APPLICATIONS OF NITROGEN ISOTOPES AND OTHER TRACERS OF ANTHROPOGENIC INPUT TO MODERN REEFS

APPLICATIONS OF NITROGEN ISOTOPES AND OTHER TRACERS OF ANTHROPOGENIC INPUT TO MODERN REEFS

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Abstract

I investigated the use of nitrogen isotopes as a tracer of sewage contamination on coral reefs. Sewage is isotopically distinct from marine nitrogen, allowing its use as a tracer in this environment. Emphasis was placed on sampling modern coral tissues, as modern coral reefs are in a rapid state of decline, possibly as a result of sewage contamination.

Samples were collected in Zanzibar for two separate studies. The first study involved sampling over a depth/light gradient on large coral heads. These data, when combined with data from Jamaica, show light is a controlling factor on the nitrogen isotopic composition of coral tissue. Regression of $\delta^{15}N$ of coral tissue with depth/light attenuation explains 75-90% of the variance. Results indicate increased fractionation with depth, which is related to the symbiotic nature of corals. Light-sufficient, nitrogen-limited zooxanthellae (in the coral tissue) must diffuse all available nitrogen, and there is little fractionation. At depth, light-limited, nitrogen-sufficient zooxanthellae fractionate nitrogen during assimilation processes.

These results have important implications for the use of nitrogen isotopes as a sewage tracer on reefs. In general, increased nutrients to the reef environment result in decreased water clarity, or lowered available light to the coral. This could lower the zooxanthellae's nitrogen requirements and allow fractionation (isotopic depletion). Corals have been shown to become reliant on heterotrophy in non-limiting nutrient conditions, related to loss of control over

their zooxanthellae. This suggests the $\delta^{15}N$ of their tissues would reflect their diet (and therefore the source of contamination). The counteractive effects of these two factors suggests it may not be possible to resolve the source of eutrophication to corals using the isotopic signatures of their tissue. That is, if the contamination gradient is coincident with a gradient in the symbiotic relationship (i.e. from nutrient limited to nutrient non-limited) or trophic status, there is little possibility of resolution of sewage effects.

Results from the second sample suite from Zanzibar and a sample suite from Jepara Bay, Indonesia confirm this hypothesis. Zanzibar samples were collected at a eutrophied and non-eutrophied reef, and show no significant difference between isotopic data. This may be a result of distance from source, or the counter-effects of light. At Jepara Bay, samples were collected along a contamination gradient, from 2 point source discharges. Isotopic data show significant change along the gradient, with the entire bay eutrophied. Without $\delta^{15}N$ from all potential nitrogen sources it is impossible to adequately conclude whether sewage was the contaminant using isotopes. Other data collected from these reefs do support the interpretation of anthropogenic contamination. These data include decline in coral and fish communities (determined using biological assays, Zanzibar), and increased heavy metal and chlorophyll-a concentrations (Jepara).

One control on nitrogen isotope composition of modern corals was identified, and others suggested. Results will remain enigmatic until sample suites including complete water chemistry, source chemistry, and light regime are interpreted.

Preface

This thesis consists of three manuscripts currently submitted to various academic journals. These works were completed as a direct result of research conducted for, or as part of, my Master's thesis.

Chapter Two, **Evidence for Light as a Control of $^{15}N/^{14}N$ of Coral Tissue: Examples from Jamaica and Zanzibar**: a model of the controlling factors on nitrogen isotopes in coral tissues (specifically light). Written in conjunction with Ph.D. candidate Jeff Heikoop (also supervised by Dr. Mike Risk). Primary writing was conducted by Jeff Heikoop who also supplied data from Jamaica; Zanzibar sample suite, figures and editing by myself; Mike Risk provided scientific input, collected Zanzibar samples, and provided funding for analysis; Ian Sandeman provided data and scientific input; Henry Schwarcz provided scientific input. This manuscript is also contained in Jeff Heikoop's Ph.D. thesis.

Chapter Three, **Anthropogenic Effects on a Coral Reef Community, Zanzibar, Tanzania**: an assessment of reef health in Zanzibar. This was a preliminary attempt to apply nitrogen isotopes in a natural setting. This manuscript was written in conjunction with Mike Risk, who also funded the project, and collected samples; sampling, drafting, interpretation of results, statistical and laboratory analysis were conducted by myself; data on Zanzibar's fish community and its interpretation were provided by Chris Horrill. Raw data are included in Appendix One.

Chapter Four, **Tracing Anthropogenic Contamination in Jepara Bay, Indonesia**: was a second attempt to apply isotopes in a natural setting. This data set was collected after consideration of the Zanzibar results, and was the primary focus of my Master's research. Primary writing, data analysis, laboratory work, field sampling, and drafting were conducted by myself; Mike Risk provided scientific input; Tonny Bachtiar provided grain size and heavy metal analysis; Bill Morris provided scientific input and computer access. The complete data set is provided in Appendix One.

In addition to these manuscripts, forewords and additional chapters have been added in an attempt to keep these works in a logical progression and give them context.

Acknowledgements

Thanks to: Dr. Mike (Mtemi) Risk for the opportunity to do this research, the occasional near death experience, and his support throughout; Dr. Henry Schwarcz for use of his lab and equipment (most importantly, the mass spectrometer), and scientific contributions; Dr. Bill Morris for scientific contributions, computer access, countless discussions, review of manuscripts and moral support; Dr. R. G. Walker and Dr. P. M. Clifford for patiently reviewing manuscripts; Mr. (soon to be Dr.) Jeff Heikoop for endless hours of scientific (or not) discussion, editing, proofing and co-authoring; Mr. Martin Knyf, without whom my samples would be waiting for a mass spec. to this very day; Karen Edmundson from CCIW for help with chlorophyll analysis; Jodie Smith, Tonny Bachtiar and Tom Tomascik who were of great assistance during field work in Indonesia; Gwenn French and Daude Mukaka for their help in Zanzibar; Cam Tsujita for teaching me the wonders of time management and sharing the bitterness that grad students inevitably seem to develop; Richard McLaughlin for selflessly lending his glue stick at a critical time; and Mr. Lawrence West for helping me gain the confidence to complete this work. There are many other members of the McMaster geology community whom have greatly contributed to the quality of my life and my sanity in the last few years, not the least of whom are the women in the department office, who also deserve acknowledgement.

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Dedication

... to the African Savannah, the reefs of Northern Sulawesi, and the Canadian Rocky Mountains, places which have shown me the wonders of our world and the simplicity of life

... to David Blais, who has shared everything with me throughout this adventure, your support has been invaluable.

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Appendix One

Chapter One - Introduction and Background

Coral reefs are vital to this Earth. With ecosystems declining and biodiversity dropping rapidly, it has been said that we are currently in the greatest mass extinction event in the Earth's geologic history (Ward, 1994). It is almost inevitable that reefs' existence will be adversely affected in the future.

The habitat of coral reefs, composed primarily of hermatypic scleractinian coral, is generally oligotrophic shallow tropical and subtropical water (e.g. Darwin, 1842; Muscatine and Porter, 1977; Hallock and Schlager, 1986; Sammarco et al., 1995). Intrinsic to their habitat, many reefs are located offshore from Lesser Developed Countries (LDC's). LDC's direct considerable effort to achieving the technological advancements prevalent in many first world countries. This has often come at the expense of the environment. Through geologic time corals have evolved to cope with natural disturbances, however, they are often unable to cope with the increasing influence of man. The current rate of evolution of their habitat has left them susceptible to destruction if not extinction (Connell, 1978; Salvat, 1989).

Declining health of coral reefs is not a new issue; reef scientists have been observing it for decades. What is new is the recognition of *Homo sapiens* sapiens' role in reef demise. In 1993 a conference held in Miami discussed 'Global Aspects of Coral Reefs', and involved reef scientists from around the world. When asked to reach a consensus on the major threats facing the world's coral reefs, they arrived at this list of the top three

threats: siltation, sewage and overfishing. These 'threats' are all anthropogenic, and all are prevalent in LDC's.

Siltation occurs mainly as a result of run-off and excess sediment in rivers. This is largely a result of deforestation, construction and agriculture. The impacts on reefs of siltation include: smothering of corals by sediment, reduced water clarity, increased incidence of coral disease, and lowered coral recruitment. Many studies have shown these deleterious effects (for example: Cortés and Risk, 1985; Rogers, 1990).

Overfishing is a serious problem which disrupts the natural balance of the marine community. It may result in increases of bioeroders (e.g. urchins) which weaken coral, or algae which may overgrow it (Sammarco, 1982; McClanahan and Shafir, 1990; McClanahan and Mutere, 1994). Many LDC's depend on reefs as a major source of protein (Risk et al., 1993), making it difficult to see this situation improving through time. In fact, if fishing practices were to mirror population trends in these countries, fishing pressure on reefs could only be expected to rise.

This study focuses on the third of the three major concerns facing coral reefs, sewage. Sewage acts to kill coral by reducing water clarity, promoting fast growing organisms which smother the more slowly growing coral, and shifting the community structure to favour organisms which tend to harm coral or increase mortality, such as urchins and coral associates (Smith et al., 1981; Rose and Risk, 1985; Tomascik and Sander, 1985; Hallock, 1988; Bell, 1991; Lapointe and Clark, 1992).

Previous works have focused on biological and chemical assessments of reefs to reach conclusions on the level and effect of sewage contamination. We

sought to use geochemical indicators, specifically stable isotopes, to trace sewage contamination. This technique has successfully been used in sediments (e.g. Sweeney and Kaplan, 1980; Sweeney et al., 1980), and in reconstructions of food webs and trophic status (Rau et al., 1981; Mariotti et al., 1984; Keegan and DeNiro, 1988; Cifuentes et al., 1988). Applications of isotopes in a reef setting have been limited, even more so the applications of isotopes to trace sewage or eutrophication stress. Notably, Risk et al. (1989) and Allison et al. (1991) linked increases in $\delta^{15}N$ to contributions of human effluent to the reef environment.

Stable Isotopes as Tracers

Stable isotopes are atoms of the same element with different atomic masses resulting from varying number of neutrons (ex. ^{12}C and ^{13}C). These isotopes are naturally occurring and are stable throughout geologic time. Characteristic ratios of stable isotopes occur in different environmental settings, these ratios are measured relative to defined standards for each element. Measurements are generally reported in delta notation in units of per mille, such that: $\delta^{15}N = (((^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{standard}) -1)*1000$.

Isotopes have been used as tracers of fluxes in natural and disturbed environments. Their use as tracers is dependent upon a number of intrinsic properties. First, physical and chemical processes produce measurable differences in stable isotope ratios (Smith and Epstein, 1971). These differences are maintained or increased as they pass up the food chain (DeNiro and Epstein, 1978, 1981). In the case of carbon isotopes, there is little change between levels

of the trophic pyramid, with a typical fractionation of 1 ‰ at each step (e.g. DeNiro and Epstein, 1981; Keegan and DeNiro, 1988; Michener and Schell, 1994). Nitrogen isotopes fractionate by approximately 3-5 ‰ with each step up the food chain (Wada, 1980; DeNiro and Epstein, 1981; Minagawa and Wada, 1984). This fractionation is related to the retention by the animal of heavy isotopes in its body, with light isotopes preferentially excreted.

Relative differences in isotopes, characteristic of their source, are preserved within the tissues of animals or, more generally, in organic matter. Physical mixing of distinct or end member sources then determines the isotopic distributions of stable isotope ratios (see Sweeney and Kaplan, 1980, for an application in sediments; Owens, 1987, for a box model approach; and LeBlanc et al., 1989, for an application in an estuarine environment). The assumption that isotopic signatures can be related to source allows stable isotopes to be used as environmental tracers. The different relationships between individual isotopes and food webs make the use of isotopes as tracers more powerful if a number of isotopes are used in conjunction (Owens, 1987; Michener and Schell, 1994). The benefits of this have been shown repeatedly (for example: Rau et al., 1981; Sweeney et al. 1981; Cifuentes et al., 1988; LeBlanc et al., 1989). In general, carbon and nitrogen isotopes can be used to determine diet, trophic status, and variation in the natural environment.

Nitrogen

Nitrogen isotopes, specifically the ratio of $^{15}N/^{14}N$, vary significantly in the natural environment. They are reported in values of per mille (‰) relative to the standard for nitrogen, the atmosphere (i.e. atmospheric nitrogen is assigned a value of 0 per mille). A review compiled by Owens (1987) shows the natural

variation in $\delta^{15}N$ content from all major ecosystems (Table 1, see also Owens, 1987, Fig. 5). Although overlap exists between systems, much of this can be attributed to varying experimental design and technique. Noticeably, the terrestrial and marine systems have distinct signatures, suggesting any terrestrial contributions to the marine environment would be distinguishable. A review of nitrogen in the terrestrial environment has been given by Létolle (1980), and in the marine by Wada (1980).

