsed in distilled water, centrifuged, then freeze-dried to ensure preservation. All tissue samples represent combined tissue and zooxanthellae.

Table 5.1. Jamaica carbon isotope data

SAMPLE[1]	$\delta^{13}C$ (‰) TISSUE	DEPTH (m)	Λ PAR[2] (%)	GENUS	$\delta^{13}C$ (‰) SKELETON	$\delta^{18}O$ (‰) SKELETON	$\delta^{13}C$ (‰)[3] TRANSFORMED
ap1	-12.7	1	9	*Porites*	-3.1	-4.4	-2.3
ap2	-10.2	1	9	*Porites*	-1.5	-4.3	-0.9
ap3	-10.7	1	9	*Porites*	-1.6	-4.3	-1.2
aag	-9.9	1	9	*Agaricia*	-3.5	-4.0	-3.9
1p1	-9.9	1	9	*Porites*	-2.4	-3.9	-1.7
1ag2	-12.3	1	9	*Agaricia*	-2.3	-3.5	-3.0
1ag3	-13.1	1	9	*Agaricia*	-2.8	-3.9	-2.2
1ma1	-10.8	1	9	*Montastrea*	-1.4	-4.2	0.2
1ma2	-9.8	1	9	*Montastrea*	-1.2	-4.0	-0.3
bp1	-10.7	5	36	*Porites*	-1.3	-3.9	-1.9
bp2	-11.0	5	36	*Porites*	-0.3	-3.3	-2.7
bag1	-13.9	5	36	*Agaricia*	-4.0	-4.4	-3.1
bag2	-11.3	5	36	*Agaricia*	-2.1	-4.0	-2.4
5p1	-12.7	5	36	*Porites*	-2.1	-3.7	-2.2
5p2	-11.7	5	36	*Porites*	-1.3	-3.4	-2.1
5p3	-10.5	5	36	*Porites*	-2.3	-4.1	-1.2
5p4	-12.2	5	36	*Porites*	-2.9	-4.4	-0.8
5ag1	-13.9	5	36	*Agaricia*	-3.0	-3.8	-2.7
5ag2	-13.0	5	36	*Agaricia*	-3.7	-4.2	-2.3
5ag3	-12.9	5	36	*Agaricia*	-1.9	-2.6	-5.2
5ag4	-12.5	5	36	*Agaricia*	-2.4	-4.0	-1.5
5ma1	-11.7	5	36	*Montastrea*	-3.6	-4.3	-1.9
5ma2	-12.3	5	36	*Montastrea*	-3.1	-4.4	-1.0
5ma3	-10.0	5	36	*Montastrea*	-0.3	-3.4	-1.1
5ma4	-12.8	5	36	*Montastrea*	-2.6	-4.2	-1.1

Table 5.1. Jamaica carbon isotope data (continued)

SAMPLE[1]	$\delta^{13}C$ (‰) TISSUE	DEPTH (m)	Λ PAR[2] (%)	GENUS	$\delta^{13}C$ (‰) SKELETON	$\delta^{18}O$ (‰) SKELETON	$\delta^{13}C$ (‰)[3] TRANSFORMED
cp	-13.5	12	62	*Porites*	-2.6	-4.1	-2.7
cag1	-15.5	12	62	*Agaricia*	-4.5	-4.5	-3.5
cag2	-13.4	12	62	*Agaricia*	-3.1	-4.0	-3.5
cag3	-14.4	12	62	*Agaricia*	-3.4	-3.9	-4.1
12p1	-10.9	12	62	*Porites*	-2.0	-4.0	-1.1
12ag1	-13.7	12	62	*Agaricia*	-3.8	-4.1	-2.7
12ag3	-14.2	12	62	*Agaricia*	-0.3	-3.0	-2.4
12ag4	-13.4	12	62	*Agaricia*	0.9	-2.8	-1.9
12ma1	-12.4	12	62	*Montastrea*	-2.3	-4.5	0.1
12ma2	-12.9	12	62	*Montastrea*	-1.1	-4.0	-0.2
dp	-13.4	22	77	*Porites*	-0.8	-3.5	-2.7
dag1	-13.3	22	77	*Agaricia*	-4.1	-4.3	-3.4
dag2	-14.0	22	77	*Agaricia*	-2.3	-3.8	-3.1
dag3	****	22	77	*Agaricia*	-4.2	-4.0	-4.4
dag4	-13.2	22	77	*Agaricia*	-2.9	-4.3	-2.4
22p2	-13.3	22	77	*Porites*	-2.8	-4.2	-1.2
22p3	-14.3	22	77	*Porites*	-2.2	-3.7	-2.3
22ag2	-14.8	22	77	*Agaricia*	-2.2	-2.9	-4.6
22ag3	-14.7	22	77	*Agaricia*	-2.1	-3.0	-4.2
22ma1	-13.9	22	77	*Montastrea*	0	-3.1	-1.9
ep	-14.1	30	82	*Porites*	-3.0	-4.1	-3.0
ema1	-13.8	30	82	*Montastrea*	-1.9	-3.8	-2.8
ema2	-12.0	30	82	*Montastrea*	-4.1	-4.6	-2.6
30p1	-12.9	30	82	*Porites*	-1.8	-3.5	-2.3
30p2	-12.4	30	82	*Porites*	-3.3	-4.3	-1.6
30ag1	-13.1	30	82	*Agaricia*	-2.2	-3.2	-3.8

Table 5.1. Jamaica carbon isotope data (continued)

SAMPLE[1]	$\delta^{13}C$ (‰) TISSUE	DEPTH (m)	Λ PAR[2] (%)	GENUS	$\delta^{13}C$ (‰) SKELETON	$\delta^{18}O$ (‰) SKELETON	$\delta^{13}C$ (‰)[3] TRANSFORMED
30ag2	-14.1	30	82	*Agaricia*	-3.0	-3.1	-4.7
30ag3	-12.7	30	82	*Agaricia*	-2.5	-3.5	-3.1
30ma2	-12.6	30	82	*Montastrea*	-2.3	-4.1	-1.1
30ma3	-12.0	30	82	*Montastrea*	-1.4	-3.9	-0.8

1 - some samples (e.g.,1ag1) were not analysed due to partial bleaching or contamination with filamentous algae or bioeroding sponges.
- samples names beginning with letters were collected in January, 1993, whereas sample names starting with numbers were collected in January, 1994.

2 - decrease in PAR as a percentage of surface irradiance; values are calculated from PAR data given in Fig. 3.1. -- e.g., at 1 m depth % of surface PAR is 91, therefore decrease in PAR is 9 %.

3 - please note that small discrepancies between transformed data in this table relative to the formula given in the text are due to rounding errors (corections were originally done on data to three decimal places)

***sample lost during preparation

Table 5.2. Zanzibar carbon isotope data

SAMPLE	DEPTH (M)	TISSUE δ^{13}C (‰)	SKEL δ^{13}C (‰)	SKEL δ^{18}O (‰)	TRANSFORMED δ^{13}C (‰)
Head 1					
GH1105	1.75	-11.9	-2.3	-5.2	-1.1
GH1106	2.00	-11.9	-2.2	-4.6	-2.8
GH1108	2.25	-14.0	-3.7	-4.7	-4.0
GH1107	2.50	-13.3	-2.8	-4.8	-2.8
GH115	2.75	-12.2	-3.5	-4.9	-3.2
GH1116	3.00	-13.6	-1.4	-4.3	-2.9
Head 2					
GH2112	1.50	-11.2	-1.8	-5.0	-1.5
GH2111	1.75	-11.3	-1.3	-4.8	-1.6
GH2110	2.00	-11.7	-2.4	-5.2	-1.5
GH2109	2.25	-11.9	-2.6	-5.2	-1.7
GH2104	2.5	-12.0	-2.9	-5.1	-2.3
GH2103	2.75	-12.4	-2.7	-4.8	-3.0
GH2102	3.00	-12.9	-3.0	-4.9	-3.0
GH2101	3.25	-11.5	-1.6	-4.4	-3.1
Head 3					
Z7	7.00	-11.7	-2.6	-5.1	-2.6
Z6	7.25	***	-3.4	-5.4	-2.5
Z5	7.50	-11.9	-3.2	-5.3	-2.6
Z4	7.75	-12.9	-3.1	-5.2	-2.8
Z3	8.00	-12.1	-2.3	-4.8	-3.2
Z22	8.25	-12.6	-2.8	-5.3	-2.2
Z2	8.50	-13.6	-3.1	-4.9	-3.7

***sample lost during preparation

Samples for skeletal isotopic measurements were treated with 6% sodium hypochlorite to remove organic matter following Carriquirry (1991). Coral skeletons were then filed to remove a sample of aragonite. We attempted to sample equivalent amounts of time from the upper growth surfaces of skeletal samples within each data set. For Jamaica samples, growth rate patterns measured by Huston (1985) were utilised. Samples about 0.1 mm deep were taken from the surface of Agaricia samples, about 0.6 mm deep from shallow Porites samples and 0.3 mm from deeper colonies which showed more plating morphology. Similarly, Montastrea samples were filed to a depth of about 0.7 mm for shallow samples and 0.2 mm for deeper colonies when they showed plating morphologies. Samples were taken over all skeletal elements (corallite walls, septa, etc.) and over several corallites in order to minimise isotopic variation (cf. Land et al., 1975a; Patzold, 1992). Zanzibar samples were taken 1mm deep into the skeleton. In all cases, this sampling should represent about 10% of one year's growth or a time period a little longer than one month (Huston, 1985; Dunn, 1995). Sample size was approximately 5 mg for all samples.

Tissue samples were combusted at 550 ° C for 2 hours, one day prior to analysis. The gases produced were cryogenically separated on a vacuum line, yielding pure CO_2. Carbonate samples from the first year of collection at Jamaica were reacted with >100% H_3PO_4 in sealed and vacuumed tubes at 25 ° C. Aliquots of 0.3 mg taken from original 5 mg samples were used for corals from Zanzibar and the second year of collection at Jamaica. These samples were

analysed using an automated carousel in which samples are reacted in >100% H_3PO_4 at 90 °C in a common acid vessel. All analyses were conducted on a VG SIRA mass spectrometer. In-house and international standards were analysed during runs to assure data quality. Repeatability for $\delta^{13}C$ of organic matter was tested using gelatine and glutamic acid organic standards and was 0.1 ‰ (± one standard deviation). Precision for $\delta^{13}C$ of carbonates was tested using our laboratory coral standard (Koodhoo -- a coral from a remote Maldivian atoll homogenised to < 4 ϕ) and was 0.04 ‰ for carbon and 0.03 ‰ for oxygen. All results are reported in standard delta notation in per mille units, relative to the PDB standard.

Following McConnaughey et al. (in press), isotopic equilibrium for Jamaica and Zanzibar corals was calculated using the equations of Romanek et al. (1992) for carbon and Grossman and Ku (1986) for oxygen:

$$\delta^{13}C_{aragonite} = \delta^{13}C_{\ dissolved\ bicarbonate} + 2.7$$

and

$$(\delta_c - \delta_w) = 4.75 - 0.23t$$

δ_w and δ_c are the $\delta^{18}O$ of CO_2 gas equilibrated with water at 25° C and generated by reaction of aragonite with phosphoric acid at 25° C, respectively. These values are expressed on the same isotopic scale. *t* is temperature in ° C. For Jamaica we used a temperature of 27.7 °C which adequately represents the temperature at the time of collection for both sampling seasons (data from

CARICOMP and the Caribbean Marine Research Center Water Temperature Network). The oxygen isotopic composition of the water at Discovery Bay has been measured at 0.7 ‰ (Rachel Eustice, personal communication). Since the value of $\delta^{13}C$ of seawater is unknown for Discovery Bay, literature values from other sites were used and $\delta^{13}C$ was set at 0.5 -1 ‰. The lower value (Swart et al., 1996) represents waters of Biscayne National Park, Florida, measured early in the year (corresponding to the time of Jamaica sample collection). The higher number is a typical sea surface value from other regions (e.g., McConnaughey, 1989a; Kroopnick, 1985). For Zanzibar, a temperature of 29 °C (diver measured) was utilised. The isotopic composition of the seawater was taken to be 0 ‰ and 1 ‰ for $\delta^{18}O$ and $\delta^{13}C$, respectively, typical of seawater at normal salinity. Although environmental conditions have been largely assumed, an approximation of isotopic equilibrium is adequate for the purpose of this paper.

Light intensities below the surface at Discovery Bay, Jamaica, used for comparison with tissue and skeleton $\delta^{13}C$ (Table 5.1), were measured by Ian Sandeman using a LI-Cor, LT-185 Quantum Radiometer/Photometer with a LI-192SB Cosine Collector (see Sandeman, 1996) at mid-day under calm sunny conditions during the winter of 1986. These data are described in more detail in Chapter 3.