Table 1. Mean $\delta^{15}N$ (‰) content of all materials, collected from the major ecosystems, as compiled by Owens, 1987.

Ecosystem	mean ± 1 s.d.
Atmosphere	1.6±1.4
Terrestrial	3.9±3.1
Freshwater	4.3±2.7
Estuarine	4.6±2.0
Marine	7.2±2.6

The effect of sewage contamination has been reported traceable in the marine environment using nitrogen isotopes (for example, Sweeney and Kaplan, 1980; Sweeney et al., 1980; Risk et al., 1989; Allison et al., 1991). Interestingly, Sweeney and Kaplan (1980) report effluent lighter than marine values, leading to a decrease in $\delta^{15}N$ of nearshore marine suspended particulate matter, whereas Risk et al. (1989) and Allison et al. (1991), show an enrichment. As Owens (1987) observes, each ecosystem should be considered on an individual basis, with relative differences between trophic levels noted, as well as the isotopic composition of the source.

Corals

The corals we are concerned with are scleractinian hermatypic corals. They have an aragonitic skeleton, are sedentary in their environment and lead to the development of reefs. These animals have developed a symbiotic relationship with microalgae (zooxanthellae, *Symbiodinium microadriaticum*), which inhabit scleractinian tissues (Oliver and Coates, 1987).

The relationship between zooxanthellae and corals is controversial. Zooxanthellae are dinoflagellates contained within coral tissues that survive through exchange of metabolic products with the coral. The relationship is puzzling because exactly what the two parties get out of it is not fully known. There is little doubt that corals benefit from the relationship: they have adapted to nutrient poor waters, by utilising light and harvesting photosynthates from the zooxanthellae (Lewis and Smith, 1971); developed an internal nutrient recycling facility (Szmant-Froelich and Pilson, 1977); and benefited from increased rates of calcification and a broadening of habitat ranges (Muscatine and Porter, 1977; Hinde, 1988). The zooxanthellae, however, are not necessarily as lucky, with energy budgets sometimes in their favour (Davies, 1984; Wilkerson and Trench, 1986), and sometimes suggesting they are captive slaves of the coral (Szmant-Froelich and Pilson, 1984; Cook and D'Elia, 1987; Muscatine et al., 1989a). In any case, the symbiosis has resulted in corals which may behave as sometimes plant and sometimes animal, or more technically, autotophs or heterotrophs. Recently, a workshop was conducted on the relationship between zooxanthellae and reef-building coral (1991). The results of this workshop (published by Jokiel

et al., 1994) indicate that translocation of carbon and metabolic products only occurs in nutrient-limited conditions, eutrophication allows zooxanthellae to outgrow their coral host and stop translocating. The implications of this are that the coral animal will become reliant on heterotrophy as a nutritional strategy in nutrient enriched conditions.

The sedentary nature of corals in a marine environment puts them at risk of environmental perturbations and also makes them scientifically interesting. The coral becomes a recorder of its environment, both in its skeleton and its tissues. Small-polyp corals, such as *Porites* sp. (studied here), are thought to function mainly as autotrophs (cf. Porter, 1976; Edmunds and Spencer Davies, 1986, 1989). In nutrient enriched conditions (e.g. increased particulate and dissolved organic matter), however, corals have been shown to rely on heterotrophy (Jokiel et al., 1994; Risk et al.,1994), using direct assimilation and absorption of DOM/POM as a potential feeding strategy. It is therefore possible to expect corals to record a variety of environmental perturbations in their tissues, with their isotopic composition reflecting their diet.

Purpose

If corals are in a nutrient enriched environment, then they can be assumed to be absorbing dissolved organic matter (DOM) directly, as a heterotrophic feeding strategy. Isotope ratios can be used to trace the source of this material, potentially allowing identification of sewage contamination on reefs.

One purpose of this thesis is to establish the use of nitrogen isotopes in coral tissues, with the specific aim of attempting to pinpoint sewage stress on coral reefs. A model assessing the geochemical factors affecting isotopic fractionation first needs to be derived. This model could then be applied in the form of two case studies, one from Zanzibar Island, Tanzania and one from Jepara Bay, Indonesia. This is achieved through three manuscripts, which are presented as chapters of this thesis.

Chapter Two-Modelling of Nitrogen Isotopes in Coral

Nitrogen isotopes in coral tissues had not previously been a large field of research, with few publications existing. Risk et al. (1989) and Allison et al. (1991) noted increased $\delta^{15}N$ in areas of elevated nutrient concentrations, but did not provide a mechanism. Sammarco et al. (1995) used isotopes to infer coral diet, suggesting terrestrial input influenced corals across the entire Australian continental shelf. In 1994, Muscatine and Kaplan reported a depletion of ^{15}N with increasing depth, and suggested a mechanism. They attributed their observations to "...light-sufficient, nutrient-limited zooxanthellae in shallow water and light-limited, nutrient-sufficient zooxanthellae in deep water." That is, fractionation increases with increasing depth, or the coral tissue will become isotopically lighter (depleted) in low light conditions (given the relationship, increasing depth equals an exponential increase in light attenuation).

Other previous research on nitrogen in corals had focused on tracing chemical fluxes within the tissues, with works of note including: D'Elia and Webb, 1977, explaining diffusion of nitrogen into coral tissues; Domoter and D'Elia, 1984, suggesting that zooxanthellae prefer ammonium; Szmant-Froelich and Pilson, 1984, demonstrating that the degree of translocation and recycling of nitrogen within the coral tissues is related to the amount of available nitrogen; Wilkerson and Trench, 1986, suggesting nitrogen recycling is efficient enough to supply all the zooxanthellae needs, and also that zooxanthellae are *not* dependent upon one form of nitrogen; and Muscatine et al., 1989, a study suggesting the symbiotic zooxanthellae contained in coral tissue are nitrogen limited.

Two sample suites were collected independently for this study: Heikoop collected a series of coral over a depth trend in Jamaica, attempting to understand the influence of light on the nitrogen signature. This suite involved multiple samples of multiple species. The second sample suite was collected in Zanzibar and consisted of a collection of three heads (of the same species) with multiple samples from each head over a depth trend. By collecting these samples over a single head, between species and between coral variability was removed, and the sole influencing factor on the nitrogen isotopes in these heads was light. Additional data were provided by Ian Sandeman, also collected from Jamaica, with incident light values collected from the tops and sides of coral heads.

Evidence for Light as a Control of $^{15}N/^{14}N$ of Coral Tissue: Examples from Jamaica and Zanzibar

Heikoop, J.M., Dunn, J.J., Sandeman, I., Risk, M.J., Schwarcz, H.P.

Abstract

Nitrogen isotope values are reported from two sample suites, from Jamaica and Zanzibar, composed of coral tissue collected over a depth/light gradient. The Jamaica suite consists of multiple samples from three species of coral (*Montastrea annularis*, *Porites astreoides*, and *Agaricia agaricites*) sampled at increasing depths. Nitrogen values are regressed against light attenuation, with each species showing a significant decrease in $\delta^{15}N$. The Zanzibar sample suite is comprised of three coral heads (all *Porites lobata*) with multiple samples collected along a depth gradient from the top to the base of each head. This suite also shows a significant decrease in $\delta^{15}N$ of the isotopic values with increasing depth. Light is interpreted as a control of the nitrogen isotopic composition in corals containing symbiotic zooxanthellae.

Introduction and Background:

Measurements of the natural abundance of nitrogen isotopes have been utilised to trace sewage (Risk et al., 1989; Allison et al., 1991; Risk et al., 1993) and other terrestrial inputs (Sammarco et al., 1995) to coral reefs and aid in the identification of nutritional sources in reef settings (Schoeninger and DeNiro, 1984; Keegan and DeNiro, 1988; Sammarco et al., 1995). Nitrogen

isotopes are useful in such studies because terrestrial and marine nitrogen sources are often isotopically distinct (Table 2 in Owens, 1987; Michener and Schell, 1994). Nitrogen isotopes can also be assumed to fractionate in a consistent manner along food chains, with enrichments of approximately 3‰ per trophic level identified (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). These fractionations result from excretion of isotopically light nitrogen.

The study of nitrogen fractionation during nutrient uptake and assimilation by primary producers (e.g. Wada and Hattori, 1978; Marriotti et al., 1984; Muscatine and Kaplan, 1994) has implications for the nitrogen isotopic composition of hermatypic corals. Corals thrive in nutrient limited conditions, largely due to the 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. Zooxanthellae assimilate carbon and nitrogen which can be translocated to the coral host (e.g. Muscatine et al., 1984) to supplement available exogenous food sources, including dissolved organic matter (DOM) and zooplankton (Muscatine et al., 1989b; Risk et al., 1994). The algal symbionts assimilate dissolved inorganic nitrogen (DIN) from the external environment (e.g. D'Elia et al., 1983; Wafar et al., 1985) and also assimilate nitrogenous wastes excreted by the coral (Szmant-Froelich and Pilson, 1977).

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 invoke increasing fractionation with increasing depth and light attenuation as a possible

control on $\delta^{15}N$ of the zooxanthellae. They propose that low rates of nitrogen assimilation under low light conditions could promote large fractionations (depletions) when sufficient nitrogen is available. This fractionation pattern would be analogous to that observed in cultured marine diatoms under similar conditions (Wada and Hattori, 1978).

The isotopic signature of the tissue component of the coral tissue/zooxanthellae symbiosis should generally be related to its diet (dissolved/particulate, inorganic/organic sources). Since corals (in combination with their symbionts) tend to conserve nitrogen (Szmant-Froelich and Pilson, 1977) stepwise enrichment of $\delta^{15}N$ relative to diet may not be obvious. In general, however, $\delta^{15}N$ of coral tissue will reflect weighted $\delta^{15}N$ of the relative contributions from both autotrophic and heterotrophic dietary food sources. The tissue trend with depth observed by Muscatine and Kaplan (1994) suggests either a change in trophic status or a change in $\delta^{15}N$ of diet. Increasing reliance on heterotrophy as a nutritional strategy has been confirmed for these samples using $\delta^{13}C$ determinations (Muscatine et al.,1989b).

This study expands on the work of Muscatine and Kaplan (1994) using combined coral tissue/zooxanthellae from two study areas. We analysed replicate samples of three species of coral over a 30 m depth range at Discovery Bay and were able to demonstrate a strong relationship between mean $\delta^{15}N$ and light attenuation. In addition, we examined $\delta^{15}N$ variation along a gradient 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 minimised. The results in both settings suggest that light is a dominant control on coral $\delta^{15}N$.

Materials and Methods:

Coral skeleton and adhering tissues (approximately 50 cm^2 per sample) were chiselled from the upper surfaces of coral heads of three species growing on the forereef at Discovery Bay, Jamaica (Table 1). Sampling was performed utilising SCUBA on two occasions, 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 before shipment to Canada. Similar sampling was performed in April 1993, on large heads of *Porites lobata* collected in shallow water (1.5 - 8.5 m depth from two small fringing reefs offshore Zanzibar, Tanzania (Table 2). These specimens (also of coral skeleton and tissue) were taken in 25 cm depth increments from the tops to the bases, of three large coral heads, and frozen prior to and during shipment.

All samples were divided into two aliquots. Aliquot one was prepared following LeBlanc et al. (1989) and Risk et al. (1994). The sample was placed in dilute hydrochloric acid (always less than 10% by volume) 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 skeleton which contained the endolithic algal layer directly beneath the coral surface. A second aliquot was placed in 6% sodium hypochlorite (bleach) to remove the tissue layer but preserve the skeleton. This second aliquot was taken as part of a companion study to be presented at a later date.

When the skeleton was completely dissolved from the first aliquot, the resulting tissue mat was thoroughly rinsed in distilled water and collected by straining the material through a fine nylon mesh. Repeated centrifugation removed excess water. Samples were then freeze-dried to ensure preservation. Attempts were made on several Jamaica samples to separate the zooxanthellae and tissue components of aliquot one. This material did not prove amenable to obtaining separated fractions of the required purity, largely as a result of the initial drying required to ensure preservation between the time of collection and sample preparation. Insufficient sample was available to attempt separation on Zanzibar samples. As such, 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 flame sealed. Samples were combusted at 550° C for 2 hours, one day prior to being analysed. 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 (gelatine and glutamic acid), as well as atmosphere standards for nitrogen, were analysed to estimate precision. Based on these samples, precision was ± 0.1‰ for carbon and ± 0.07 ‰ for nitrogen. The precision obtained from four replicates of one of the corals studied yielded ± 0.2‰ for carbon and ± 0.1 ‰ for nitrogen (all precisions are reported as ± 1 σ error).