5.4 Results and discussion

5.4.1 Oxygen isotope disequilibrium

Both Jamaica and Zanzibar corals show substantial variability in skeletal $\delta^{18}O$ (see standard deviations in Table 5.3). For Jamaica samples, this variability exists between heads collected over a 30 m depth range. In the case of Zanzibar, this variability occurs between samples collected over a depth range from the outer growth surface of single heads.

This variability has four possible sources: 1. variation in SST and $\delta^{18}O$ of seawater between samples (i.e. changes in isotopic equilibrium conditions); 2. species dependent "vital effects" (Weber and Woodhead, 1972); 3. kinetic isotope effects; and 4. variation in the amount of time being sampled due to errors in chronological assumptions (see methods).

The first source of variation is unlikely in this data set. Temperature and salinity are nearly constant over this depth range at Discovery Bay (Land et al., 1975a; Emson and Woodley, 1987; Webber, 1993) and $\delta^{18}O$ of seawater does not vary significantly between different reef environments (Rachel Eustice, 1996; personal communication). One-way ANOVA and regression analysis indicates no significant differences exist in these parameters as a function of depth for data from each year of collection. Variation in these factors over the scale of a single large head at Zanzibar is highly unlikely.

Table 5.3. Jamaica and Zanzibar oxygen isotope data

DATA SET	MEAN $\delta^{18}O$ (‰)	N	STD DEV.
JAMAICA			
All Corals yr1	-4.1	20	0.32
All Corals yr2	-3.7	35	0.52
Porites yr1	-4.0	8	0.39
Porites yr2	-3.9	10	0.33
Agaricia yr1	-4.1	10	0.23
Agaricia yr2	-3.4	14	0.52
Montastrea yr1	-4.2	2	0.56
Montastrea yr2	-4.0	11	0.43
ZANZIBAR			
HEAD1	-4.8	6	0.31
HEAD2	-4.9	8	0.27
HEAD3	-5.1	7	0.21

yr1 -Jan. 1993
yr2 -Jan. 1994

There is almost certainly isotopic variation associated with the varying amount of time represented with each sample (and the different environmental conditions each period of time will record; see Leder et al., 1996; Swart et al., 1996; Quinn et al., 1996; Wellington et al., 1996). Each sample is however, biased strongly towards the time of collection.

The issue of "vital effects" and kinetic isotope effects are likely one and the same (cf. McConnaughey, 1989a). The vital effect may represent an average species- or genus- dependent kinetic isotope effect. At Zanzibar, we are only dealing with one species (Porites lobata) and samples from single heads. Therefore a species dependent vital effect cannot be responsible for the observed variation. In the Jamaica data set, One-Way ANOVA analysis indicates there are no significant differences between species for the first year of collection. For the second year of collection, Agaricia samples are significantly enriched in the heavy isotope of oxygen (Table 5.3). Given the slow annual increase in thickness of this coral, this could represent reduced kinetic isotope depletion relative to the other two species.

McConnaughey (1989a) has shown that coral suites exhibiting strong kinetic isotope effects, and growing under the same environmental conditions, will show strong correlation in $\delta^{18}O$ and $\delta^{13}C$, along a linear trend that passes near equilibrium values of these parameters. Correlations between oxygen and carbon for Jamaica and Zanzibar corals are given in Table 5.4. In both cases strong correlations exist and although the trends do not actually approach isotopic

Table 5.4. Jamaica and Zanzibar correlation data

CORRELATION	r	N	p
C_{skel} vs O_{skel}			
All Jamaica Corals	0.65	49	<0.01
Porites	0.69	16	<0.01
Agaricia	0.78	22	<0.01
Montastrea	0.88	11	<0.01
All Zanzibar Corals	0.49	21	<0.05
Head 1	0.42	6	NS
Head 2	0.51	8	NS
Head 3	0.67	7	NS
C_{tiss} vs C_{skel}			
All Jamaica Corals	0.32	48	<0.05
Porites	0.33	16	NS
Agaricia	0.17	21	NS
Montastrea	0.15	11	NS
All Zanzibar Corals	0.43	20	<0.05
Head 1	0.14	6	NS
Head 2	0.84	7	<0.05
Head 3	0.47	7	NS
C_{tiss} vs $C_{transformed\ skel}$			
All Jamaica Corals	0.72	48	<0.01
Porites	0.73	16	<0.01
Agaricia	0.55	21	<0.01
Montastrea	0.62	11	<0.05
All Zanzibar Corals	0.75	19	<0.01
Head 1	0.64	6	NS
Head 2	0.90	7	<0.01
Head 3	0.78	6	NS
C_{tiss} vs N_{tiss}			
All Jamaica Corals	0.50	48	<0.01
Porites	0.61	16	<0.05
Agaricia	0.10	21	NS
Montastrea	0.61`	11	<0.05
All Zanzibar Corals	0.68	19	<0.01
Head 1	0.52	6	NS
Head 2	0.88	7	<0.01
Head 3	0.82	6	<0.05

-- Jamaica samples from 1993 and 1994 have been pooled
-- transformed carbon values identified as outliers have been omitted

equilibrium, the extensions of these lines pass near that point (Fig. 5.1 and Fig. 5.2). This suggests a large part of the oxygen isotopic variation in both data sets may be due to the presence of kinetic isotope effects.

5.4.2 Correction for the presence of kinetic isotope effects

In order to correct for the presence of kinetic isotope effects in coral skeletal data it is necessary to separate the various components contributing to the observed isotopic signal. It is useful to compare the isotopic signatures of ahermatypic (azooxanthellate) corals with hermatypic corals (McConnaughey, 1989a). On $\delta^{18}O$ vs $\delta^{13}C$ plots, ahermatypic corals plot on straight lines with a slope about 0.33, extrapolating to near isotopic equilibrium (Figs. 5.1, 5.2, Fig. 13 in McConnaughey, 1989a; see also Emiliani et al., 1978; Smith et al., 1996). The isotopic composition of ahermatypic corals is thought to be controlled by a mixture of kinetic isotope effects and an equilibrium process. Slower growing corals seem to have a higher proportion of equilibrium control and plot closer to isotopic equilibrium (McConnaughey, 1989a). Zooxanthellate corals however, tend to plot to the right of this kinetic line. This is thought to be due to the effect of photosynthesis preferentially depleting the internal carbon pool in ^{12}C (McConnaughey, 1989a). Therefore, isotopic signatures of azooxanthellate corals, which lack any photosynthetic enrichment of skeletal $\delta^{13}C$, can be used to define the kinetic isotope line. We have used a line of slope 0.33, trending to equilibrium, to define the trend along which kinetic isotope effects can occur in Figs. 5.1 and 5.2 (after McConnaughey, 1989a).

Fig. 5.1. $\delta^{18}O$ versus $\delta^{13}C$ of Jamaica skeletal samples. Note overall strong correlation and trend towards equilibrium. Eq is equilibrium (see text for explanation of how equilibrium isotopic composition was calculated), KIE marks the trend along which kinetic isotope effects occur. Strong kinetic isotope effects lead to simultaneous depletions in oxygen and carbon in a proportion of approximately 1:3. P labels trend for metabolic disequilibrium isotope effects associated with photosynthesis. The average $\delta^{18}O$ values for samples from year 1(1993) and year 2 (1994) are -4.1 ‰ and -3.7 ‰ respectively. These values were used to transform the data shown in Table 5.1. Based on an equilibrium $\delta^{18}O$ of approximately -1.2 ‰, average kinetic isotope effect depletion for oxygen is 2.9 ‰ for year 1 and 2.5 ‰ for year 2. This corresponds to average carbon depletions of 8.7 ‰ and 7.5 ‰ for the first and second year respectively (based on a slope of 1/3 for the KIE trend).

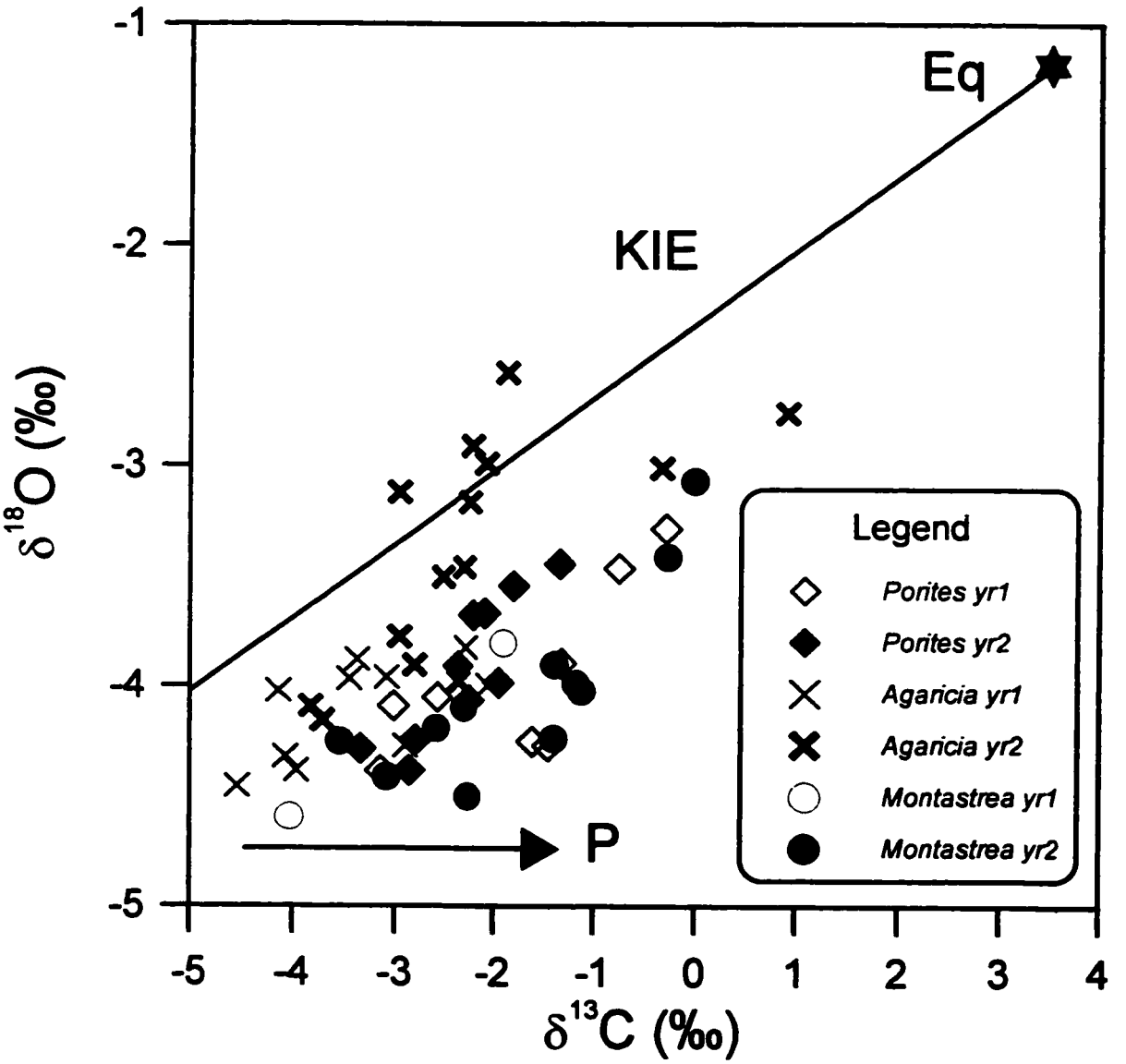
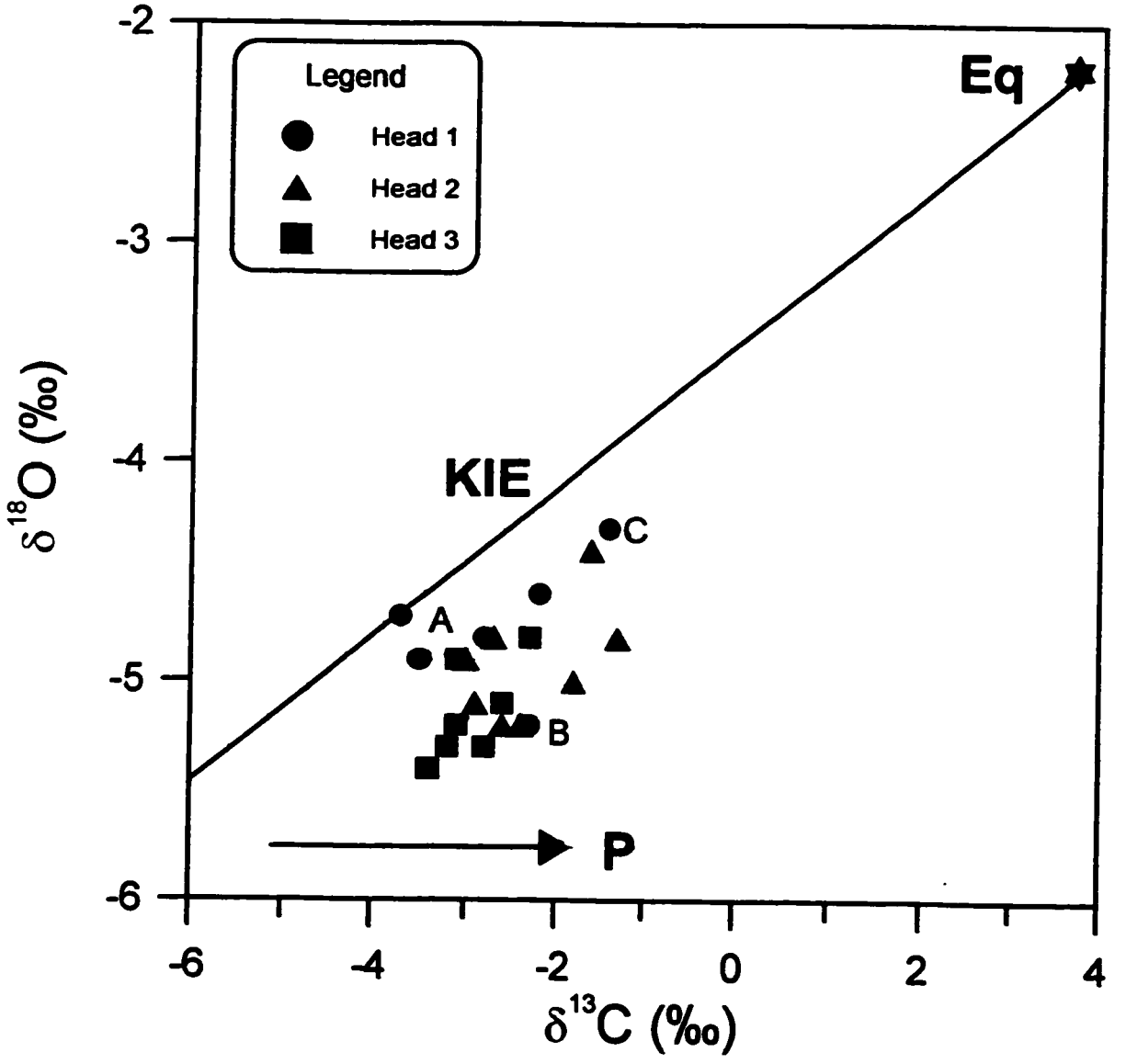

Eq
KIE
P
Legend
Porites yr1
Porites yr2
Agaricia yr1
Agaricia yr2
Montastrea yr1
Montastrea yr2
δ18O (‰)
δ13C (‰)
-1
-2
-3
-4
-5
-5
-4
-3
-2
-1
0
1
2
3
4

Fig. 5.2. $\delta^{18}O$ versus $\delta^{13}C$ of Zanzibar coral skeletal samples. The data exhibit a strong correlation trending towards equilibrium. Average $\delta^{18}O$ values for Head 1, Head 2 and Head 3 are -4,8, -4.9 and -5.1 ‰. Based on an equilibrium value of -2.2 ‰, these corals are showing kinetic isotope effects of approximately 2.7 ‰ and 8.1 ‰ for oxygen and carbon respectively. Points labelled A,B and C are identified to allow comparison with the same points in Fig. 5.10. The point labelled B, being furthest from the KIE line will transform to the most enriched $\delta^{13}C$ value.


Legend
Head 1
Head 2
Head 3
Eq
KIE
A
B
C
P
-2
-3
-4
-5
-6
-6
-4
-2
0
2
4
δ18O (‰)
δ13C (‰)

All the corals in the Zanzibar data set plot to the right of the kinetic isotope line suggesting strong photosynthetic/metabolic carbon isotope effects. In the Jamaica set, some samples plot to the left of the line. This might indicate predominance of heterotrophic feeding (e.g., zooplankton predation) as a nutritional strategy (i.e. suppression of photosynthetic activity). These are generally some of the deepest corals which also seem to have low tissue $\delta^{13}C$ and high tissue $\delta^{15}N$, relative to other samples from the depth they represent (Table 5.1, Table 3.1). Such tissues signals could support the suggestion of a strongly heterotrophic diet (Muscatine et al., 1989; Muscatine and Kaplan, 1994; Chapter 3).

According to the model of McConnaughey (1989a), the carbon isotopic composition of a zooxanthellate coral is therefore composed of three components: a) an equilibrium component, b) a kinetic isotopic depletion completion, with carbon being three times more depleted in the heavy isotope than oxygen, and c) photosynthetic enrichment in ^{13}C (Figs. 5.1, 5.2). It is this last component that we will try to resolve.

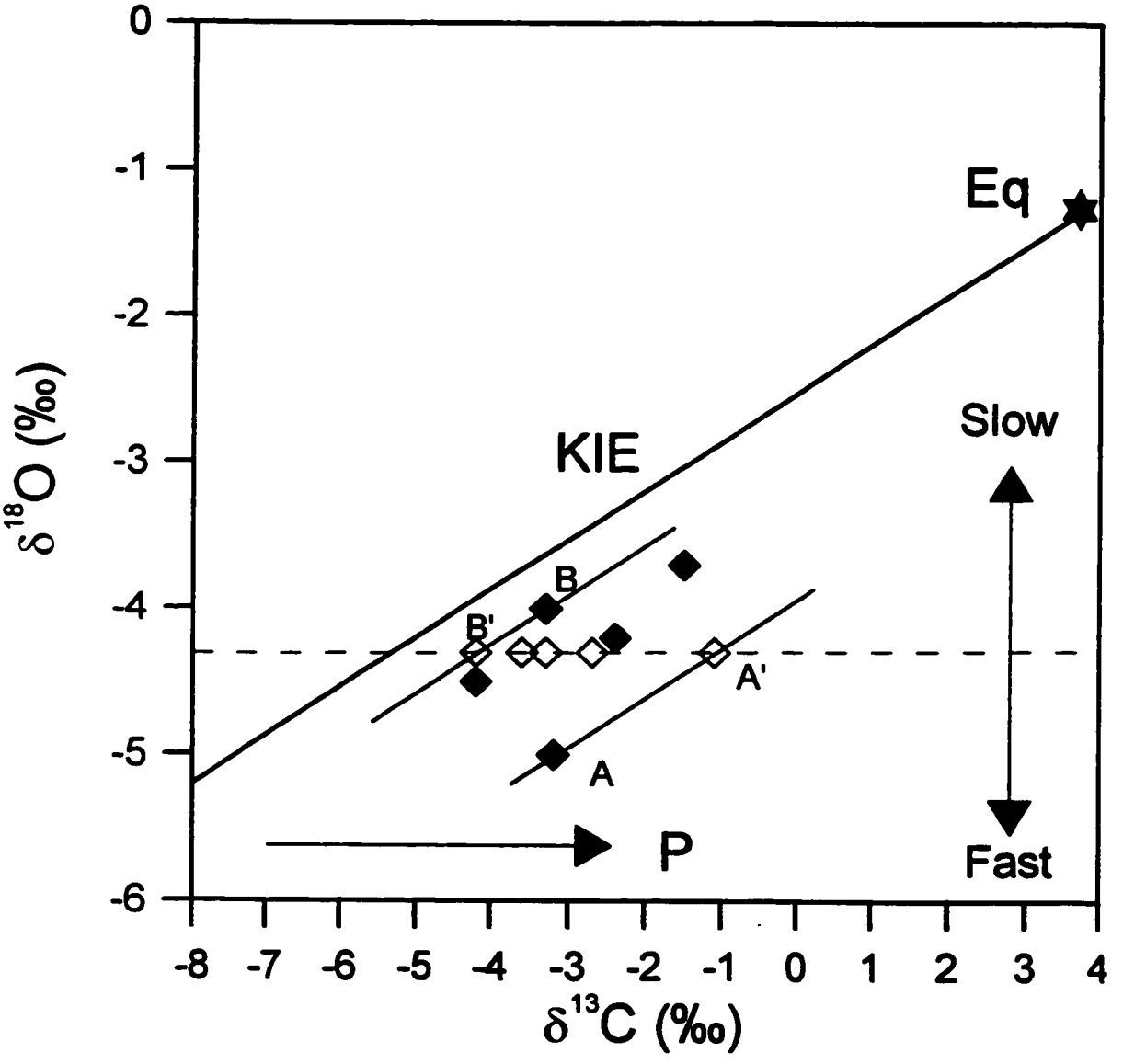
If we assume that all of the $\delta^{18}O$ variation in both data sets is due to the magnitude of kinetic isotope effects, then we should be able to account for these influences. If the magnitude of the kinetic isotope effect was constant within each data set (each year of sampling for Jamaica data or each large head for Zanzibar data), then samples within each set should have the same $\delta^{18}O$, since

they were deposited under identical environmental conditions (i.e isotopic equilibrium is the same for each sample). In addition, photosynthesis does not directly affect coral skeletal $\delta^{18}O$ (McConnaughey, 1989a).

For each data set, we calculate an average $\delta^{18}O$ and transform the oxygen and carbon data along a line representing kinetic isotope effects to that average value. A hypothetical example is shown in Fig. 5.3. The average $\delta^{18}O$ of the five points is -4.3 ‰. All of these coral samples were precipitated under identical environmental conditions and therefore share a common isotopic equilibrium. In the absence of variable magnitudes in kinetic isotope effects, we propose that all these samples would have a common $\delta^{18}O$ value (we have chosen to use the average value of -4.3‰). We therefore transform the data along lines parallel to the kinetic isotope line (oxygen decreasing at a rate approximately one third that of carbon) to the common oxygen value. We term the new carbon values obtained in this way "transformed" skeletal $\delta^{13}C$. We have transformed to an average $\delta^{18}O$ value rather than to equilibrium $\delta^{18}O$, to yield transformed $\delta^{13}C$ values close to the actual values observed in the coral skeleton. The choice of a $\delta^{18}O$ value has no influence on the relative differences between carbon values following transformation.

It is interesting to note that the interpretation of the data changes dramatically, following the transformation. Before transformation of the data, points A and B in Fig. 5.3 are in the middle of the range of $\delta^{13}C$ values. After

Fig. 5.3. Plot of hypothetical data showing graphically, how carbon isotope data are transformed. Data have been transformed to a constant oxygen value of -4.3 ‰ along slopes parallel to the kinetic isotope effect trend (see discussion in text). The trend from slow to fast is marked to emphasise that in general, slower growing/calcifying samples will tend to be enriched in the heavy isotopes of carbon and oxygen. This is best recognised in oxygen data since metabolic effects (P) act on skeletal carbon values, in addition to kinetic isotope effects. See also Fig. 13 in McConnaughey (1989a). We lack growth rate data for Jamaica and Zanzibar samples, but in general, deeper samples often have enriched $\delta^{18}O$ relative to other samples. This trend is not consistent throughout the data set however.


0
-1
-2
-3
-4
-5
-6
$\delta^{18}O$ (‰)
-8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4
$\delta^{13}C$ (‰)
Eq
KIE
Slow
Fast
P
B
B'
A
A'

transformation, however, points A' and B' have the heaviest and lightest carbon values, respectively. Rather than reading graphs of untransformed data in oxygen-carbon space from left to right to determine which samples have the heaviest $\delta^{13}C$ (i.e. most photosynthesis) we must look from the upper left to the lower right hand corner along a line perpendicular to the kinetic isotope trend. Those points closest to the kinetic isotope line will transform to the lightest values. Conversely, those points furthest from the kinetic line along this perpendicular trend will transform to the heaviest values. Although there is considerable overlap, shallowest samples from both Jamaica and Zanzibar tend to be the furthest offset from the kinetic isotope effect trend.

Mathematically, the transformation can be represented as follows;

$$\delta^{13}C_{transformed} = \delta^{13}C_{original} - (3 \cdot (\delta^{18}O_{original} - \delta^{18}O_{average}))$$

Data from the two years of Jamaica sampling were transformed separately to account for possible interannual differences in temperature or $\delta^{18}O$ of seawater. Similarly, each Zanzibar coral colony was transformed separately. Average $\delta^{18}O$ values for these groups are given in Table 5.3.