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 acid strengths of 0, 5, 10 and 30 % hydrochloric acid (the 0% 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 analyses in the case of material dried prior to shipment). The first three treatments yielded identical results within measurement error. The 30% acid treatment resulted in a value nearly 1 ‰ depleted relative to the other tests. Aliquots of a second coral were left in 10% hydrochloric acid for 5, 10 and 15 days. All treatments yielded identical nitrogen isotopic ratios within measurement error.

$\delta^{15}N$ results are reported relative to 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. One-way ANOVA and simple linear regression analyses were performed in order to characterise the variations with depth and light recorded in the two data sets. Non-parametric Kruskal-Wallis tests were also performed as an alternative to One-way ANOVA. Statistical tests were considered significant when probabilities of $p<0.05$ were obtained.

Results:

Jamaica

$\delta^{15}N$ values of all corals examined in this study are reported in Tables 1 and 2, with a regression summary in Table 3. Nitrogen isotopic ratios of

Jamaica corals range from 4.3 to 0.1 ‰. A One-way ANOVA and Kruskal-Wallis test of the entire Jamaica data set reveals significant differences in the mean values of the five depths studied (both tests $p<0.001$). A two factor crossed design ANOVA was also performed to determine if species effects were significant in addition to depth effects; results revealed significant species and depth effects ($p<0.001$) and no significant interaction. As a result of this analysis, it was decided to examine the data on a species basis rather than as pooled data. *Porites* and *Agaricia* samples were collected in both 1993 and 1994, while most of the *Montastrea* data were from 1994 alone. Regression coefficients were determined for $\delta^{15}N$ versus decrease in available light (see below) for both *Porites* and *Agaricia* with data separated out by year and also combined. In each case the regression coefficients determined on data separated out by year did not differ significantly from one another or from combined data (based on the overlap of intervals defined by two standard errors about the regression coefficient estimates). Data for each species were therefore not separated out by year of collection but rather were combined for further analysis.

A summary of the Jamaica data(Fig. 1) indicates an overall decrease in $\delta^{15}N$ with depth. One-way ANOVA and Kruskal-Wallis tests indicate there are significant differences in the mean $\delta^{15}N$ values at the five depths for each of the coral species studied (all tests $p<0.05$). Qualitative observation of the data suggests that, with the exception of *Agaricia*, the rate of change decreases with depth. The data were also plotted against a yearly average of light attenuation measured in-situ (light available to a horizontal surface, Sandeman, unpublished

data) on the forereef of Discovery Bay (Fig. 2); $\delta^{15}N$ decreases with decreased light availability.

Since replicates were available for each depth of the Jamaica data set, pure error tests were performed on each regression to test the appropriateness of the simple linear regression model (all other statistical tests pertaining to regression of the Jamaica data ignored the presence of replicates). In each case the linear model was adequate so higher order polynomial regressions were not performed. Coefficients of determination (R^2) and tests for goodness of fit of the regression were also calculated (Table 3). With the exception of *Agaricia*, $\delta^{15}N$ data have a slightly stronger relationship with depth than light availability (R^2=46.6 % with depth as dependant variable).

The decrease in light availability explains between 40 to 80% of the variation in $\delta^{15}N$ of corals from Discovery Bay (Table 3). The decrease in $\delta^{15}N$ with light attenuation is strongly significant in each case. If simple linear regression of the mean $\delta^{15}N$ values with light is performed, then 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. In general, it is not possible to say that there are any differences between the slopes of the regressions of *Porites*, *Montastrea* and *Agaricia*. Intervals defined by two standard errors (or 95% confidence intervals) about the slopes all overlap. Of the three species studied, however, the data suggest that $\delta^{15}N$ of *Montastrea* decreases the least rapidly with decreasing light availability. The *Porites* $\delta^{15}N$ intercept (0 % decrease in light availability) is significantly higher than that of *Montastrea* or *Agaricia*. 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*) (Figs. 1,2) is a function of differences in the year of collection (despite the fact regression slopes do not vary significantly between the two collection years).

A summary diagram of $\delta^{13}C$ values of Jamaica corals (Fig. 3) shows an overall trend similar in form to that of the $\delta^{15}N$ (Fig. 2). Individual species studied follow trends similar to that of the combined data, with the carbon isotopic data become more depleted in ^{13}C over the upper half of the depth range (closest to the surface) before levelling out at greater depths. There is a slight increase in average $\delta^{13}C$ at 30 m relative to 22 m depth (increase is significant, p=0.016,Student's t-test).

Zanzibar

$\delta^{15}N$ data from the three Zanzibar *Porites lobata* coral heads (Fig. 4) all display an overall decrease with the log of depth. The regressions shown are highly significant and explain between 75 - 90% of the variation in $\delta^{15}N$ (Fig. 4, Table 3). Heads 1 and 2 were collected from the same fringing reef, while Head 3 was collected from a fringing reef approximately 10km distant. The regression coefficients for Head 1 and Head 2 are not significantly different (see standard errors on these values in Table 3). Given the large standard error for Head 3, it is not possible to state that the slope of the $\delta^{15}N$ versus log depth regression is significantly greater than that of Heads 1 and 2. The intercept term of the Head 3 regression equation does appear to be significantly higher, but this head was collected at a greater depth than Heads 1 and 2 (Table 2). Since we are more

interested in variation of $\delta^{15}N$ over each coral head than sample distance from the surface, it is useful to compare $\delta^{15}N$ values estimated from the regression corresponding to the depth of the top of each large head. The $\delta^{15}N$ values calculated in this manner are 5.24, 5.34 and 5.18 ‰ for Head 1, Head 2 and Head 3 respectively. The 95% confidence intervals on these regression estimates all overlap indicating the tops of the heads all have essentially identical $\delta^{15}N$.

The $\delta^{13}C$ data corresponding to these heads will be presented as part of the companion study mentioned previously. In general, the data show a decrease (depletion of ^{13}C) with depth. When the data are regressed against the logarithm of depth, the simple linear regression model explains 40 - 65 % of the observed $\delta^{13}C$ variance. The decrease in $\delta^{13}C$ with depth is only significant at the 10% significance level for Head 2 and Head 3 and is not significant for Head 1.

Figure 1. $\delta^{15}N$ versus depth for samples from Discovery Bay, Jamaica. Means are plotted as closed squares, and the data ranges are as indicated. Individual graphs are plotted for each species and the combined data.

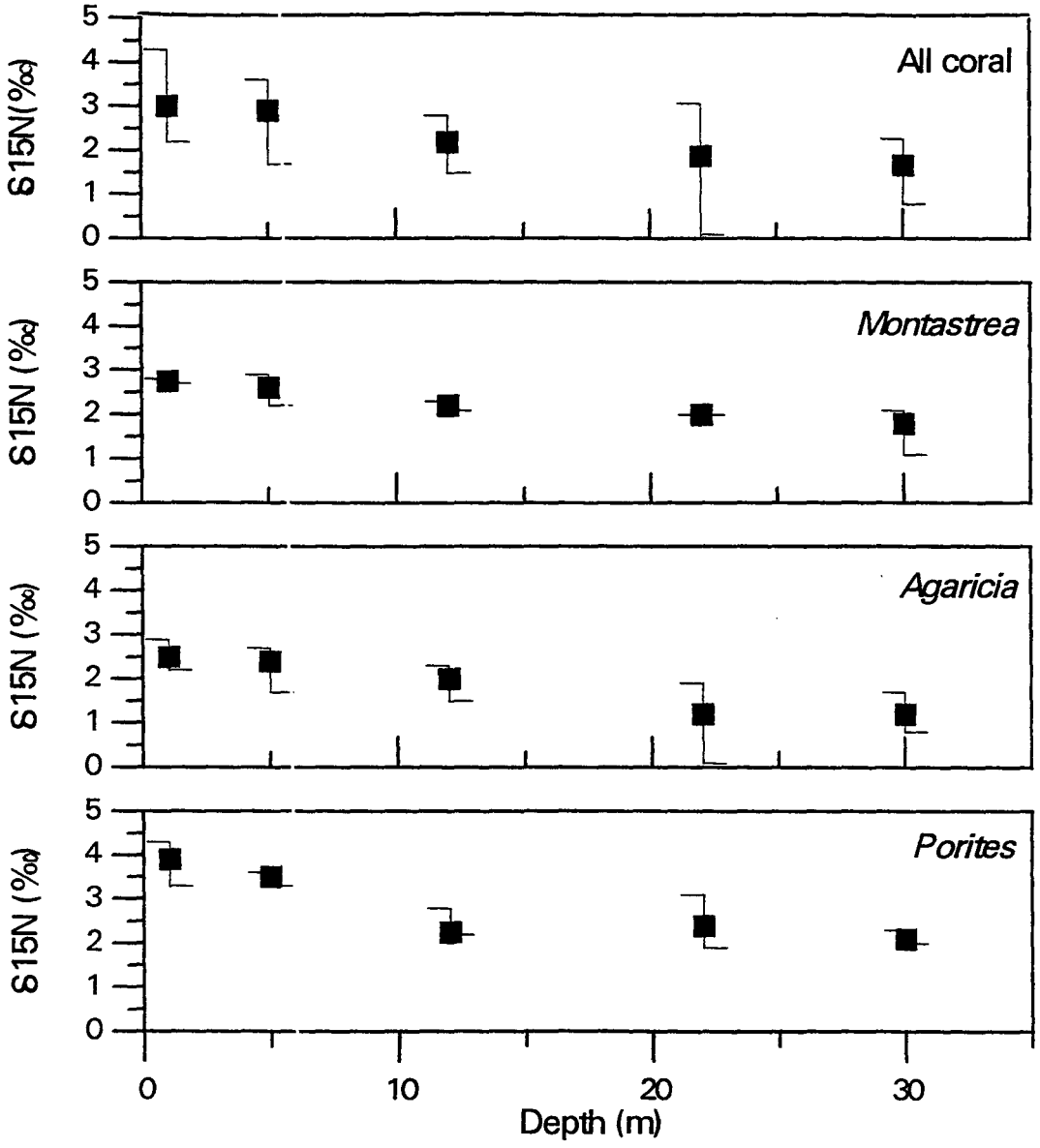


Figure 2. $\delta^{15}N$ versus decrease in light (%) for samples from Discovery Bay, Jamaica. Means are plotted as closed squares, data ranges are shown. Regression lines are shown, plotted for mean values. Note the decrease in isotopic values with depth.