The effect of these transformations should be to reveal the effect of photosynthetic enrichment of the internal DIC pool (P in Figs. 5.1, 5.2, 5.3) (c.f. McConnaughey, 1989a) . The only other source of variation might be

depth-related variation in $\delta^{13}C$ of reef DIC (cf. Kroopnick, 1985). The Discovery Bay environment is so well mixed, however, that we assume any such variation is minimal and such variation will not be a factor over the scale of Zanzibar coral colonies.

Even given the fact that there will be error in the transformed carbon values associated with varying degree of time averaging between samples (i.e. all samples do not represent the exact same growth interval and therefore the same equilibrium conditions) we might expect to find significant relationships between transformed $\delta^{13}C$ and depth/light availability. In other words, if the transformation is appropriate, and we have isolated the photosynthetic enrichment component of skeletal $\delta^{13}C$, a strong relationship with light should exist.

Coral tissues show decreasing $\delta^{13}C$ with depth, related to light availability (Land et al., 1975b; Muscatine et al., 1989). As light availability decreases, zooxanthellar fractionation of DIC increases, leading to isotopically lighter zooxanthellae and lighter organic carbon being translocated to the host. In addition, corals in lower light conditions appear to rely more on heterotrophic feeding on isotopically depleted food sources such as zooplankton, leading to a net depletion in coral tissue $\delta^{13}C$ with depth (Muscatine et al., 1989). The light-related magnitude of photosynthesis affects the degree of fractionation by the zooxanthellae and hence, $\delta^{13}C$ of coral tissue/zooxanthellae. It also simultaneously affects the isotopic composition of the internal DIC pool from

which CO_2 for calcification is derived (Goreau, 1977a; also see Fig. 1 in McConnaughey et al., in press). As such, we might expect to find a strong relationship between coral skeletal $\delta^{13}C$ and $\delta^{13}C$ of associated tissue (see Fig. 15 in McConnaughey, 1989a). Ratios of photosynthesis to respiration tend to decrease with depth for Discovery Bay corals, emphasising the effect of light on coral metabolism in this setting (Muscatine et al., 1989).

5.4.3 Jamaica tissue and skeletal $\delta^{13}C$

Before comparing transformed carbon values of Jamaica coral skeletons to tissues and light availability we take a closer look at tissue $\delta^{13}C$ for these samples. Jamaica corals show a highly significant linear relationship with light (Fig. 5.4. Table 5.5) and a highly significant curvilinear relationship with depth (Fig. 5.5). The overall linear relationship with depth is consistent with the results of Muscatine et al. (1989) for separated zooxanthellae and coral tissue. The slight increase in $\delta^{13}C$ of coral tissue at 30 m depth (or decrease at 22 m) is more difficult to explain. This phenomenon may involve increased heterotrophic feeding on isotopically depleted zooplankton at 22 m depth. Alternatively, corals at 30 m might be heavier due to photoadaptation, or heterotrophic feeding on an unidentified isotopically enriched food source (perhaps dissolved organic matter). Jamaica coral tissue $\delta^{13}C$ show a strong correlation with tissue $\delta^{15}N$ (Fig. 5.6, Table 5.4). Although dissolved inorganic nitrogen is taken up only indirectly as a result of photosynthesis (e.g., Summons et al., 1986), both variables

Fig. 5.4. $\delta^{13}C$ of Jamaica coral tissue versus decrease in light availability. See Table 5.1 for light data. Solid squares represent means for each depth. Error bars give range of data from minimum to maximum values. Best fit lines are shown. Linear regression data are given in Table 5.5.

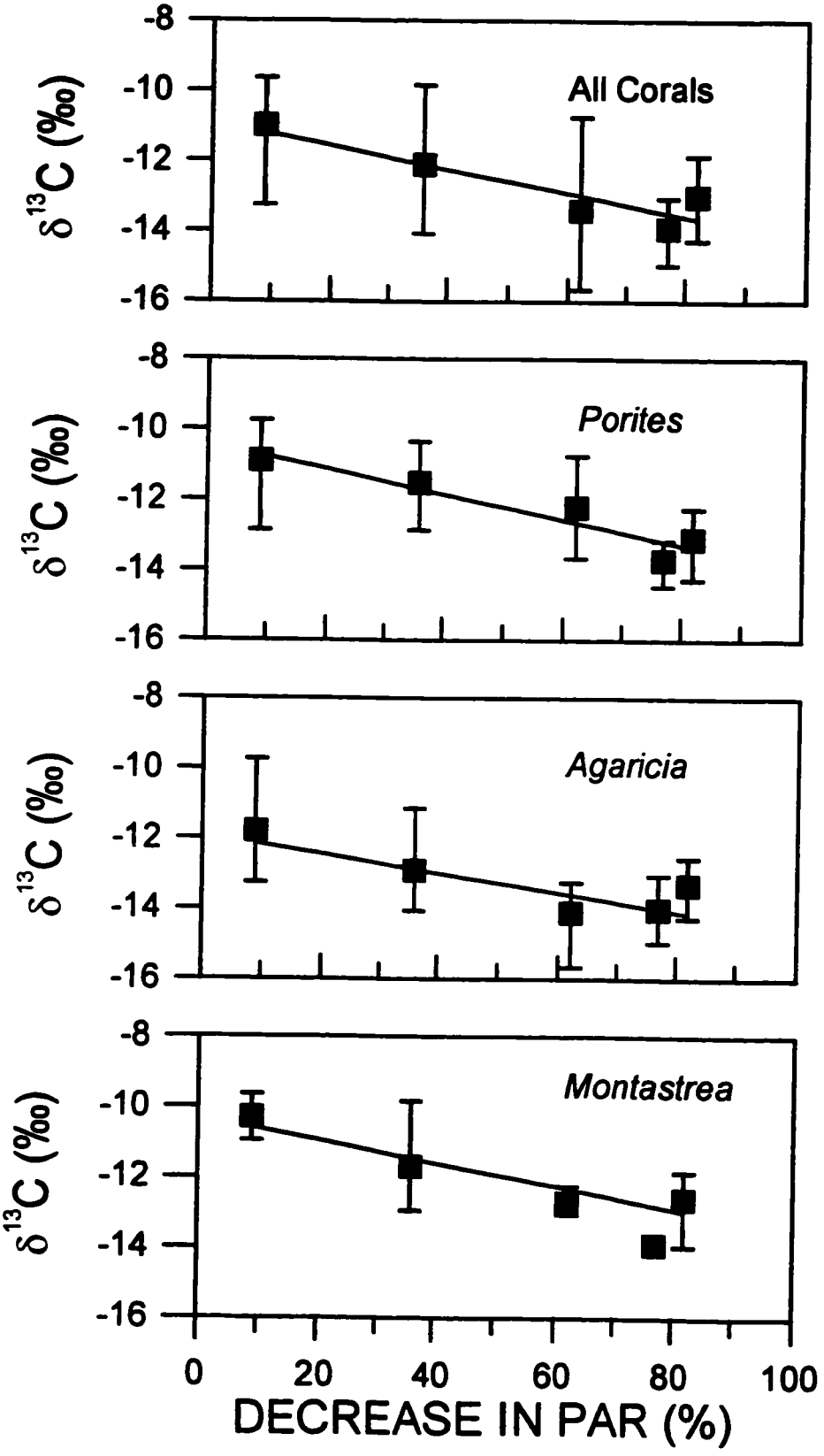

All Corals
Porites
Agaricia
Montastrea
δ13C (‰)
-8
-10
-12
-14
-16
0
20
40
60
80
100
DECREASE IN PAR (%)

Table 5.5. Regression data[1] for Jamaica and Zanzibar corals

y ($\delta^{13}C$ of)	x	m (slope)	b	p	R^2
JAMAICA TISSUE					
All Corals	%ΔPAR[2]	-0.0336	-10.9	<0.001	0.389
Porites	%ΔPAR	-0.0361	-10.4	0.001	0.524
Montastrea	%ΔPAR	-0.0325	-10.3	0.007	0.502
Agaricia	%ΔPAR	-0.0274	-11.9	0.004	0.325
JAMAICA SKELETON					
untransformed					
Porites yr1	%ΔPAR	-0.0019	-1.70	0.895	0.003
Porites yr2	%ΔPAR	-0.0054	-1.89	0.545	0.064
Montastrea	%ΔPAR	-0.0040	-1.77	0.783	0.009
Agaricia yr1	%ΔPAR	-0.0077	-2.90	0.701	0.022
Agaricia yr2	%ΔPAR	0.0086	-2.74	0.555	0.033
transformed					
Porites yr1	%ΔPAR	-0.0201	-1.38	0.020	0.625
Porites yr2	%ΔPAR	-0.0028	-1.65	0.712	0.024
Montastrea	%ΔPAR	-0.0207	-0.237	0.021	0.466
Agaricia yr1	%ΔPAR	-0.0134	-2.47	0.376	0.113
Agaricia yr2	%ΔPAR	-0.0233	-1.75	0.027	0.371
ZANZIBAR TISSUE					
Head 1	DEPTH	-0.994	-10.5	0.311	0.251
Head 2	DEPTH	-1.09	-9.47	<0.001	0.960
Head 3	DEPTH	-1.06	-4.19	0.055	0.643
ZANZIBAR SKELETON					
untransformed					
Head 1	DEPTH	0.171	-3.06	0.861	0.009
Head 2	DEPTH	-0.395	-1.35	0.354	0.144
Head 3	DEPTH	0.086	-3.59	0.796	0.015
transformed					
Head 1	DEPTH	-1.10	-0.09	0.305	0.257
Head 2	DEPTH	-1.11	0.423	0.001	0.876
Head 3	DEPTH	-0.806	3.28	0.006	0.877

1 - for linear regression in the form y=mx +b

2 - % Δ PAR refers to percentage decrease in PAR relative to surface PAR as in Table 5.1.

-- outliers identified in the text have been omitted from this analysis

Fig. 5.5. Plot of data from Fig. 5.4 but versus depth. Symbols as in Fig. 5.4. Quadratic regression fits and regression data are shown.