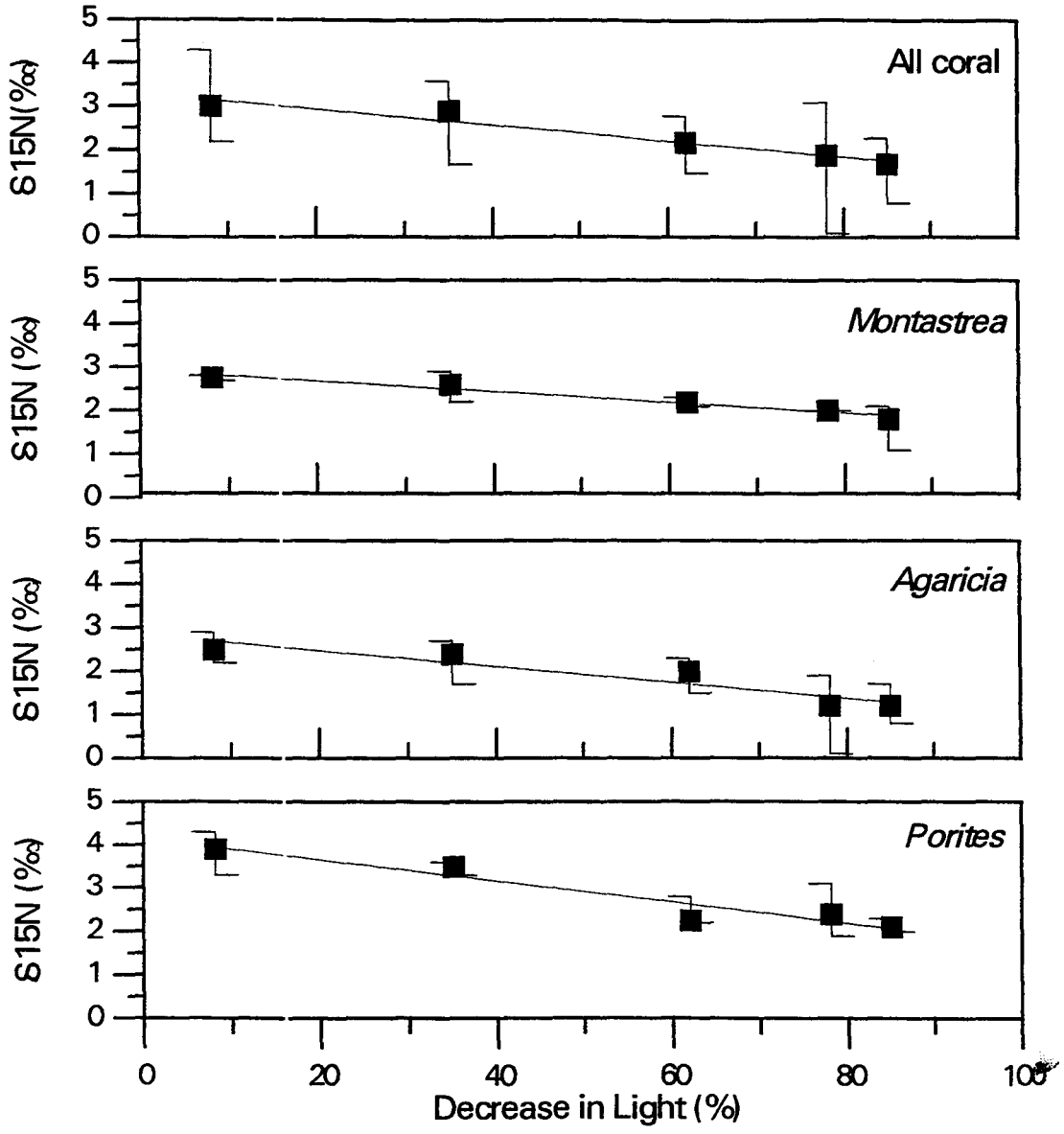


Figure 3. $\delta^{13}C$ versus depth for all corals from Discovery Bay, Jamaica. Note the similarities between the C and N trend. Means are plotted as closed squares, data ranges are shown.

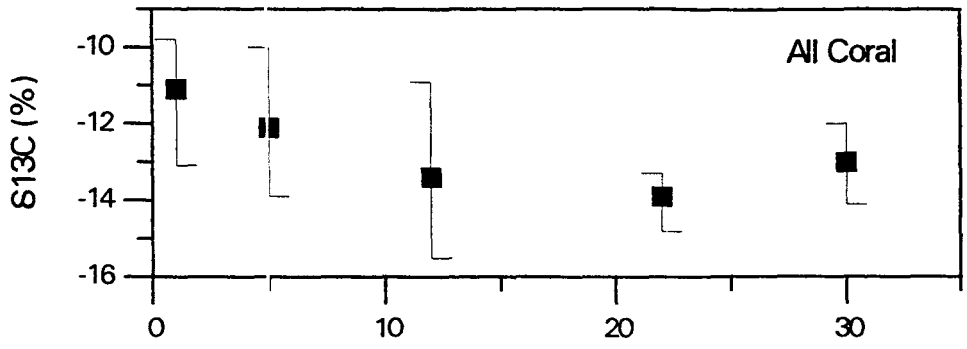


Figure 4. $\delta^{15}N$ versus log of depth for samples from Zanzibar. All samples are from the same coral species, regression lines are shown.

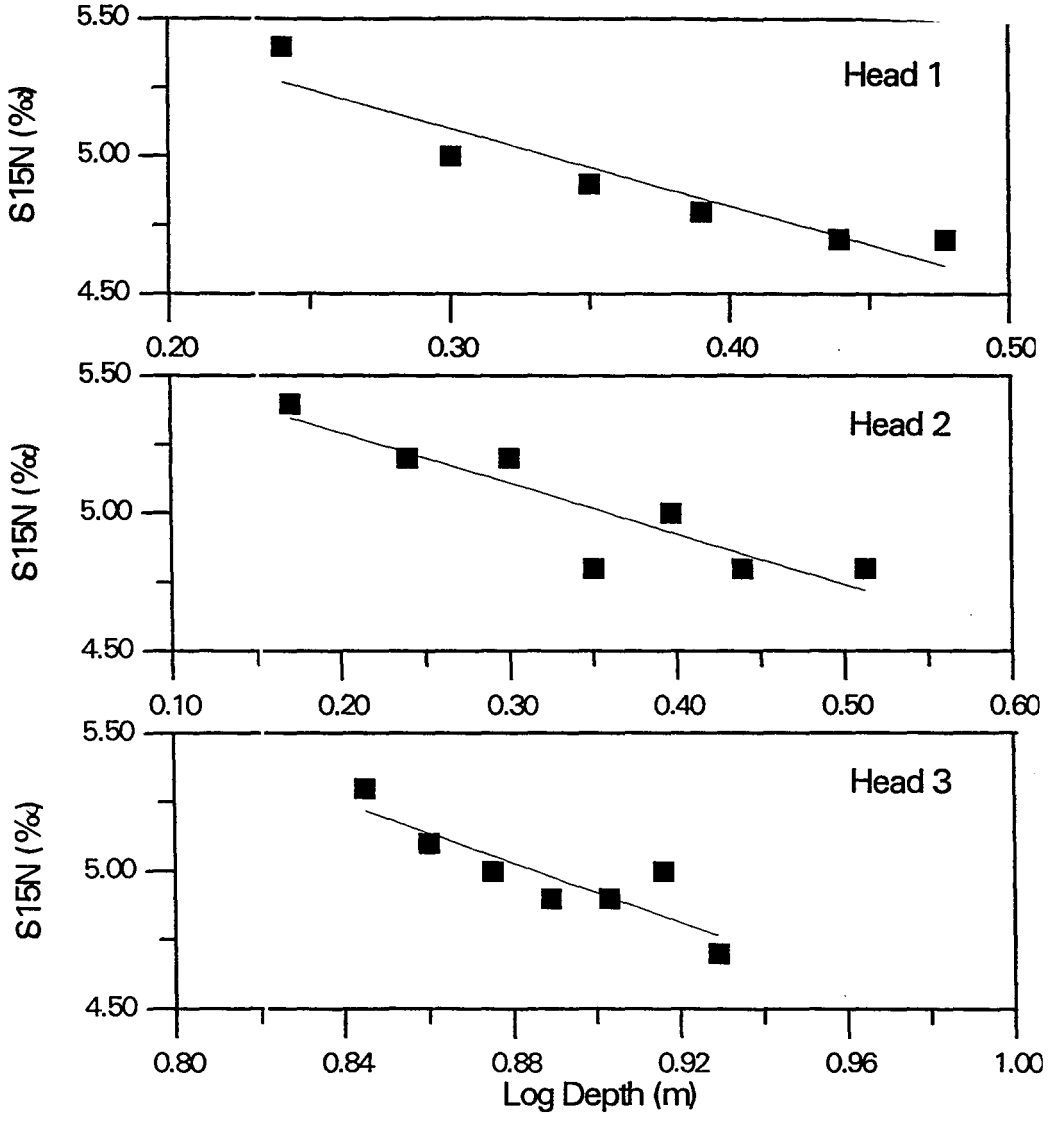


Table 1. Data from Discovery Bay, Jamaica

Sample	$\delta^{15}N$ (‰)	Depth (m)	Genera	Year of Collection
ap1	4.3	1	*Porites*	1993
ap2	4.1	1	*Porites*	1993
ap3	3.8	1	*Porites*	1993
aag	2.2	1	*Agaricia*	1993
1p1	3.3	1	*Porites*	1994
1ag2	2.9	1	*Agaricia*	1994
1ag3	2.4	1	*Agaricia*	1994
1ma1	2.7	1	*Montastrea*	1994
1ma2	2.8	1	*Montastrea*	1994
bp1	3.6	5	*Porites*	1993
bp2	3.3	5	*Porites*	1993
bag1	1.7	5	*Agaricia*	1993
bag2	2.3	5	*Agaricia*	1993
5p1	3.6	5	*Porites*	1994
5p2	3.6	5	*Porites*	1994
5p3	3.6	5	*Porites*	1994
5p4	3.5	5	*Porites*	1994
5ag1	2.5	5	*Agaricia*	1994
5ag2	2.6	5	*Agaricia*	1994
5ag3	2.7	5	*Agaricia*	1994
5ag4	2.7	5	*Agaricia*	1994
5ma1	2.2	5	*Montastrea*	1994
5ma2	2.9	5	*Montastrea*	1994
5ma3	2.6	5	*Montastrea*	1994
5ma4	2.8	5	*Montastrea*	1994
cp	2.2	12	*Porites*	1993
cag1	2.2	12	*Agaricia*	1993
cag2	1.6	12	*Agaricia*	1993
cag3	1.5	12	*Agaricia*	1993
12p1	2.8	12	*Porites*	1993
12ag1	2.2	12	*Agaricia*	1993
12ag3	2.0	12	*Agaricia*	1993
12ag4	2.3	12	*Agaricia*	1993
12ma1	1.9	12	*Montastrea*	1994
12ma2	0.1	12	*Montastrea*	1994

continued...

dp	1.6	22	*Porites*	1993
dag1	1.9	22	*Agaricia*	1993
dag2	1.6	22	*Agaricia*	1993
dag3	1.9	22	*Agaricia*	1993
dag4	1.0	22	*Agaricia*	1993
22p2	3.1	22	*Porites*	1994
22p3	2.6	22	*Porites*	1994
22ag2	2.1	22	*Agaricia*	1994
22ag3	2.5	22	*Agaricia*	1994
22ma1	2.0	22	*Montastrea*	1994
ep	2.0	30	*Porites*	1993
ema1	1.1	30	*Montastrea*	1993
ema2	2.1	30	*Montastrea*	1993
30p1	2.0	30	*Porites*	1994
30p2	2.3	30	*Porites*	1994
30ag1	0.8	30	*Agaricia*	1994
30ag2	1.7	30	*Agaricia*	1994
30ag3	1.0	30	*Agaricia*	1994
30ma2	2.0	30	*Montastrea*	1994
30ma3	2.0	30	*Montastrea*	1994

Table 2. Data from Zanzibar

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
GH1115	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	2.50	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	8.5	4.7

Table 3. Results of Statistical Analyses from Jamaica and Zanzibar.

Y ($\delta^{15}N$)	X	Slope (m)	Std Error of (m)	Y-Intrcpt (b)	Std Error of (b)	p	R^2 (%)
Jamaica							
All Corals	% Δ light	-0.0212	0.0032	3.50	0.19	<0.001	45
Porites	% Δ light	-0.0240	0.0032	4.23	0.17	<0.001	78
Montastrea	% Δ light	-0.0141	0.0033	3.03	0.20	0.001	62
Agaricia	% Δ light	-0.0178	0.0046	2.92	0.28	0.001	40
Zanzibar							
Head1	log depth	-2.80	0.47	5.93	0.18	0.004	90
Head2	log depth	-1.93	0.41	5.68	0.15	0.005	82
Head3	log depth	-4.47	1.13	8.96	1.00	0.011	76

Discussion:

The decrease in $\delta^{15}N$ of the Jamaica data (Fig. 1), is consistent with that found by Muscatine and Kaplan (1994) for separated tissue and zooxanthellae. It is likely, therefore, that the observed shifts in $\delta^{15}N$ (Figs. 1,2) reflect simultaneous depletions in the coral animal and algal symbiont. Several factors could potentially explain the decrease in $\delta^{15}N$ with depth, including variations in $\delta^{15}N$ of the external seawater DIN pool, changes in the concentration of DIN with depth, trophic level shifts, and changes in zooxanthellar fractionation related to light attenuation (Muscatine and Kaplan, 1994).

The strong correlation between $\delta^{15}N$ of Jamaica corals and light availability between 0-30 m depth (approximately 200-1200 $\mu Ein/m^2/s$) (Fig. 2, Table 3) supports the hypothesis that light is an important factor. Assimilation of DIN by phytoplankton involves discrimination against the heavier isotope of nitrogen, leading to a fractionation relative to source (e.g. Wada and Hattori, 1988, Marrioti et al., 1984). For example, during light limited, but nitrogen sufficient conditions, assimilation by cultured marine diatoms leads to large nitrogen isotopic fractionations (Wada and Hattori, 1988). When the available DIN substrate is limiting however, fractionation decreases. In marine diatoms, this is seen during rapid growth associated with high irradiance but nitrogen limiting conditions (Wada and Hattori, 1988). Muscatine and Kaplan (1994) suggest that zooxanthellae behave similarly to phytoplankton to explain the increase in zooxanthellar fractionation with depth (or decreased light availability). Slower zooxanthellar nitrogen assimilation (see discussion of nitrogen specific growth rates in Muscatine and Kaplan, 1994), associated with decreases in light availability, would promote fractionation under nitrogen sufficient conditions.