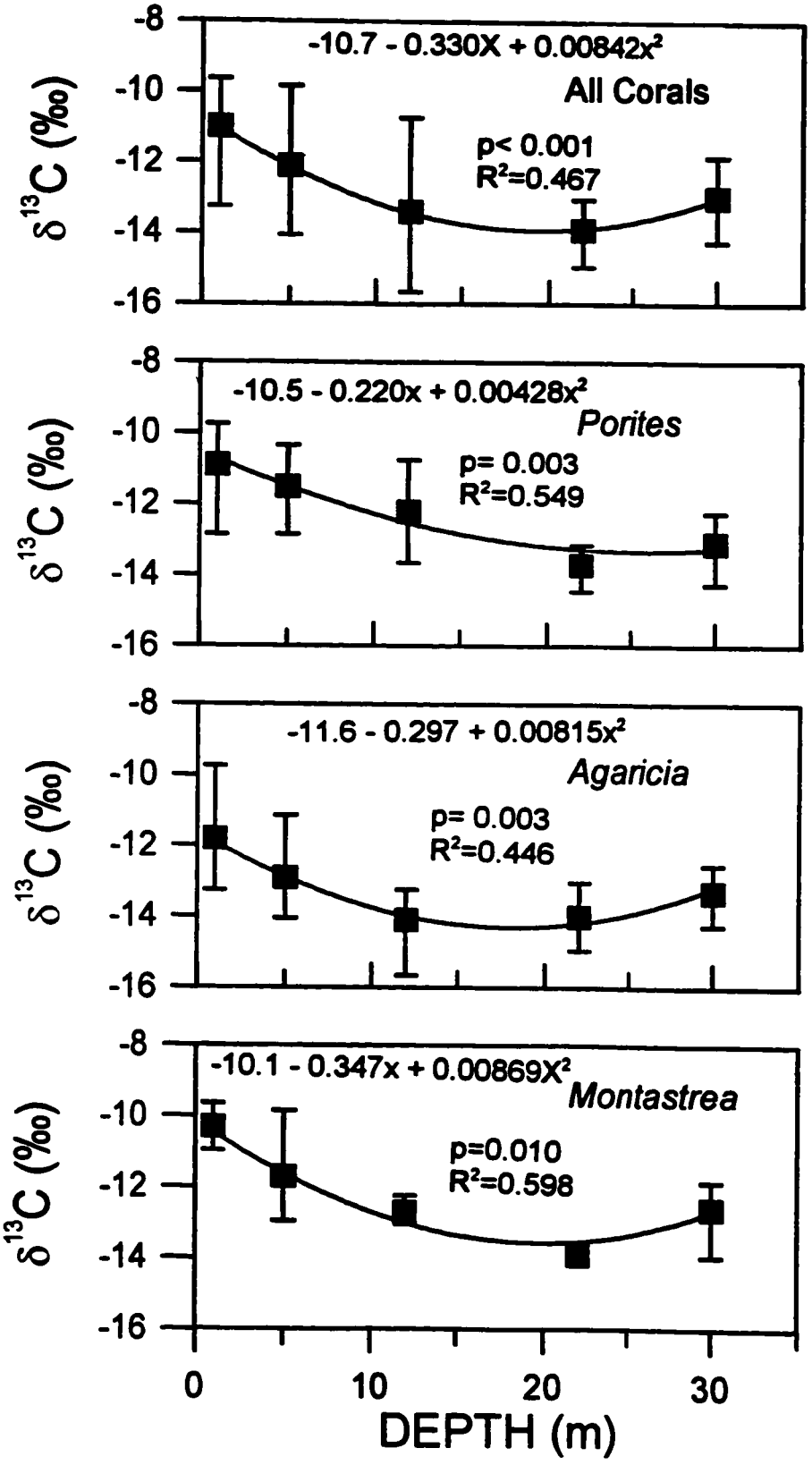
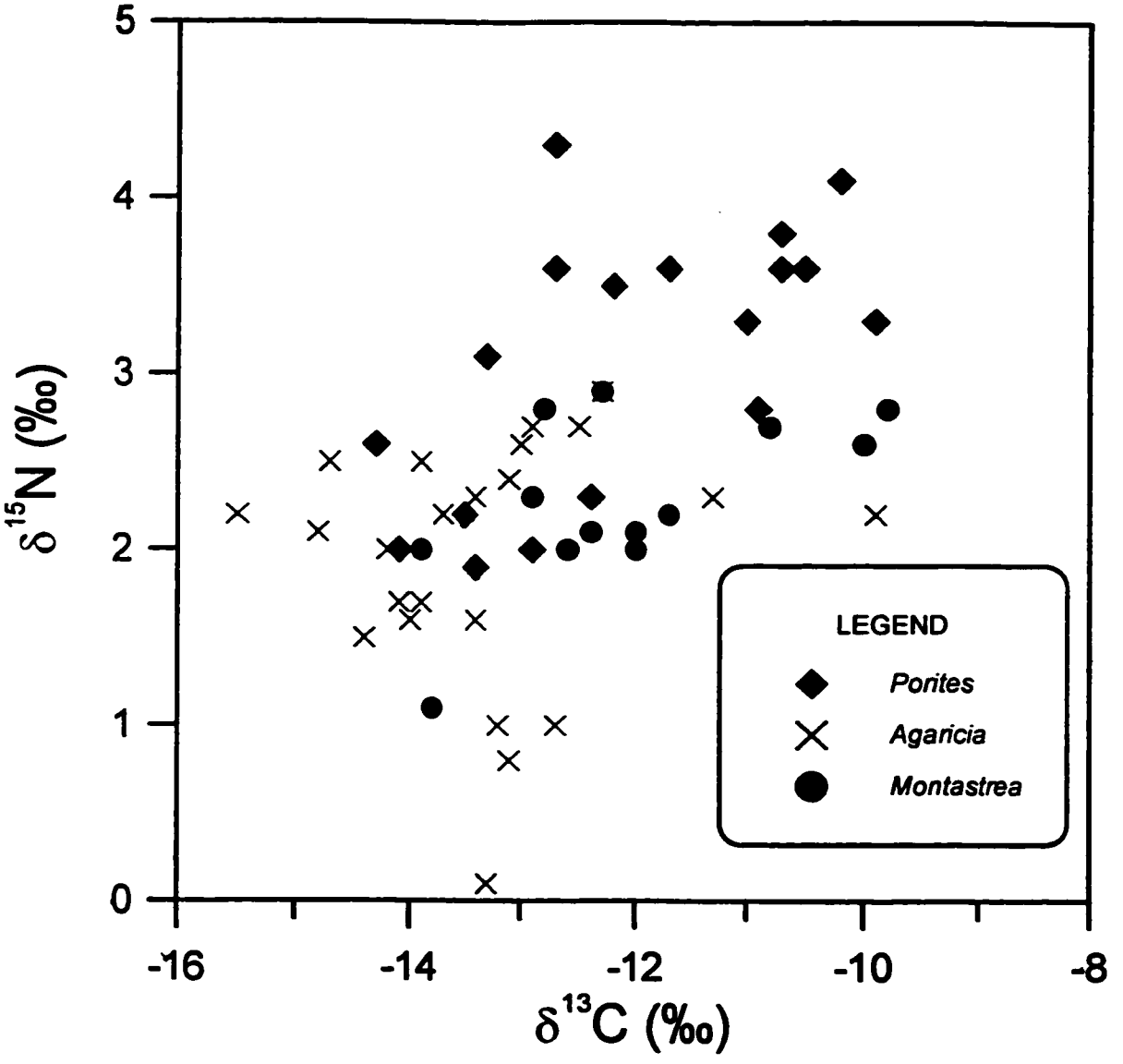

-10.7 - 0.330X + 0.00842x²
All Corals
p< 0.001
R²=0.467
-10.5 - 0.220x + 0.00428x²
Porites
p= 0.003
R²=0.549
-11.6 - 0.297 + 0.00815x²
Agaricia
p= 0.003
R²=0.446
-10.1 - 0.347x + 0.00869X²
Montastrea
p=0.010
R²=0.598
δ13C (‰)
-8
-10
-12
-14
-16
0
10
20
30
DEPTH (m)

Fig. 5.6. $\delta^{15}N$ versus $\delta^{13}C$ of Jamaica coral tissue. Correlation data are found in Table 5.4.


5
4
3
2
1
0
-16
-14
-12
-10
-8
δ15N (‰)
δ13C (‰)
LEGEND
Porites
Agaricia
Montastrea

are thought to be affected by light availability (Muscatine et al., 1989; Muscatine and Kaplan, 1994; Chapter 3).

Fig. 5.7 demonstrates correlations between coral tissue $\delta^{13}C$ and untransformed and transformed skeletal $\delta^{13}C$. With the exception of a few points, the transformed carbon data are better correlated with tissue $\delta^{13}C$ (Table 5.4). Without transformation it would be difficult to identify a meaningful relationship between carbon isotopic signatures of the skeleton and tissue. After transformation, however, a positive correlation is evident, as would be predicted due to the influence of light on the isotopic composition of both variables.

Untransformed and transformed carbon data are plotted versus light availability in Fig. 5.8. Regression data are given in Table 5.5. In each case, little or no relationship between untransformed skeletal $\delta^{13}C$ and light availability is evident. In one case (Agaricia samples from 1994) the trend appears to be one of increasing $\delta^{13}C$ with depth. The transformed data show significant correlations, as would be expected based upon recorded responses of coral skeletal $\delta^{13}C$ to depth/light availability and prevailing models of metabolic carbon fractionation in corals (Weber and Woodhead, 1970; Weber, 1974; Weber et al., 1976; Land et al., 1975a; Goreau, 1977a,b; Fairbanks and Dodge, 1979; Weil et al., 1981; Swart, 1983; McConnaughey, 1989a).

There are one or two outliers on Fig. 5.8. These correspond to samples which were outliers in Fig 5.7. In the case of Montastrea , samples

Fig. 5.7. Tissue $\delta^{13}C$ versus untransformed and transformed skeletal $\delta^{13}C$ of Jamaica samples. Upper graphs represent data from year 1, lower graphs data from year 2. Symbols are as in Fig.5.1. Note significant improvements in correlation (see Table 5.4). Points for which transformation appears to yield outliers have been marked (see text for explanation). Point *5p4* is also considered an outlier. This point is hidden by other points on the graph (at transformed skeletal $\delta^{13}C$ = -0.8 ‰ and tissue $\delta^{13}C$ = -12.2 ‰).

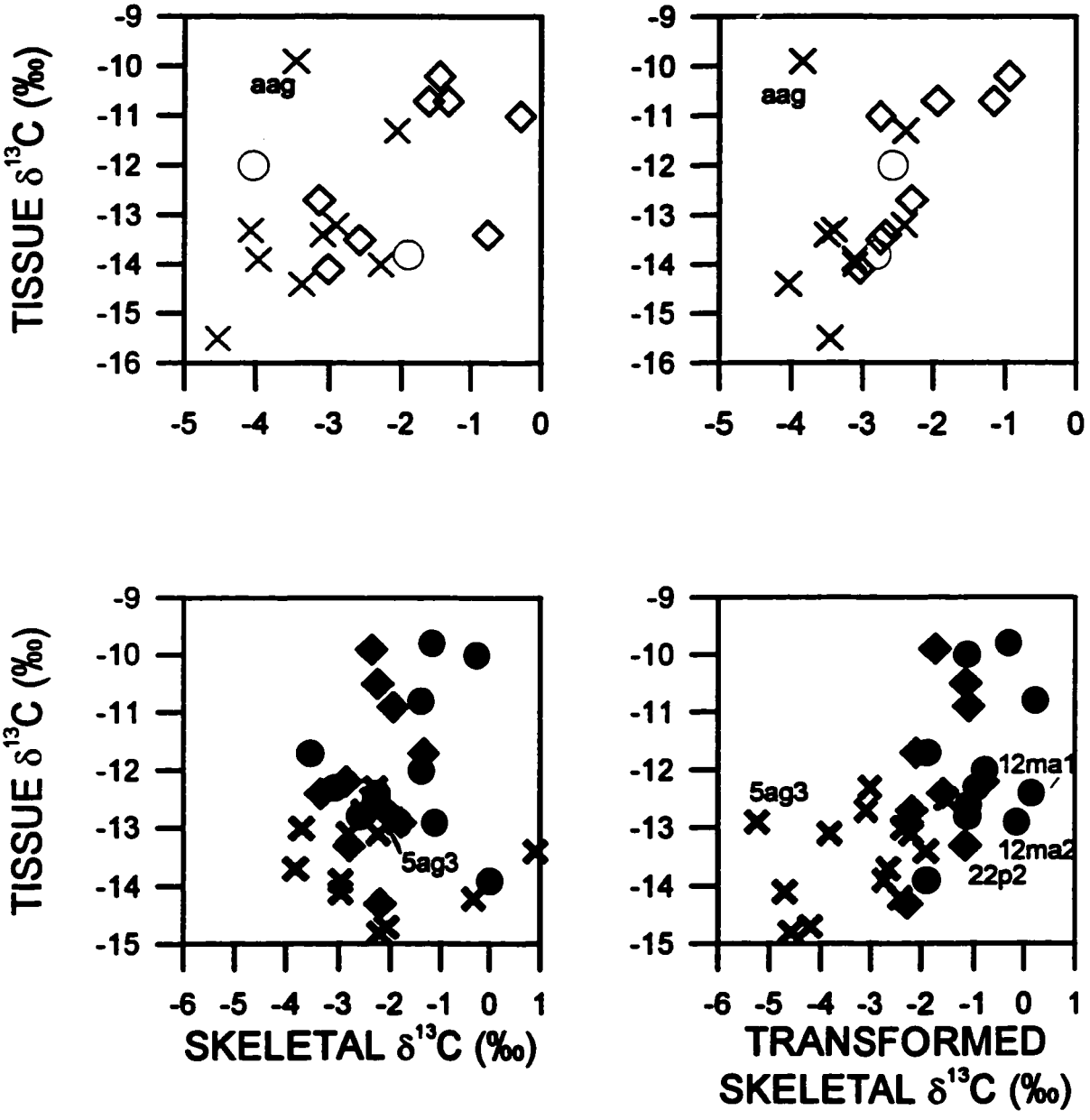
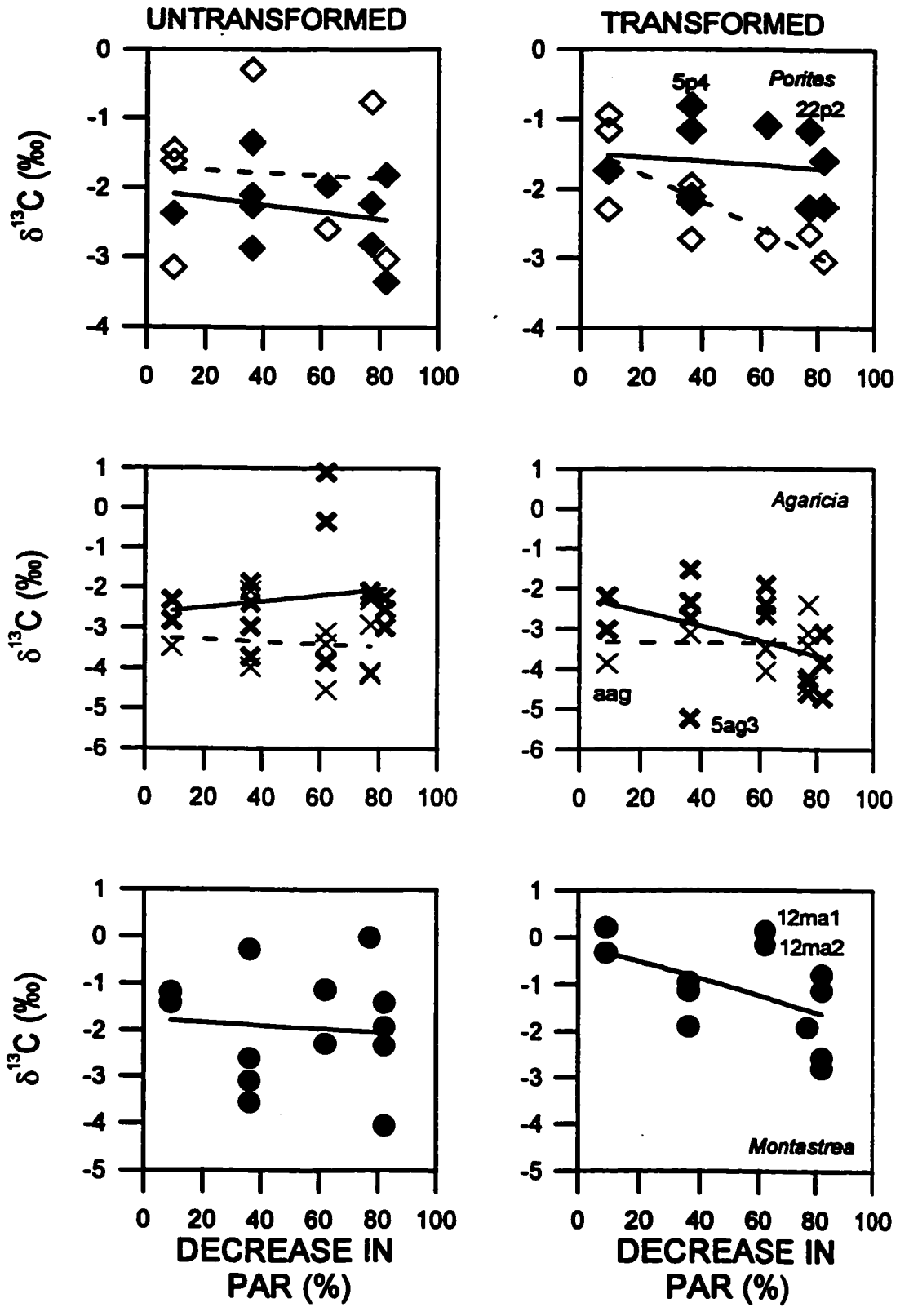

TISSUE δ13C (‰)
-9
-10
-11
-12
-13
-14
-15
-16
aag
-5 -4 -3 -2 -1 0
aag
5ag3
-6 -5 -4 -3 -2 -1 0 1
SKELETAL δ13C (‰)
5ag3
12ma1
12ma2
22p2
-6 -5 -4 -3 -2 -1 0 1
TRANSFORMED
SKELETAL δ13C (‰)

Fig. 5.8. Plot of untransformed and transformed Jamaica skeletal $\delta^{13}C$ versus decrease in light availability. Linear regression lines for all data are shown. Dashed lines for samples from year 1, solid lines for samples from year 2. Symbols as in Fig. 5.1. See Table 5.5 for regression coefficients. Transformed skeletal outliers have been marked as in Fig. 5.7. Outliers have been ignored in plotting regression lines.


UNTRANSFORMED
TRANSFORMED
δ13C (‰)
5p4
Porites
22p2
Agaricia
aag
5ag3
12ma1
12ma2
Montastrea
DECREASE IN PAR (%)
DECREASE IN PAR (%)

12ma1 and *12ma2* (12 m depth, 62% decrease in PAR, Table 5.1) seem to transform to values which are too enriched to fit well in either trend. It is quite likely that there is error in these transformed values associated with time averaging over a shorter time period than other samples. Montastrea from near this depth at Discovery Bay have the highest growth rates (Huston, 1986) for this species. The assumption that all samples could be transformed to the same $\delta^{18}O$ would be in error. Because we transform carbon data at a rate of three times that of oxygen data, such errors would be greatly compounded in transformed carbon data. In addition, the light-related carbon isotopic signal for Montastrea would be recording a shorter and presumably different integrated light history. With these points removed from the analysis, regression of transformed skeletal $\delta^{13}C$ with light availability becomes highly significant (Table 5.5). Montastrea annularis may actually consist of three sibling species and have three strains of zooxanthellae (Rowan and Knowlton, 1995). It is possible that the Montastrea data set does not represent a single sibling species/zooxanthellae strain combination, and that different light-related fractionation patterns may have led to the presence of points we have identified as outliers.

For Agaricia data, the trend towards heavier carbon with decreasing light availability is reversed in the transformed data set. Removal of point *5ag3*, which also appears as an outlier in Fig. 5.7, leads to a highly significant trend (Table 5.5). The trend for Agaricia samples from the first year of sampling does not improve with transformation of data. This may largely be due to the

presence of point *aag* from 1 m depth. Removal of this point, however, leaves data from only the three intermediate depths, making evaluation of trends difficult. In the case of Agaricia, an extremely slow growing coral (Huston, 1986) it is possible that sampling has led to time averaging over a greater period of time than most samples.

Transformed carbon data for the first year of Porites samples show a highly significant trend with decrease in light availability. The trend is not significant for data from the second year. Samples *5p4* and *22p2* from 5 m and 22 m respectively (36% and 77 % decrease in PAR, Table 5.1) appear enriched relative to the expected trend. These samples are also furthest from the best fit line for the correlation between coral tissue and transformed carbon for this species. Removal of these points, however, still results in a trend which does not show significant depletion in $\delta^{13}C$ with decreasing light availability. It is interesting that these same samples show a very steep positive correlation with tissue $\delta^{13}C$ (Fig. 5.7). While tissue $\delta^{13}C$ changes sharply over depth, there is only a small variation in skeletal $\delta^{13}C$. This may reflect efficient photoadaptation in this highly autotrophic species, reflected in the uniform values of transformed skeletal $\delta^{13}C$.

5.4.4 Zanzibar tissue and skeletal $\delta^{13}C$

Before examining transformed carbon data from Zanzibar corals we will look at tissue trends with depth. All Zanzibar samples were collected at 25 cm increments from the tops to the bases of the near vertical sides of large coral

colonies. Light availability to vertical surfaces is greatly reduced relative to horizontal surfaces (see Fig 3.1). As such $\delta^{13}C$ of coral tissue would be expected to decrease with depth over the head. All three coral heads from Zanzibar show evidence of this trend (Fig. 5.9, Table 5.5). The trend is not significant for Head 1 but is very nearly significant for Head 3. If the deepest point from Head 2 is removed the trend is highly significant. We can offer no explanation for this heavy tissue value and have treated it as an outlier in subsequent statistical analyses. These same samples showed a decrease in $\delta^{15}N$ with depth (Chapter 3). For nitrogen, however, the trend was more significant with ln (depth). The fact that $\delta^{13}C$ of these samples shows a stronger relationship with depth may reflect a degree of photoadaptation in deeper samples or resource sharing with respect to carbon between different depth zones of the colony.

Aside from possible time averaging problems associated with sampling, the assumption of a uniform value for isotopic equilibrium for all Zanzibar samples is stronger than in the case of Discovery Bay corals. Figure 5.10 demonstrates an improvement in correlation between tissue $\delta^{13}C$ and skeletal $\delta^{13}C$ following transformation. Correlations improve for each head and the data set as a whole (Table 5.4). Figure 5.11 shows the relationships between untransformed and transformed carbon values and depth for each of the heads. None of the untransformed carbon trends are significant. In two of the cases however, the data trend towards heavier $\delta^{13}C$ with depth. This is opposite to the expected

Fig. 5.9. $\delta^{13}C$ of Zanzibar coral tissue versus depth. Best fit regression lines are shown. See Table 5.5 for regression coefficients. The deepest point from head 2 has been omitted from regression analysis as an outlier (see text). See dicussion on point *gh1108* in text.

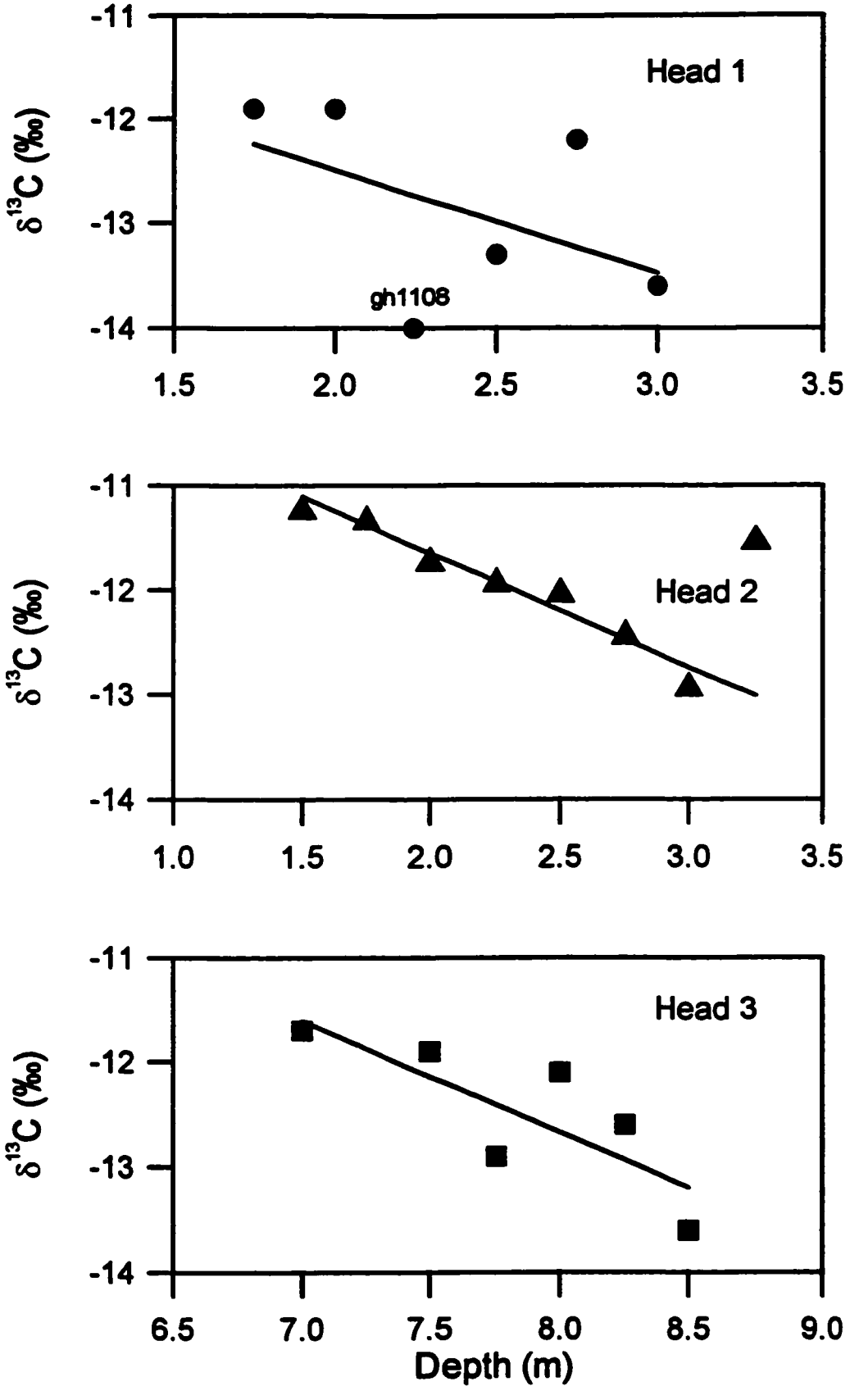

Head 1
gh1108
Head 2
Head 3
δ13C (‰)
-11
-12
-13
-14
1.5
2.0
2.5
3.0
3.5
1.0
6.5
7.0
7.5
8.0
8.5
9.0
Depth (m)

Fig. 5.10. $\delta^{13}C$ of Zanzibar coral tissue versus untransformed and transformed skeletal $\delta^{13}C$. See Table 5.4 for correlation data. Points *gH2101* (diamond) and *z22* were omitted from statistical analysis. Points A,B and C can be compared to show the effect of carbon transformation. Note how the relative positions of these points have changed in carbon space (compare to Fig. 5.2).