Since zooxanthellae are contained within the microenvironment of the coral tissue, their immediate source of DIN is contained within the internal tissue pool (we will refer to this nitrogen source as the internal DIN pool, analogous to the internal dissolved inorganic carbon (DIC) pool (e.g. Muscatine et al., 1989b). The initial $\delta^{15}N$ of the internal DIN pool reflects $\delta^{15}N$ of contributions from the larger external (seawater) DIN pool. This initial value could then be modified by inputs of nitrogenous wastes from the coral animal and/or fractionation during uptake or assimilation of DIN by zooxanthellae. Corals in higher light regimes, with greater dependence on autotrophy and smaller zooxanthellar fractionation of nitrogen, will tend to have values closest to the external DIN pool (assuming conservation of nitrogen and minimum fractionation during diffusion of DIN into the coral tissue). It follows that, the external DIN pool at Jamaica would have a $\delta^{15}N$ signature of about 3-4 ‰ (refer to intercept values for regressions in Table 2), well within the range of $\delta^{15}N$ of marine dissolved nitrogen compiled by Owens (1987).

Muscatine et al. (1989) found depletions in ^{13}C of both tissue and zooxanthellae samples at Discovery Bay with depth. The authors argued that under the high irradiance conditions of shallow water, photosynthetic uptake of carbon was more rapid than the rate of diffusion of bicarbonate into the internal DIC pool, effectively eliminating the potential for fractionation. With increasing depth and decreasing irradiance however, fractionation towards lower $\delta^{13}C$ values could occur. This model was based on the diffusion/depletion hypothesis by which zooxanthellae are thought to acquire nutrients (phosphate, nitrate, ammonium) (D'Elia and Webb, 1977; D'Elia et al., 1983). Since DIN assimilated by zooxanthellae must first diffuse through the tissue barrier the rate of

assimilation versus rate of diffusion could impose another constraint on the magnitude of nitrogen fractionation by zooxanthellae under different light regimes.

It is more difficult to understand how decreasing light could cause depletion of $\delta^{15}N$ in the tissue component of corals from Discovery Bay. To explain this phenomenon, it is useful to examine the separated tissue $\delta^{15}N$ measurements generated by Muscatine and Kaplan (1994). To understand the trends observed in $\delta^{15}N$ of the tissue component it is also useful to examine the variation in $\delta^{13}C$ as an indicator of trophic status (this study, corresponding samples Muscatine et al., 1989b). Muscatine et al. (1989b) found that the tissue component of corals from Discovery Bay, Jamaica, became increasingly depleted in ^{13}C with depth at a greater rate than for the zooxanthellae. They attributed this to increased reliance on isotopically depleted heterotrophic food sources (eg. plankton) under decreased irradiance conditions, corresponding to decreased zooxanthellar activity. Our $\delta^{13}C$ data (Fig. 3) suggest an overall decrease with depth which is similar to that found by Muscatine et al. (1989b) for separated coral tissue and zooxanthellae. With increasing reliance on exogenous food sources at greater depths, the tissue component of the corals would become increasingly enriched in ^{15}N. We base this assertion on the fact that zooplankton (we will assume, for the sake of argument, that the exogenous food source is zooplankton) tend to be enriched relative to phytoplankton upon which they feed and to DIN (Minagawa and Wada, 1984). In other words, in deeper water, the coral animal will be acting more as a secondary consumer rather than a primary consumer (herbivore feeding on translocated photosynthate) and should be correspondingly enriched (cf. DeNiro and Epstein,

1981, Minagawa and Wada, 1984). If the coral zooxanthellae symbiosis is excreting nitrogen, then the coral would tend to be isotopically enriched relative to dietary zooplankton.

To test this assertion, we measured $\delta^{15}N$ of two zooplankton samples taken from the Discovery Bay forereef at 10 m depth in March, 1993. The samples were collected at 7:30 a.m. utilising a plankton net with an effective mesh size of approximately 100 µm. The zooplankton consisted largely of 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. Minagawa and Wada, 1984). This low value may reflect an isotopically light source of DIN at the base of the plankton food chain (e.g. nitrogen fixation, Wada and Hattori, 1976). It is unknown whether these samples are representative of the $\delta^{15}N$ of the zooplankton being consumed by corals at depth on the Discovery Bay forereef, or whether the zooplankton actually being utilised would be enriched relative to these samples. Different size classes of zooplankton have been shown to have different isotopic signatures (Minagawa and Wada, 1984). In either case it seems unusual that a diet increasingly reliant on zooplankton with depth could yield a negative slope of $\delta^{15}N$ versus depth (Figs. 1 and 2). It must be acknowledged that there could be some other exogenous food source such as dissolved organic matter, isotopically depleted relative to DIN, which becomes an increasingly important part of coral diet with depth. Any such food source would also have to have a low $\delta^{13}C$ signature to reconcile the carbon data.

Muscatine and Kaplan (1994) also reported the $\delta^{15}N$ of the tissue component becoming isotopically lighter over most of depth range, not heavier

as would be expected from increasing reliance on an enriched heterotrophic food source. This fact can be reconciled by the very large depletions in the zooxanthellae $\delta^{15}N$ with depth (see Table 1 in Muscatine and Kaplan, 1994). It would appear that the autotrophic contribution to the diet of the coral animal, although perhaps not large at depth, is sufficiently depleted to result in low $\delta^{15}N$ tissue values. By affecting $\delta^{15}N$ of zooxanthellae (and hence $\delta^{15}N$ of nitrogen translocated to the host), light is having an influence of $\delta^{15}N$ of coral tissue.

Enriched tissue values were recorded by Muscatine and Kaplan (1994) at 50 m depth for *Montastrea annularis* and *Montastrea cavernosa*. These values could reflect dominance of a heterotrophic dietary signal (Muscatine and Kaplan, 1994). That is, in very low light conditions, where zooxanthellae are less active, the relationship between light and the nitrogen isotopic compostion of the coral may not be as significant of a control of $\delta^{15}N$ of the tissues (i.e. we can only assert the strong relationship between coral $\delta^{15}N$ and light over the range 200-1200 μEin/m^2/s). These data also supports the contention that a heterotrophic diet source would tend to be enriched. 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). This suggests that *Porites* relies more on autotrophy as a nutritional strategy at any given depth than *Montastrea*. This is consistent with polyp size and morphology of these corals (cf. Porter, 1976, 1985; Edmunds and Spencer Davies, 1986, 1989).

It should be noted that, based on the above argument, zooxanthellae in deeper water corals cannot be obtaining all of their nitrogen from catabolism of a zooplankton diet. If this were the case, isotopic mass balance would require that the combined tissue/zooxanthellae $\delta^{15}N$ closely resemble that of zooplankton

and the trend with depth would be expected to be one of enrichment rather than depletion. It has been demonstrated that zooxanthellae do not receive all of their nitrogen requirements from the coral host (Szmant-Froelich and Pilson, 1977, 1984). Based on this fact it has even been suggested that the zooxanthellae coral relationship is not a true symbiosis. The fact that there is a second source of nitrogen available to zooxanthellae (seawater DIN) helps account for the observed trends.

The recognition of variation in external DIN as a factor to the $\delta^{15}N$ must be considered (Muscatine and Kaplan, 1994), despite the strong correlation with light in the Jamaica samples. Variation could arise from phenomena including groundwater inputs (D'Elia et al., 1981), Ekman upwelling, and anthropogenic inputs. Additionally, with all light utilising organisms depleting the external DIN pool, the remaining DIN and the total photosynthetic biomass will become enriched. This could create a gradient in DIN over the reef associated with the corresponding gradient in photosynthesis (i.e. DIN could be enriched near surface because of photosynthesis). Evaluating any of these potential scenarios is complicated by factors such as the degree of mixing over the reef (temperature and salinity are nearly constant over the reef suggesting efficient mixing) and the time of the day the corals are assimilating the majority of their nitrogen either from DIN or exogenous sources. Until $\delta^{15}N$ measurements of seawater at various depths and nitrogen inputs to the reef at Discovery Bay are available it will not be possible to properly test these hypotheses.

Concentration of DIN is another potential factor (Muscatine and Kaplan, 1994) that could cause isotopic shifts in opposing directions. If greater concentrations of DIN were available in surface waters (perhaps as a result of

eutrophication), and nitrogen was no longer a limiting nutrient to corals, then the coral/zooxanthellae symbiosis would be more likely to excrete isotopically depleted nitrogen, yielding isotopically enriched tissues by mass balance. Alternatively, however, zooxanthellae might fractionate nitrogen more strongly under conditions of nutrient enrichment, leading to depleted coral $\delta^{15}N$ (Muscatine and Kaplan, 1994). It is difficult to evaluate the effect concentration would have on $\delta^{15}N$ of corals until controlled experimental work is performed.

Notwithstanding the strong correlation between $\delta^{15}N$ of Jamaica coral tissue and light attenuation, we cannot conclude certainly that light is the dominant factor responsible for the observed variations (Fig. 2, Table 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, $\delta^{15}N$ or concentration of DIN in seawater can be assumed constant, with the main factor varying over a coral head being light. Although we lack light data from the reefs studied in Zanzibar, we can use appropriate data from Discovery Bay (Sandeman, unpublished data) 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 at the surface is about 80 %. Therefore, based on Discovery Bay data, we can say that the variation in light over a 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. The samples ranged in each case 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. 4) likely reflect variations in coral head morphologies. To understand the exact relationship between $\delta^{15}N$ and orientation of coral surfaces, light values would have to be measured for each sampling location over a coral head.

In general, 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 also show an overall decrease in $\delta^{13}C$ with depth, analogous to the situation at Discovery Bay. Both sites experience decreasing $\delta^{15}N$ with decreasing light availability, but in the case of Zanzibar, light is the most obvious causative factor. Samples taken from the very tops of Head 1 and Head 2 (receiving maximum insolation), collected from the same reef at the same depth, both yield very similar $\delta^{15}N$ values. although we cannot use this 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 causes a large part of the observed variation.

If we accept that light is a major factor in both the Zanzibar and Jamaica trends, nitrogen fractionation during assimilation of DIN is not the only potential light related mechanism that may be a factor. All zooxanthellae are currently classified as *Symbioclinium microadriaticum* , even though there are numerous

types of zooxanthellae with vast morphological variation. Different strains of zooxanthellae are found both between and within individuals of a host species. In addition, zooxanthellar physiology in corals changes in response to different light regimes (Sandeman, unpublished data). If these different assemblages and physiologies are associated with different degrees of nitrogen fractionation, a light related $\delta^{15}N$ trend could develop. Another potential factor is the relative proportion of zooxanthellae to coral tissue over a light gradient. If zooxanthellae are isotopically depleted relative to tissue (this should certainly be true at greater depths if zooplankton are being consumed by the coral), then an increase in the proportion of zooxanthellae with decreasing light could lead to the observed trends for combined tissue/zooxanthellae. At Discovery Bay, zooxanthellar concentrations do not increase with depth, but rather the amount of pigment in the zooxanthellae increases (Sandeman, unpublished data). Such changes in pigment concentration could also affect zooxanthellar physiology and nitrogen fractionation. Temperature can also affect zooxanthellar activity, but since temperature is relatively constant over the depth range of interest at Discovery Bay, it is likely not a factor in this case.

The final point we will consider is some of the other sources of variation in the Jamaica $\delta^{15}N$ data. There is considerable variation in the range of values about the means at each depth (Figs. 1,2). Some of this variation will simply be a function of physiological variability between corals, within both the plant and animal (zooxanthellae) components of the symbiotic relationship. Light could also be a factor in this variability. $\delta^{15}N$ was regressed against the decrease in light being received by the tops of coral heads (i.e. a horizontal surface) (Fig 2). Not all of the coral samples obtained, however, were oriented exactly horizontal,

especially the *Agaricia* samples. There will also be small scale light variation related to the type of substrate surrounding the coral, with different substrate types absorbing or reflecting light in different manners (Brakel, 1979).