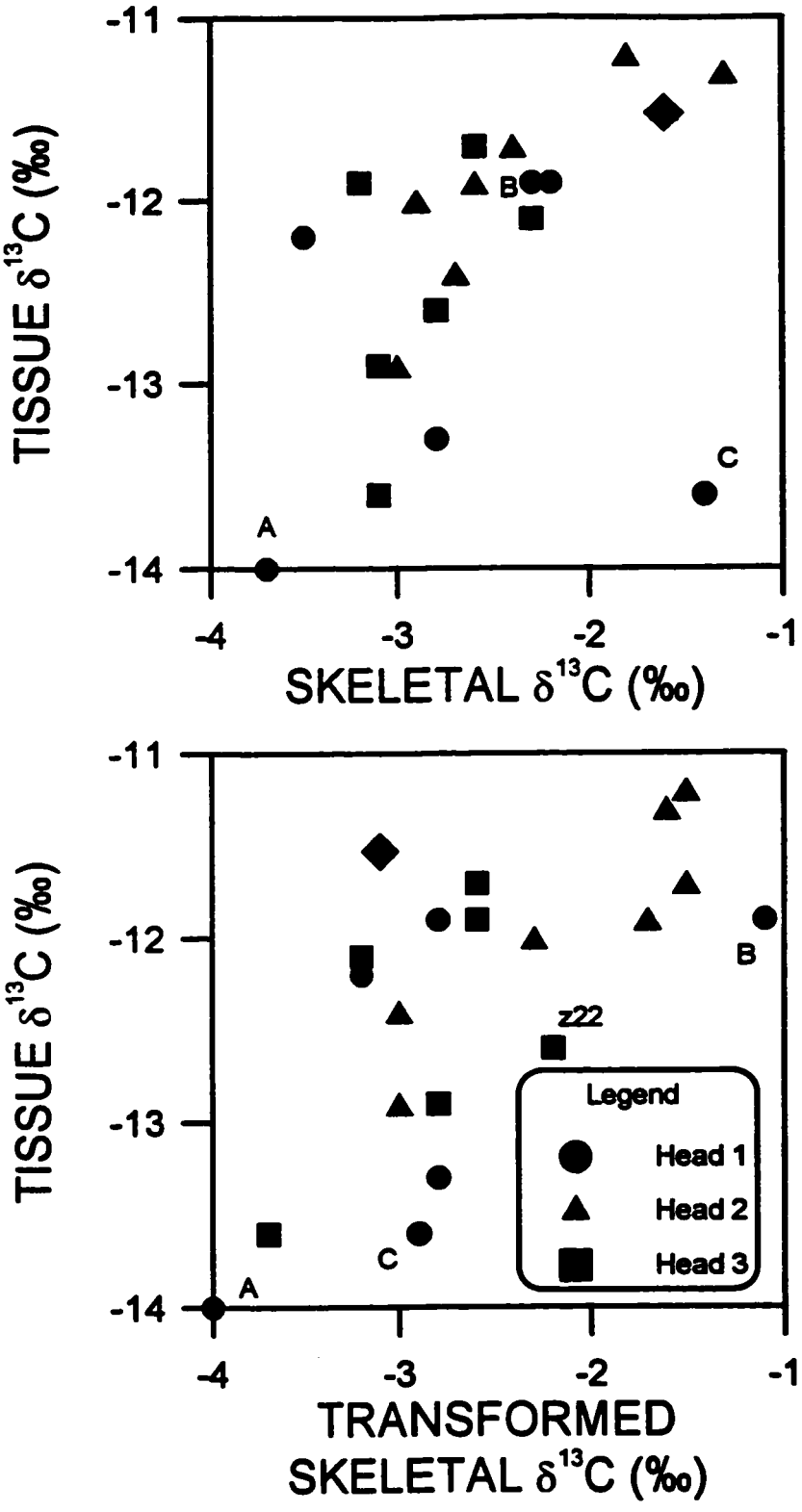
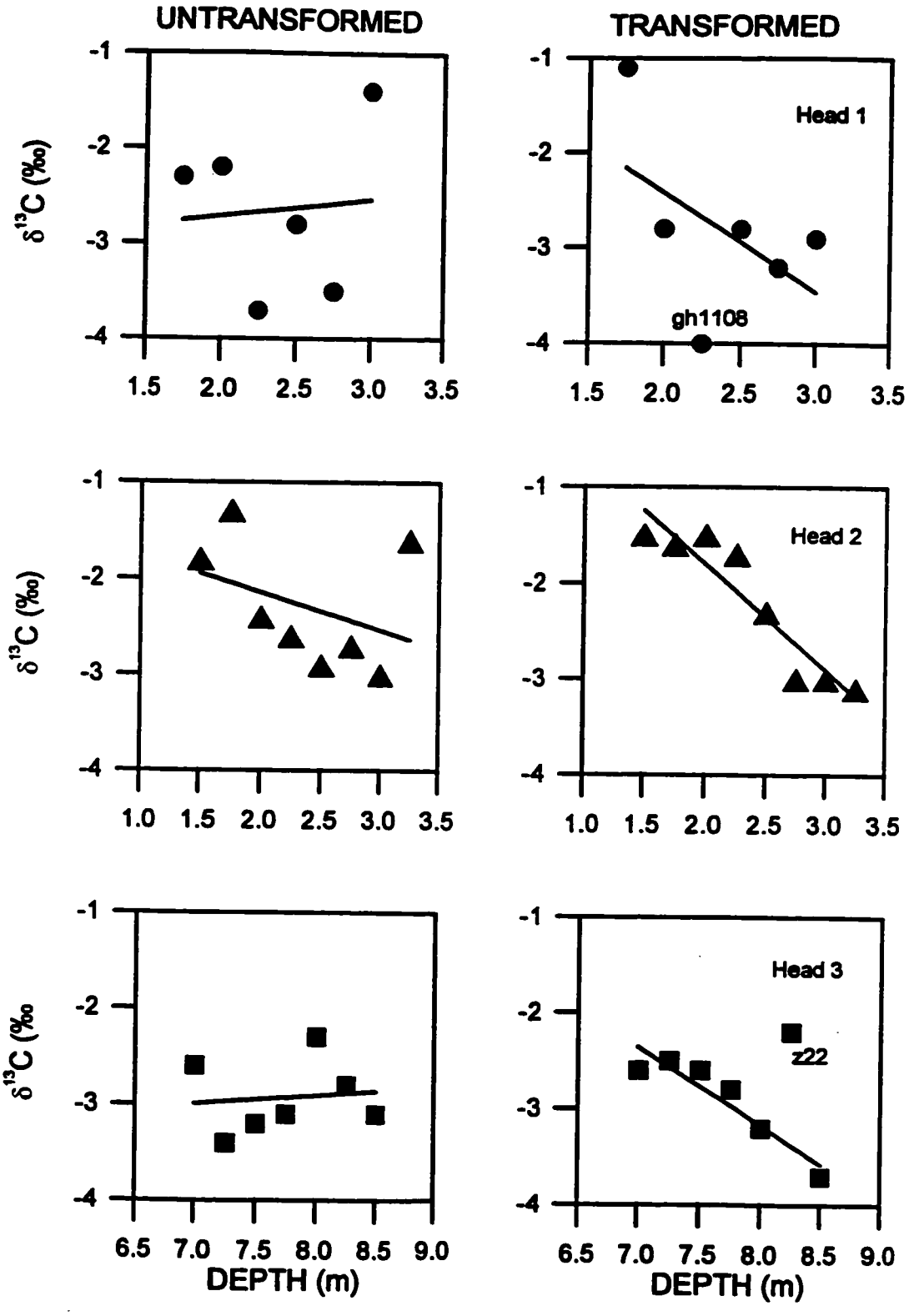

-11
-12
-13
-14
TISSUE δ13C (‰)
A
B
C
-4
-3
-2
-1
SKELETAL δ13C (‰)
-11
-12
-13
-14
TISSUE δ13C (‰)
A
B
C
z22
Legend
Head 1
Head 2
Head 3
-4
-3
-2
-1
TRANSFORMED
SKELETAL δ13C (‰)

Fig. 5.11. $\delta^{13}C$ of untransformed and transformed Zanzibar skeletal data versus depth. Best fit regressions lines are shown. Regression data can be found in Table 5.5. Point *z22* was omitted from regression analysis.


UNTRANSFORMED
TRANSFORMED
Head 1
gh1108
Head 2
Head 3
z22
δ13C (‰)
DEPTH (m)

trend, given patterns of light availability. After transformation all three colonies show trends towards lighter carbon with increasing depth/decreasing light availability. For Head 1, the trend is not significant, largely due to the point *gh108* at 2.25 m depth. This depth, however, also has anomalously low tissue $\delta^{13}C$ (Fig. 5.9) so the transformation may be appropriate. The transformed data yield a highly significant trend for Head 2 and Head 3 if the outlier noted in the caption is ignored.

5.5 Conclusions

Despite exceptions represented by a few points, overall, transformed data shows significant relationships with associated tissue and depth/light availability. This suggests that it is possible to correct for or to average out these kinetic isotope effects, yielding transformed data which strongly reflects a coral's light related carbon metabolism. Without transformation, kinetic isotope effects obscure or even reverse predicted trends.

While sampling resolution over intervals representing different extension rates can certainly have a profound effect on coral skeleton $\delta^{13}C$ and $\delta^{18}O$ (Swart et al., 1996; Leder et al., 1996; Wellington et al., 1996; Quinn et al., 1996), it is unlikely that differences in the length of the growth interval represented by different samples have seriously affected this analysis. These effects would tend to be random and cause some spread in $\delta^{18}O$. If all the $\delta^{18}O$ variation was due to this phenomenon, then it is improbable that the

transformations would have had any value and that significant trends related to light availability would be obtained from transformed data. Transformation of the carbon data allows us to see more clearly how photosynthesis leads to enrichment of coral skeleton $\delta^{13}C$.

It could be possible to extend this technique back though time in coral reconstructions. Accurate chronologies would need to be established and independent measures (e.g., Sr/Ca thermometry; e.g., Beck et al., 1991; DeVilliers et al., 1994) or reasonable assumptions of equilibrium conditions (based on historical data such as SST, salinity or rainfall measurements) would be required. Such a technique could be particularly important in reconstructing phenomena such as coral bleaching events. When corals lose their zooxanthellae they are forced to rely solely on isotopically depleted heterotrophic food sources. This in combination with the loss of photosynthetic enrichment of the internal DIC pool has been shown to lead to strongly depleted coral skeleton being deposited during bleaching events (Carriquiry, 1991; Carriquiry et al., 1994). Leder et al. (1991) however, found no obvious depletions associated with bleached corals, which survived and continued growing through the Carribean wide bleaching event of 1987. These authors concluded that $\delta^{13}C$ enrichment caused by slow growth may have offset the isotopic effect of loss of photosynthesis. Isotopic enrichment during bleaching events was also noted by Allison et al. (1996). Techniques presented in this paper suggest a way of

correcting for any growth-related phenomenon under such conditions, potentially allowing for retrieval of a meaningful bleaching signal.

It is not absolutely necessary that environmental conditions through time be known in order to employ this technique in at least a relative sense. If several colonies were collected in close proximity to one another, it should be possible to apply this type of data transformation for a given point in time. As long as accurate chronologies can be established, isotopic records could be compared. Any variation in $\delta^{18}O$ between the different colonies at a single point in time should be due to kinetic isotope effects since environmental conditions were identical. $\delta^{13}C$ data from the colonies could therefore be averaged for that point in time, carbon transformations could be applied, and the relative carbon isotopic response of each coral to environmental conditions could be determined. It would also be interesting to utilise this technique on bulk coral samples from ancient, unrecrystallized reefs (cf. Stanley and Swart, 1995). Corals of different species growing in the same position on a reef could be analysed to determine their relative carbon isotopic response to light availability.

It would be worthwhile to apply this technique to a suite of samples for which the chronology was very well established by methods such as alizarin red staining (e.g., Leder et al., 1996; Swart et al., 1996) in order to more accurately assess its potential. Coeval points within or between corals could be compared to measured light data. Despite potential chronological error in the present data set, transformation of carbon data has proven to have value for most samples.