Another source of variability is the differences between species. In general, the regression coefficients are similar for all three species, suggesting that there is not a lot of variability attributable to this source. One exception however, is the significantly higher surface value (y- intercept) for *Porites* (Table 3). The $\delta^{15}N$ of *Porites* at each depth tends to be higher than that of the other two species. The small polyps of this coral may imply a strong reliance on autotrophy as a nutritional strategy (cf. Porter, 1976). High rates of nitrogen assimilation, together with a thick tissue layer (Muscatine et al., 1989), would tend to promote strong depletion of the internal DIN pool and inhibit diffusion, thereby reducing zooxanthellar fractionation. While the platy morphology of *Agaricia* also implies a high degree of reliance on autotrophy, this coral has a very thin tissue layer which will be less of a barrier to diffusion of DIN. *Montastrea annularis* has a thick tissue layer but is likely not as efficient an autotroph as the other two coral species in shallow water. The differences between the three species could also be a function of different proportions of zooxanthellae to coral animal or different zooxanthellae suites with different fractionation factors. Although the slopes of the three coral species are not significantly different it is interesting to note that the coral regarded as the best carnivore of the three has the lowest slope. If *Montastrea annularis* obtains more of its diet from heterotrophy than the other two species, the tissue component will tend to be relatively more enriched in ^{15}N at any given depth. It is not obvious why $\delta^{15}N$ data for *Agaricia* correlate more strongly with depth than

light. It is possible that the platy morphology of this coral is so efficient at optimising available light that it is less sensitive to light variation with depth. Changes in relative orientations of *Agaricia* heads (in an attempt to optimise photosynthesis) with depth could also confound any correlation with light availability to a horizontal surface.

Conclusions:

Symbiosis, conservation of nitrogen 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. The Jamaica data is consistent with $\delta^{15}N$ of separated zooxanthellae and tissue collected from the same site (Muscatine and Kaplan, 1994). By analysing 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. Although we cannot conclude that light is the sole factor leading to the observed trends, the strong correlation suggests it is certainly a variable which must be considered in future coral $\delta^{15}N$ studies, as well as $\delta^{15}N$ of all DIN sources. Data from Zanzibar coral, collected over single heads, show variation in $\delta^{15}N$ of the same sense and appropriate magnitude. The absence of other factors, strongly supports this contention that light is an important control on $\delta^{15}N$. Light variation 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.

Acknowledgements:

The authors wish to thank Dr. J. Lott from University of Guelph for collection of plankton samples, and Martin Knyf for his invaluable assistance during stable isotope analysis. Funding was provided by NSERC operating grants to MJR and an ICOD grant (Zanzibar) held jointly by University of Guelph and McMaster University.

Chapter Three - Zanzibar

Nitrogen isotopes are recognised as being able to trace sewage contamination (Sweeney and Kaplan, 1980; Sweeney et al., 1980). Initial research (Risk et al., 1989; Allison et al., 1991) applying this in a coral reef setting reported enrichments of $\delta^{15}N$, related to inputs of human effluent. Preliminary research in Zanzibar was conducted in the summer of 1992 (addressed in my bachelor's thesis, Dunn, 1993), in an attempt to further the use of isotopes as tracers of sewage contamination on reefs. Although an elevation of $\delta^{15}N$ was found at a eutrophied site, the difference was not statistically significant. These results raised questions concerning the interpretation of nitrogen isotopic signatures from coral tissues.

Follow-up research was conducted in April 1993 with the collection of two sample suites. The first suite was utilised in Chapter 2, to develop a model for controlling factors on nitrogen isotopes. The second suite, utilised here, allowed confirmation of preliminary results from Zanzibar, and an extended interpretation.

Two reefs with different levels of anthropogenic impact were surveyed, using biological and geochemical techniques. Biological field techniques (quadrats and transects) were used to assess ecosystem 'health'. This type of assay is the commonly used method of determining impacts on coral reefs (Smith et al., 1981; Tomascik and Sander, 1985). While these methods of assay allow inference into the state of decline, they do not allow speculation about the source of any possible contaminants. Geochemistry was conducted in an

attempt to pinpoint a source, using the assumption that distinct isotopic signatures are preserved (see Michener and Schell, 1994). The combination of these two methods of assay should allow construction of a complete picture of both 'health' and possible source of contamination.

Anthropogenic Effects on a Coral Reef Community, Zanzibar, Tanzania

Michael J. Risk[1], Jennifer J. Dunn[1], Christopher Horrill[2]

Abstract

Two reef communities offshore Zanzibar Island, Chumbe and Chapani, are described, with anthropogenic stress more evident on the reef in close proximity to the town of Zanzibar (Chapani Island). Both the coral and fish communities show a decline in species diversity and shift in species composition at Chapani. An increase in numbers is seen in the sea urchin community and the coral associate community (associates are defined as the sessile organisms occupying coral heads). The coral community at Chapani shows signs of stress with decreased live coral. Stable isotope data show an increase in $\delta^{15}N$ at Chapani, interpreted as a potential indicator of eutrophication. The changes in the reef community are seen as a direct result of human impacts.

Introduction

Zanzibar Island is the largest of Tanzania's coastal islands, about 80 km by 35 km (Fig. 1). One of the major tourist attractions on Zanzibar is the fringing reefs, which occur mostly on the eastern coast of the island. Massive tourism

developments are projected, almost all of which will be on the east coast, featuring the reefs.

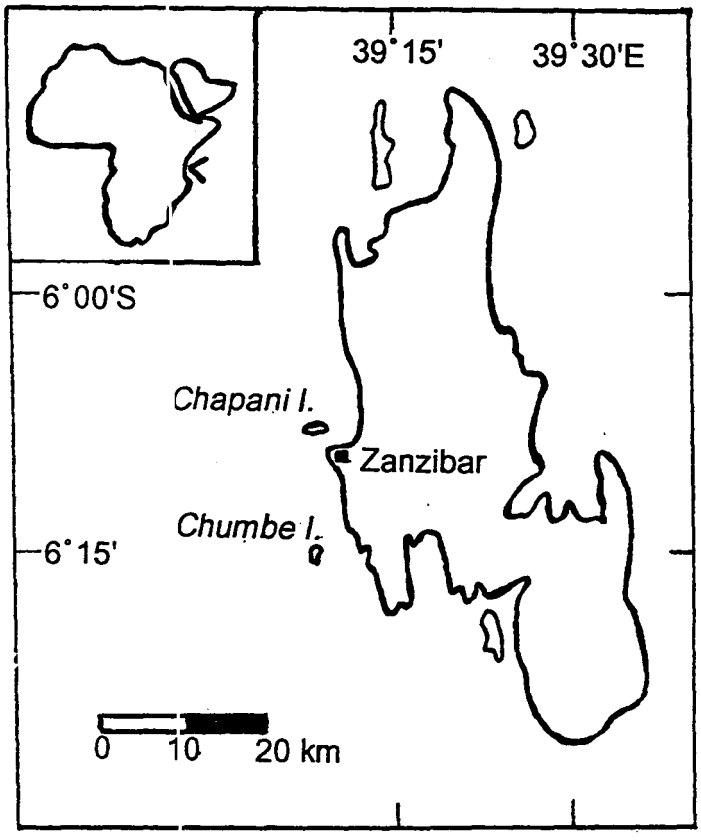
Zanzibar Town is the largest habitation centre on the island with a population of some 300 000. All the town's sewage is discharged, untreated, through several pipelines. Due to deterioration of the sewage lines through time, a large amount of the total sewage now leaks into the ground beneath the town with the remainder reaching the ocean. The harbour of Zanzibar is moderately busy, berthing a variety of coastal steamers, tankers and ferries. Consumption of fish is high on the island accounting for 80% of the animal protein consumed (Risk et al., 1993). Most of the fish are caught on and around local fringing reefs using traditional methods of trapping, beach seining, and hook-and-line. Blast fishing also is said to occur but has not reached the problem proportions of the mainland.

Relatively flat, with a maximum elevation of just over 100 m, Zanzibar has no large rivers, only small scale agriculture, and very little industry. Siltation from deforestation, one of the major threats to reefs worldwide (Cortés and Risk, 1985; Rogers, 1990), can therefore be eliminated as a possible cause of any degradation in the reefs of Zanzibar.

Heavy reliance on reefs for food and income made it important to assess the extent of anthropogenic disturbance on Zanzibari reefs efficiently. We combined immediate community assessments, with the more time intensive isotopic and growth rate analysis. In addition, many of the techniques are easily continuable in a monitoring program by local scientists. Emphasis was placed on combining accessable methods with scientifically sound results.

Zanzibar is a coral island, depends on reefs for its food, and looks to reef-oriented tourism for its future. In 1992, McMaster University and the University of Guelph entered into a joint project with the Institute of Marine Sciences, Zanzibar, to assess the impacts of human activities on Zanzibar's coastal resources. The results of one such study are reported herein.

Figure 1. A map of Zanzibar, showing the position of the two fringing reefs assessed in this study. Current direction is from south to north, scale is as indicated. Inset map of Africa indicates relative position of Zanzibar Island along the East African coast.



Materials and Methods

Site Selection

Field work was carried out during July, 1992. Several small islands occur off the coast of Zanzibar itself. Fringing reefs are developed on these islands, mostly on the sides away from the island of Zanzibar. One of these islands, Chapani (Grave) Island, is located approximately 1 km from Zanzibar Town (Chapani Island is usually called by its alternative name, Grave Island, which is derived from the siting on the island of the British garrison's cemetery). Chumbe Island (Fig. 1), about the same size as Chapani Island, has recently been gazetted as a marine reserve. It is located approximately 10 km south of the Town, off a sparsely inhabited part of the coastline. The predominant current direction is from south to north (Wyrtki, 1973), as such Chumbe is less impacted by sewage discharge from the city. The physical regime at the two sites is similar and within parameters for optimal coral growth. Both islands are fished intensively, but the pressure is less at Chumbe, not only because of its distance from Zanzibar Town, but also because it has been (theoretically) closed to fishing since 1992. We chose to compare the reef and fish communities of these two islands, as an approach to estimating the degree of anthropogenic disturbance.

Evaluation of Reef Communities

Transects

At each island, 6 sample sites were randomly located, situated within the shallow fringing reef. At each site, at least two randomly located 20 m line-

intercept transects were conducted, at a depth of 3-4 m with orientation parallel to the dominant wind/wave orientation, representing a minimum of 240 m of transect at each island.

Transects were all performed by the same operator (MJR), using a plumb bob to reduce parallax error. Point intersect accuracy was to the nearest centimeter. Each transect was archived on videotape, with the camera operator swimming (as well as possible) at a constant speed and distance above the reef, with the transect line included for scale. Each evening, the videotape was replayed to check field notes and coral identifications. Corals were identified to the genus level, except *Porites* (to species) and *Acropora* (to species group). The main references for identification were Wood (1983) and Veron (1985). The videotapes are archived and copies may be obtained from the Department of Geology, McMaster University.

Urchin counts

The long spined sea urchin, *Diadema setosum*, is common on reefs of East Africa. McClanahan and Shafir (1990) and McClanahan and Mutere(1994) have demonstrated that high densities of urchins may have a deleterious effect on these reefs, just as its congeneric relative, *Diadema antillarum*, was shown to affect Caribbean reefs (Sammarco, 1980, 1982).

Densities of urchins at each sample site were estimated using 1 m square quadrats constructed from PVC pipe. Quadrats were randomly placed at each sample site using a patterned series of diver movements. At least 10 of these quadrats were counted at each study site, with numbers recorded and agreed upon by two operators simultaneously. All urchins with at least half their test

inside the frame (when viewed from directly above) were counted. All counts were conducted at the same time every day (early to mid-morning) to avoid introducing error related to behavioural patterns.

Associate counts

For this study, coral associates were defined as sessile organisms living on coral heads, including internal bioeroders (Edinger and Risk, 1994). Increased numbers of these organisms have been linked to declining coral health, or physically weakened coral. Associates commonly occurring on these reefs are hydroids, boring and nestling polychaetes, tunicates and bivalves. We counted coral associates that were visible on the live surface of randomly chosen heads of *Porites lobata*, using quadrats 25x25 cm, placed directly on the corals. At least 2 square meters of coral surface were surveyed at each site. All species lying completely within or half within the quadrat, when viewed from above, were considered. Numbers and species were recorded by one operator.

Coral growth rates

At each island, several colonies of *Porites lobata*, diameter 30-50 cm, were collected from a depth of 3m. The tops of all heads were marked underwater before the heads were collected. Corals were washed and cut by hand into slabs approximately 5 cm thick, using a 24 inch carbide power hacksaw blade. These slabs were transported back to Canada. Sections 2-3 mm thick were cut from each of the larger slabs, along the axis of maximum growth, including the marked top of the coral head. Growth rates were determined by x-ray radiography, as fluorescent banding proved undependable.