Chapter 6: Conclusions

Examples of corals as potential environmental recorders/tracers are presented in Chapters 2-5. Chapter 2 documents the formation of skeletal death/regrowth surfaces and the incorporation of volcanic ash and iron precipitates into the skeleton as a result of the eruption of Banda Api and accompanying hydrothermal activity. Chapter 3 reveals that coral tissue samples collected over a depth gradient have decreasing $\delta^{15}N$ values with decreasing light availability. Models to explain the influence of light availability on nitrogen isotopic fractionation are presented. Chapter 4 extends this analysis to inter-reef variation in coral tissue $\delta^{15}N$. Although it is impossible at this time to determine the exact causes of variation between reefs, it is suggested that fractionation patterns associated with light availability may play a role. Chapter 5 demonstrates that coral tissue $\delta^{13}C$ of corals collected over a depth gradient strongly reflects light availability. The same can be said of skeletal $\delta^{13}C$ after correction for the presence of kinetic isotope effects. Specific conclusions related to each study are presented in individual chapters with broader implications for each signal discussed below.

6.1 Skeletal records of volcanic pulses

The use of corals as proxy recorders of volcanism could potentially be valuable in volcanology and hazard assessment. Records of small scale

subaerial oceanic island volcanism will be quickly eroded on land, and will be dispersed by wave action and mixed into sediment by bioturbation in the sea. Corals provide a possible means of both detecting and dating the occurrence of eruptions which otherwise might go undetected in the geological record, thereby allowing for a more accurate reconstruction of a volcano's history.

If this technique could be extended to document the timing and occurrence of larger ancient eruptions it might prove useful in climatological studies. When an eruption is of sufficient size and magnitude to deposit ash and volcanic aerosols into the stratosphere, marked climatic cooling can occur (e.g., AGU, 1992). Records of volcanism contained in corals which grew near climate-altering volcanic centres such as Tambora, Kilauea, or Pinatubo, could be precisely dated. Accurate dating of eruptions could allow for better comparison with climatic proxy records which are thought to record climatic perturbations associated with the effects of major eruptions (e.g., tree rings, ice cores, lake varves; AGU 1992).

Records of hydrothermal activity, analogous to the Banda Band, could potentially be used by ore geologists to study the evolution of hydrothermal centres through time. In all of these potential applications, the coral skeleton provides a means by which to date very precisely the timing of an event, using ^{14}C or U-series dating for older dead samples (e.g., Edwards et al., 1986) and density banding chronology for long-lived massive corals.

It is unknown at this time if the volcanically induced death surfaces and

the hydrothermally deposited Banda Band are representative features which might eventually prove to be relatively common in corals around active volcanic centres. It must be noted that these deposits were formed as a result of a coral's physiological response to stress. If the corals had not experienced partial mortality, abundant incorporation of ash may not have occurred. Similarly, if tissue retraction did not occur in response to external stress, deposition of a visible iron hydroxide band may not have resulted. The fact that volcanic stress led to the formation of visible deposits was significant for recognising the occurrence and timing of the event. Attempts to reconstruct the history of volcanism or hydrothermal activity based upon concentrations of non-visible, included materials would be much more difficult, particularly given the complex nature of skeletal deposition (Taylor et al., 1995).

Given the above factor, it is possible that the Banda coral records are "geological miracles". Extensive reef coring is being undertaken in Indonesia in the search for climate records contained in corals (Cole , 1994). This volcanically active region is also situated in the climatically important Western Pacific Warm Pool (Yan et al., 1993). As such, it is possible that similar proxy records of volcanism might be found.

Perhaps the broader implication of the Banda coral records is that very short-lived pulse events can be recorded in coral skeletons. While the volcanic ash from the eruption would have persisted in the water column, as a result of resuspension and transport from land, the records observed seem to represent

the initial event which occurred over an extremely brief period. The timing of the record, as established by sclerochronology, and the distinct nature of the Banda Band support this conclusion. If corals can faithfully record sufficiently stressful events occurring on the time scale of a few days or less, the temporal resolution with which corals are used as environmental recorders could be greatly enhanced. This might even prove valuable in recognition of the timing and effects of short-term anthropogenic discharges to reefs. Future studies of other short-term pulses will help assess the fidelity and usefulness of such records.

6.2 Records of coral tissue $\delta^{15}N$

The recognition of the effect of light availability on coral tissue $\delta^{15}N$ means that corals cannot in all cases be viewed as conservative tracers of nitrogen in the reef environment. Knowledge of this fact,however, can be used to design sampling schemes in which variation in environmental factors is minimised, thereby allowing for the use of corals as nutrient tracers. Analysis of coral tissue $\delta^{15}N$, from different species of corals collected over a broader range of light availability, should suggest which corals, under what conditions, might act as conservative tracers. Full understanding of coral nitrogen isotopic fractionation relative to dietary sources will require full isotopic characterisation of all such sources. The effect of other environmental factors on nitrogen fractionation must also be studied. It is possible, at least in the case of light, that corrections might be made to account for the magnitude of fractionation induced

by environmental factors, thereby allowing more corals to be used as nutrient tracers.

The fact that corals are not always conservative tracers of nitrogen suggests some important consequences for coral biology. The results of Chapter 3, for instance, suggest that zooxanthellae in corals may only be nitrogen limited in shallow water high light settings. Zooxanthellae in deeper corals may not be nitrogen limited at all, based on the occurrence of nitrogen isotopic fractionation. Similar conclusions have been reached by other workers (Muscatine and Kaplan, 1994; see also Jokiel et al., 1994). The results also suggest that DIN may be a more important component of coral diet at all depths than was previously recognised. Translocation of nitrogen from the zooxanthellae to the host and/or direct host assimilation of dissolved nitrogen may be more significant than previously thought. The use of heavy nitrogen as an isotopic tracer along with gas-chromatograph/isotope ratio mass spectrometry on individual compounds within coral tissues may provide a better understanding of coral nitrogen metabolism.

The large variation in $\delta^{15}N$ found between reefs suggests that coral tissue $\delta^{15}N$ studies merit further attention. Studies which seek correlation between physical and /or chemical environmental conditions and coral tissue $\delta^{15}N$ could help resolve many outstanding issues regarding the use of corals as nutrient tracers.

6.3 Records of coral tissue and skeletal $\delta^{13}C$

The results of Chapter 5 confirm that light affects the pattern of carbon isotope fractionation in corals in a predictable manner consistent with prevailing models. The most important consequence of this study, however, is that it is possible to account for disequilibrium kinetic isotope effects associated with the rate of calcification. Accounting for such effects provides a tool for obtaining more meaningful environmental information from coral skeletons. It should be noted that while we have compared records of light availability to skeletal $\delta^{13}C$, we are really recording the coral's metabolic response to light availability. In essence, we are obtaining a record of coral metabolism (amount of photosynthesis occurring in the coral as a response to light availability). The fact that we obtain meaningful metabolic information from the skeleton is emphasised by the fact that skeletal $\delta^{13}C$ correlates to tissue $\delta^{13}C$ after correcting for the presence of kinetic isotope effects. Tissue $\delta^{13}C$ strongly reflects coral metabolism and diet.

The greatest potential for this tool is in recording metabolic responses of corals to stressful events, which also affect the rate of calcification. If slower growth/calcification results from stressful conditions, enrichment of $\delta^{13}C$ and $\delta^{18}O$ could mask environmental and metabolic signals. The results of Chapter 5 suggest that corrections could be made to account for any such effect, allowing for the faithful reconstruction of such events. As stated previously, this could

prove to be an important tool in reconstructing bleaching history and in looking for underlying causes of bleaching events. Studies of bleached corals and corals affected by other stressful metabolic "events" should prove useful in addressing this hypothesis.

Although corals appear to represent very useful environmental recorders, caution must always be used in interpreting the record. It has been emphasised in all these studies that the environmental signal is often recorded as a result of a metabolic response. Because of this fact, corals will likely never be extremely faithful recorders. Any environmental factor which affects coral metabolism could potentially affect the fidelity of the signal being sought. Moreover, much variation could occur in environmental proxy records as a result of variations in metabolic response between individuals. As such, it will always be necessary to take replicate samples at any site, in order to resolve the common environmental signal.

Despite recent recognition of additional complexities associated with skeletal $\delta^{18}O$ and $\delta^{13}C$ records (kinetic isotope effects; McConnaughey, 1989), tissue isotopic records (this thesis) and sclerochronological reconstructions (Barnes and Lough, 1993; Taylor et al., 1995) corals have proven to be and will continue to be useful environmental proxies. Recognition of such complexities will improve the use of corals as environmental recorders if they are taken into account. Existing tools can be further refined and new records developed.

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IMAGE EVALUATION
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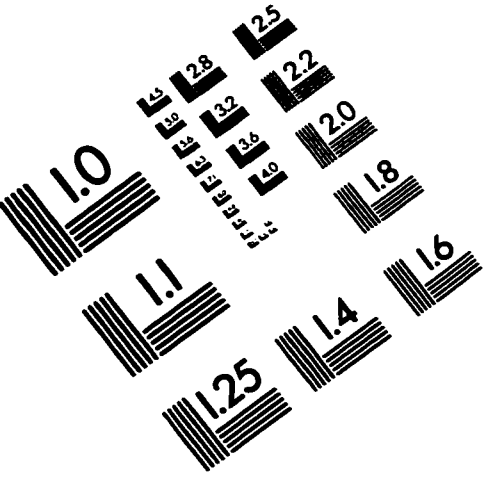

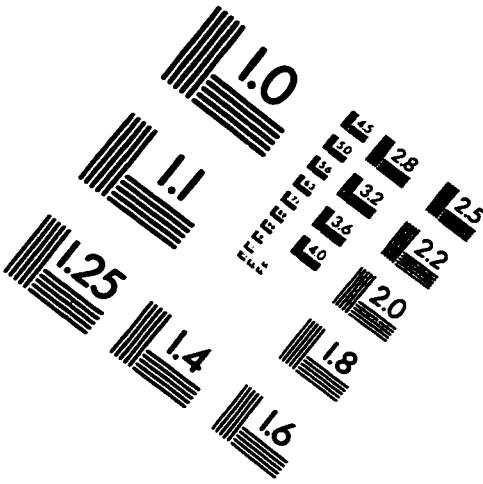

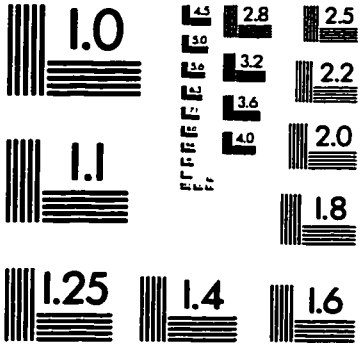

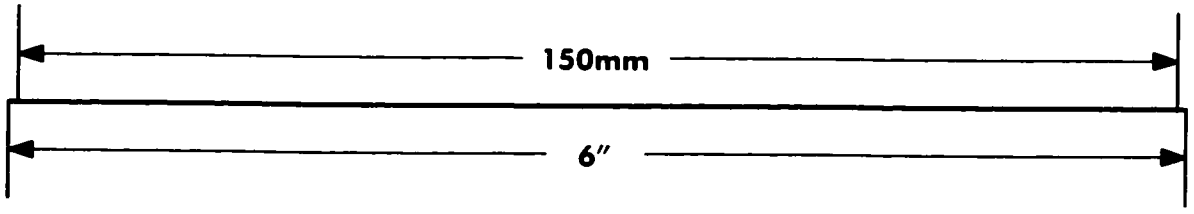

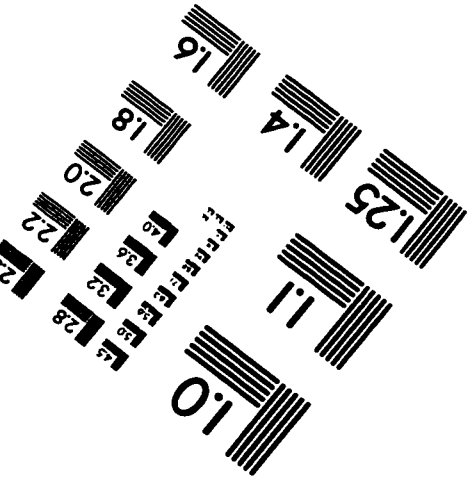

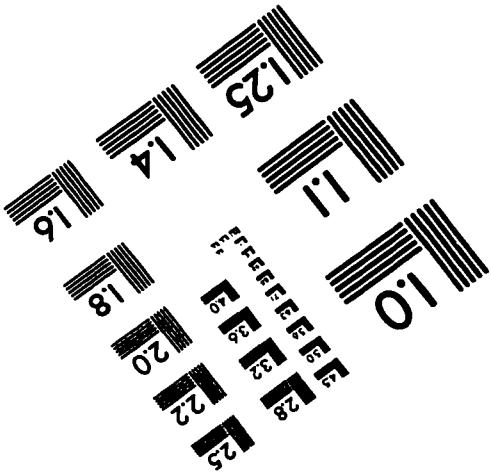

APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989