Stable isotopes in coral tissue

At each island, samples of *Porites lobata* tissue were taken for analysis of $\delta^{13}C$ and $\delta^{15}N$, some of them coincident with heads studied for growth rate analysis. Small chips of tissue and underlying skeleton were taken from the tops of coral heads using a hammer and chisel. The amount of tissue collected was approximately 50 cm^2. These chips were kept cool and dark in the field and frozen on return to shore.

On return to Canada samples were gently decalcified in dilute acid to remove any remnant skeleton. The tissue was then washed, centrifuged, freeze-dried and powdered to prepare for mass spectrometric analysis (for further detail on analytical techniques see LeBlanc et al., 1989 or Risk et al., 1994). Samples were loaded into precombusted Pyrex tubes with an excess of CuO, evacuated, and combusted at 550°C. The resulting N_2 and CO_2 gases were analysed separately on a SIRA mass spectrometer. Precision of analyses were ± 0.1 ‰.

Statistical analyses

One-way analysis of variance (ANOVA) with pooled standard deviations was performed on appropriate data.

Evaluation of Fish Communities

Several weeks after the coral assessment work had been completed, four 100 m randomly located transects were established at each island. Each line ran parallel to the reef crest and was divided into four 25 m sections. Four counts of

10 minutes duration for each section (i.e. 40 minutes per transect) were conducted by a single observer (CJH) swimming slowly along the line. Fish densities and species numbers were scored in an area 5 m on either side of the transect line, giving the total area surveyed as 250 square meters per section and 1000 square metes per transect.

Separate counts were undertaken for visually obvious families with relatively high densities, such as ballistids (triggers), chaetodonts (butterflies), and pomacentrids (damsels), and those families with low densities that were considered especially important as food fish , such as the haemulids (grunts), lutjanids (snappers), scarids (parrotfish), serranids (groupers), and lethrinids (sweetlips).

Species numbers and fish densities were compared utilising a mixed model nested ANOVA, where sites are treated as fixed effects and transects within sites and sections within transects as random effects. Count data were analysed after a square root transform, as recommended by Snedecor and Cochrane (1968).

Results and Discussion

The coral communities at Chapani and Chumbe Island were significantly different. Coral diversity at Chapani was significantly lower than at Chumbe: the average number of coral species encountered on a 20 m transect at Chapani was 6.1, while at Chumbe the figure was 14.8. The live coral cover was higher ($p<0.001$), and the ratio of live to dead coral also higher, on Chumbe (Fig. 2, Table 1).

There were also significant differences in the numbers of urchins and coral associates at both sites (Table 2). At the affected site, Chapani, the sea urchin density was 6.4 per square meter, while at the comparison site, Chumbe, the figure was 1.2 per square meter (differences are significant at $p<0.001$). At Chapani the density of coral associates on Porites lobata was 87.5 per square meter, while at Chumbe the figure was 32.3 ($p<0.001$).

Based on this community data alone, we conclude that there are significant differences between sites, resulting from anthropogenic influence at Chapani. These differences are not limited to a declining coral population, but extend to the associated community, namely the bioeroders. Large increases in bioeroders have been linked to increased nutrient availability and declining reef health (Hallock, 1988). These sorts of data were reached with minimal equipment requirements, and are well within the capabilities of most reef possessing nations.

A summary of coral growth rate data (Fig. 3, Table 3), shows the average growth rate at Chumbe is 0.7 (s.d. = 0.13) cm/yr, and there is no significant change over the last 20 years. The average growth rate at Chapani is higher 1.0 cm/yr (s.d. = 0.43) cm/yr. The differences in growth rate are highly significant ($p<0.001$), but both reefs are responding similarly, with slopes of 0.01 on best fit lines, for both data sets. Notably, the variance is much higher at Chapani. The difference in mean growth rate between the two sites may be the result of enhanced growth as a response to eutrophication at Chapani (Tomascik and Sander, 1985). The response of reefs to enhanced nutrient input has been termed the "Janus Effect" (Edinger, 1991), after the two-faced Roman god of entrances and exits. The immediate response of corals to increased nutrients is

increased growth rate, but as nutrient inputs continue to build, the corals are beset with borers, associates, and algae. At some point, Janus shows his other face, and the corals deteriorate.

Stable isotopes of nitrogen have been proposed as tracers of sewage contamination in the marine environment (Sweeney and Kaplan, 1980). There was no significant difference between the isotope data at Chapani and Chumbe, although there was an increase in $\delta^{15}N$ at Chapani (Table 2). The lack of significant difference does not imply a lack of sewage stress, but rather is interpreted as relating to a counteractive fractionation of isotopes, related to decreased water clarity. Lower light levels have been connected to a depletion of heavy isotopes, relating to zooxanthellaer assimilation (Muscatine and Kaplan, 1994; Heikoop et al., this thesis). In this study, visibility was reduced to approximately one third at the anthropogenic affected site. These results can be compared to similar reef studies which showed isotopic enrichments relating to sewage stress (e.g. Allison et al., 1991). Allison et al. (1991) compared a eutrophied and pristine site in the Maldives; notably, water clarity was not compromised at their eutrophied site. It is also possible that the distance from the source of contamination (1 km) was too great for an isotopically distinct signature to be preserved.

In addition to the differences in the coral communities, there were significant differences in the fish communities. There was no significant difference in the total numbers of fish at the two islands (Table 4). At both sites planktivorous pomacentrids made the largest overall contribution to the fish density. At Chapani, *Neopomacentrus cyanomos* was extremely abundant, making up 85% of the observed fish population. *N. cyanomos* is a small (< 10

cm) pomacentrid that is common on silted reefs and in sheltered areas. By contrast, the same species was significantly less abundant at Chumbe ($p<0.001$, T-test on arcsine transformed data), making up only 11% of the fish population. Ignoring the large numbers of *N. Cyanomos* at Chapani, the total fish density was much higher at Chumbe.

With the exception of the herbivorous pomacentrids, all the visually obvious families and the commercial family group were more abundant at Chumbe. The greater abundance of fish within the commercial family group at Chumbe was due to large differences in the densities of lethrinids, lutjanids, scarids and serranids. There was also significantly greater species richness at Chumbe.

When combining the evlauations of the fish and coral communities, it is appropriate to note the support the two data sets lend to the conclusions. For example, in East Africa, as elsewhere, sea urchins are preyed on by triggerfish (Family Ballistidae), (McClanahan and Shafir, 1990). The difference in sea urchin densities between the two reefs cannot, however, be attributed to the removal of their main predators. Ballistidae are not an esteemed food source in this region, and the surveys show no significant difference in the numbers between the two reefs. Urchin numbers are therefore attributed to increased available nutrients.

Figure 2. Benthic community data from sample sites. Mean length per transect of the subdivisions are expressed as percents. Legend for both graphs is as shown.

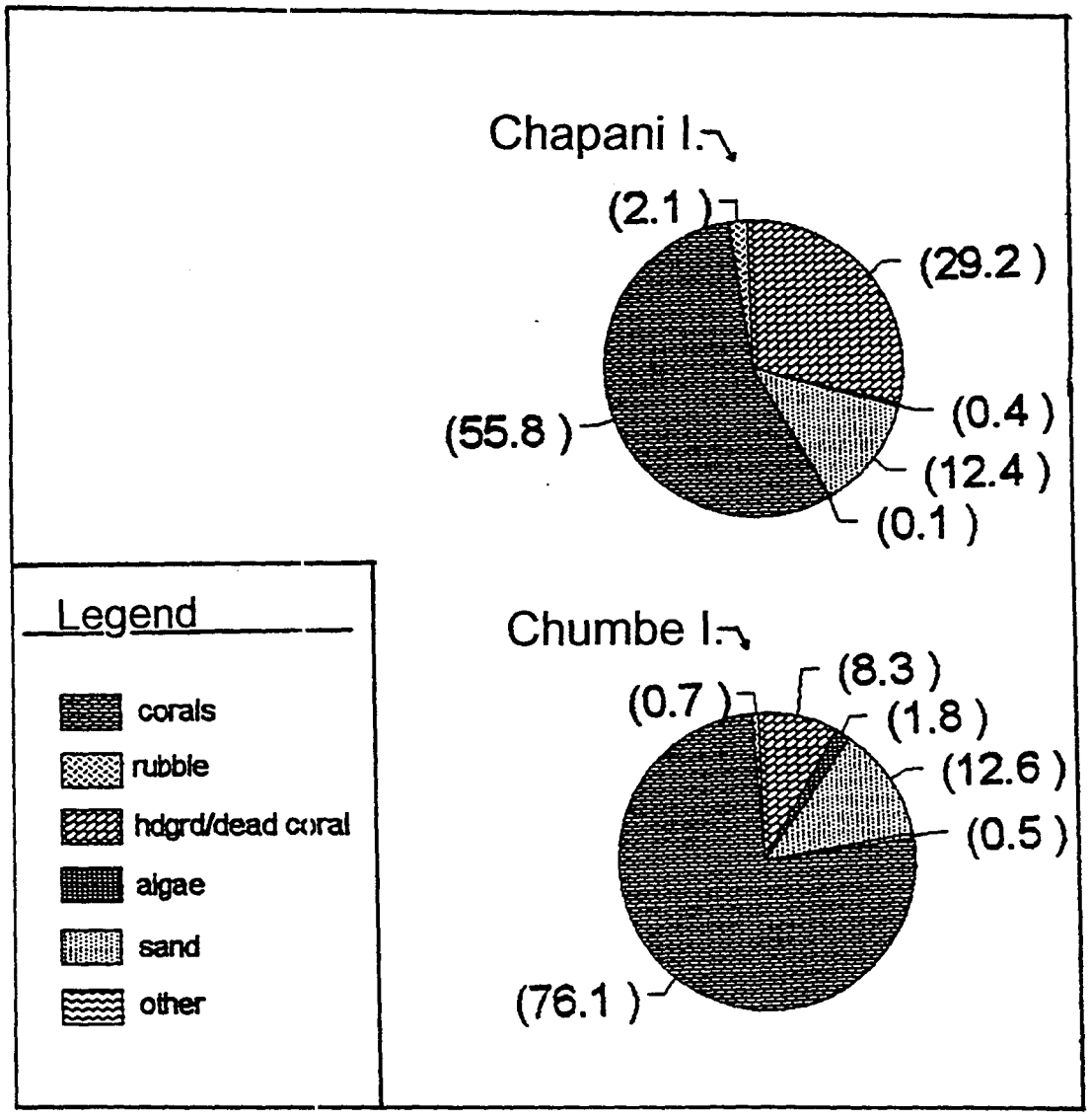


Figure 3. Mean coral growth rates in cm/yr are plotted, with 1 std. dev. illustrated. Chumbe Island (top), has an average growth rate of 0.71 cm/yr, and a best fit line with a slope of 0.01. Chapani Island (bottom), has an average growth rate of 1 cm/yr, and a best fit line with a slope of 0.01.

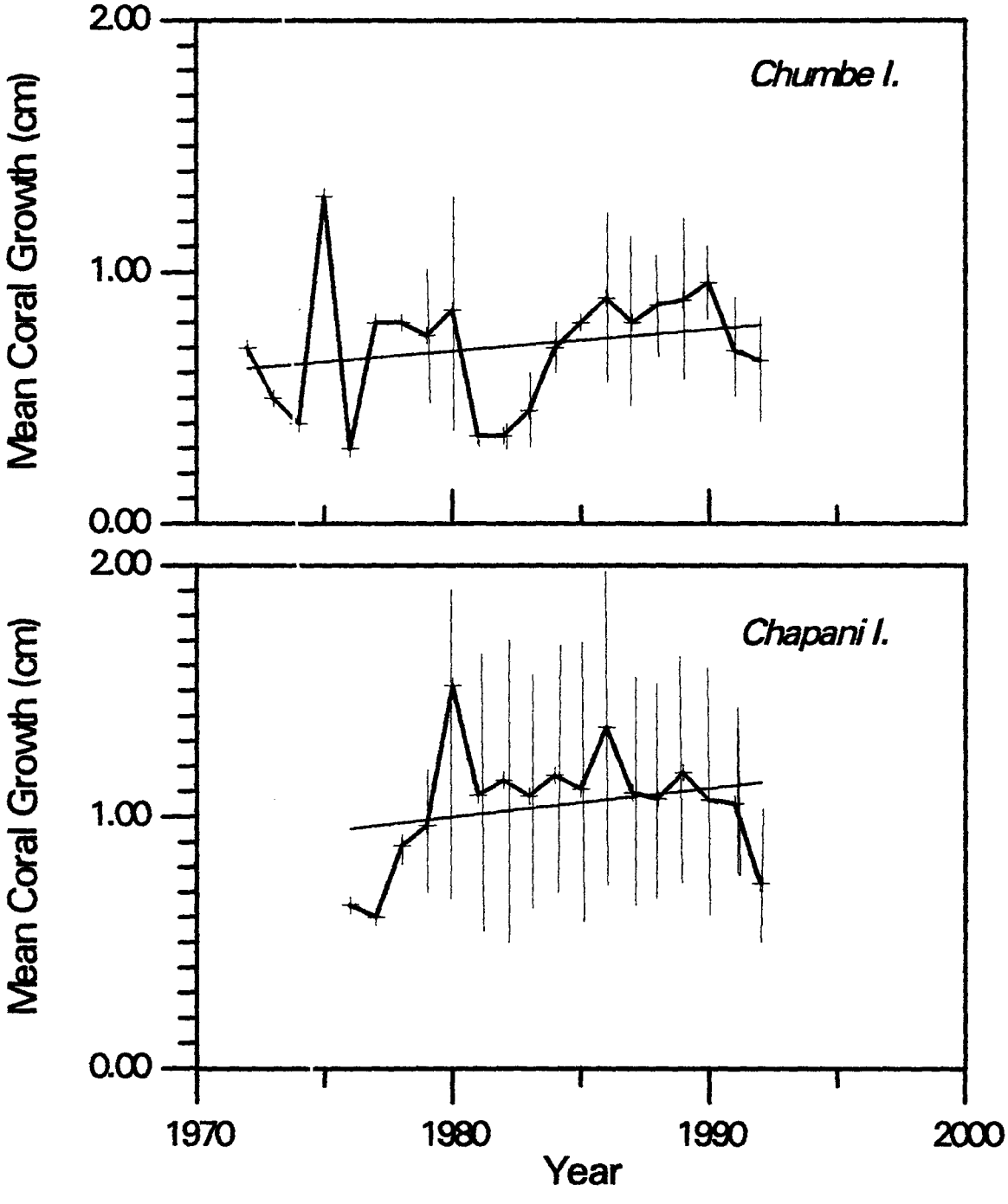


Table 1. Benthic data from transect evaluations. Data are reported as lengths of transect in cm. Mean values are displayed in Figure 2 as per cents of the total transect length.

	Transect #	Ttl Live	Ttl Dead	Hard coral	Soft coral	Algae(macro)	Rubble	Sand	Hdgd. + Dead coral
Chumbe	1-1	1371	199	1479	0	0	0	132	389
	1-2	1571	147	1553	5	0	0	276	166
	2-1	1620	0	1522	0	0	96	209	173
	2-2	1375	0	1395	0	0	0	420	185
	3-1	1484	93	1458	56	2	254	0	236
	3-2	1406	364	1160	56	57	0	322	405
	4-1	1537	147	1571	0	0	0	256	173
	4-2	1680	99	1715	0	0	0	56	229
	5-1	1580	248	1580	0	12	0	0	408
	5-2	1785	53	1785	28	32	0	0	155
	Mean	1540.90	135.00	1521.80	14.60	10.30	35.00	167.10	261.90
	Std dev	128.63	107.62	163.18	22.31	18.33	78.41	143.81	100.54
Chapani	1-1	590	0	590	20	0	0	1388	0
	1-2	872	158	898	46	0	0	923	133
	1-3	1103	102	1076	0	0	0	740	184
	1-4	765	115	765	0	0	0	1025	210
	2-1	683	96	1312	51	0	0	528	109
	2-2	512	22	1444	0	0	0	463	37
	3-1	1399	122	1533	74	0	111	91	191
	3-2	1177	9	1173	0	0	0	504	323
	4-1	1517	0	1517	0	0	0	182	301
	4-2	1206	42	1196	0	0	0	137	667
	5-1	545	511	620	64	0	0	565	741
	5-2	1134	281	1085	78	0	0	365	472
	6-1	1159	30	1174	28	35	0	590	173
	6-2	1672	0	1472	262	0	0	18	248
	Mean	1024.00	106.29	1132.50	44.50	2.50	7.93	537.07	270.64
	Std dev	356.89	135.71	306.20	66.80	9.01	28.59	372.89	210.95

Table 2. Summary table of coral community data, including quadrat studies for both urchins and associates, data are presented as mean values. As well, isotopic data are presented (mean±1 s.d.).

	Chapani I.	Chumbe I.
Associate Density per m^2	87	38
Urchin Density per m^2	6.4	1.2
Live: Dead Ratio	4.5	6
Total Number of coral Species per Transect	6.1	14.8
$\delta^{15}N$ (‰)	4.5±0.4	4.2±0.5
$\delta^{13}C$ (‰)	-10±0.8	-10.7±1.0

Table 3. Mean coral growth rate values (cm/yr ± 1 s.d.) from Chapani and Chumbe 1. Data are presented in Fig. 3.

Year	Chapani	Chumbe
1992	0.74±0.27	0.65±0.20
1991	1.05±0.32	0.69±0.19
1990	1.07±0.52	0.96±0.15
1989	1.18±0.48	0.89±0.28
1988	1.07±0.45	0.87±0.19
1987	1.10±0.49	0.80±0.34
1986	1.36±0.58	0.90±0.33
1985	1.11±0.51	0.80±0.00
1984	1.17±0.48	0.70±0.10
1983	1.08±0.47	0.45±0.15
1982	1.15±0.61	0.35±0.05
1981	1.09±0.55	0.35±0.05
1980	1.52±0.83	0.85±0.45
1979	0.97±0.24	0.75±0.25
1978	0.89±0.04	0.8±0.00
1977	0.60±0.00	0.8±0.00
1976	0.65±0.00	0.3±0.00
1975		1.3±0.00
1974		0.4±0.00
1973		0.5±0.00
1972		0.7±0.00

Table 4. Fish community data by trophic group and family group. Mean data are presented as number per 250 m^2 transect.

Group	Chapani I.	Chumbe I.
Family		
Acanthuridae (surgeonfish)	0.18	2.96
Caesonidae	0.07	0.13
Chaetodontidae (butterflies)	1.47	1.97
Labridae (wrasses)	1.23	2.55
Commercial Group[1]	1.29	2.66
Pomacanthidae (angelfish)	0.05	0.81
Pomacentridae[2] (damsels)	23.91	23.28
Sphyraenidae & Carangidae	0.11	0.05
Total	28.3	34.4
Trophic Level		
small carnivores	1.64	3.12
omnivores	2.30	2.87
planktivores	23.27	23.10
herbivores	3.29	3.01
corallivores	1.63	1.83
Total	32.13	33.93

1. includes the families: *Mullidae* (goatfish), *Scaridae* (parrotfish), *Haemulidae* (grunts), *Nemipteridae*, *Serranidae* (groupers), *Siganidae*, *Lutjanidae* (snappers), *Kyphosidae* (chubs), and *Lethrinidae* (sweetlips)

2. includes both shoaling and solitary pomacentrids

Conclusions

1. Densities of the grazing sea urchin *Diadema setosum*, and coral associates on *Porites lobata*, are higher at the more affected reef, Chapani, than at the less affected reef, Chumbe.
2. The coral community at Chapani is less healthy, and less diverse, than that at Chumbe.
3. Coral growth rates are higher at Chapani, reflecting a eutrophication effect.
4. There are significant differences in the fish fauna between the two reefs.

In combination, these observations allow us to conclude that Zanzibar reefs that experience direct anthropogenic disturbance are eutrophied. Sewage contamination is seen as the most likely cause. Although high-tech data did not provide statistically significant results, they should not be discounted in future studies.

Acknowledgements
Help in the field was provided by Gwenn French, Daude Mukaka, and Melisa. Assistance with stable isotope analysis was provided by Martin Knyf. This project was conducted jointly with the University of Guelph and the Institute of Marine Science, Zanzibar. Funding was provided by the International Centre for Ocean Development (ICOD), and NSERC operating grants to MJR.

Chapter 4: Indonesia

Similar to the Zanzibar case study, this study was devised to assess the anthropogenic impact on a nearshore reef in an embayment in Indonesia. In the Zanzibar study isotopic results proved somewhat ambiguous, with multiple variables affecting the $\delta^{15}N$ (i.e. water clarity, concentration, distance from source). Samples collected from Jepara Bay, Indonesia, were collected over a eutrophication gradient. By collecting over a concentration and distance gradient it was hoped that we would be able to resolve the component of isotopic signature reflecting anthropogenic inputs to the reef system.

Rather than using a biological, quantitative, assay of reef health, this study attempts to use a number of physical parameters as natural tracers of contamination. The use of natural tracers in the marine environment is well documented (e.g. Coakley et al., 1992; Bachtiar, 1993; Versteeg et al., 1995). The advantages of this method lie in the ease of monitoring, and assimilation of the tracers and their environment. Stable isotopes are one of many tracers used, with their main emphasis on tracing relative inputs of various sources (e.g. Sweeney et al., 1981; Owens, 1987; LeBlanc et al., 1989; Risk et al., 1994; Sammarco et al., 1995).

Research was conducted in Indonesia during the summer of 1993. Again, nitrogen isotopes were used as a potential method of tracing sewage contamination in the coral reef environment.

Tracing Anthropogenic Contamination in a Coral Reef Setting using Natural Tracers

Dunn, J.J.; Risk, M.J.; Bachtiar, T.; Morris, W.A.

Abstract

A small embayment on the north shore of central Java, Indonesia was studied using multiple natural tracers. Heavy metal analysis combined with sediment analysis suggests the extent of contamination from two point sources of discharge into the bay. Levels of Zn, Cu and Cd are all well above background levels, indicating the bay is heavily polluted. Chlorophyll-a measurements show levels of bioavailable nutrients are in excess of known thresholds, suggesting eutrophication. Attempts to use stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) as tracers of anthropogenic influence were inconclusive.

Introduction

Coral reefs have adapted to oligotrophic waters, and are unable to cope with nutrient and toxin enrichment (e.g. Darwin, 1842, Risk et al., 1994). Numerous studies tracing anthropogenic contamination have been completed in temperate climates using various tracing mechanisms including: radioactive tracers (Coakley and Long, 1990), chemical markers (Brown and Wade, 1984) including faecol sterols such as coprostanol (Coakley et al., 1992; Risk et al., 1993; Bachtiar, 1993); magnetic properties of sediments (Versteeg et al., 1995); and stable isotopes (Sweeney et al., 1980; Sweeney and Kaplan, 1980; Rau et al., 1981; Cifuentes et al., 1988). The benefits of natural tracers are mainly the

ease of monitoring, and long term presence of these materials in the environment (allowing collection over a time series).

Similar attempts to study anthropogenic impacts on coral reefs have focused mainly on biological assays of the reef (e.g. Smith et al., 1981; Tomascik and Sander, 1985). Few studies have attempted to integrate quantitative data in an attempt to establish threshold levels of contamination in the reef environment (Bell, 1991; Lapointe and Clark, 1992; Risk et al., 1993). In addition to the tracer mechanisms commonly used in temperate environments, Bell (1991) suggests the use of chlorophyll-a as perhaps the single best indicator of eutrophication on reefs. Eutrophication is defined as a nutrient excess sufficient to lead to decreased water clarity and nuisance algal growth. There is a direct correlation between levels of eutrophication (and bioavailable nutrients) and amount of chlorophyll-a in the water column.

This study evaluates several tracing mechanisms in a coral reef environment, in order to establish the degree and extent of anthropogenic contamination. We have chosen to include measurements from the water column (chlorophyll-a), the substrate (sediment and heavy metal analysis) and the biological community (isotopes). By combining recognised indicators of eutrophication, with recognised indicators of pollution (heavy metal analysis), we have attempted to assess the anthropogenic impact on this environment. Isotopic data has previously been used to suggest terrestrial/anthropogenic sources of contamination (Sweeney and Kaplan, 1980; Sweeney et al., 1980; Coakley et al., 1992; Risk et al., 1994). Isotopic data were collected from the biological community within the bay for a similar application.

Study Area

Jepara Bay, Indonesia is a small embayment /estuary on the north coast of the island of Java (Fig. 1). The bay is only 3-4 km wide and 3-4 km in length, with maximum water depths of a few meters. Two rivers act as sources of discharge to the bay, K.Jepara and K. Tambangan (Fig. 2). K. Jepara is the major source of discharge. It contains the local boat harbour in its mouth and has a course that meanders through the town of Jepara, population 300 000. The second river is too shallow for boat traffic, and its course is mainly through arable land. Jepara is a major community of north central Java, with significant boat traffic and fishing pressures on the local marine community. There is not a significant amount of heavy industry in Jepara (the main industry is furniture building), and there is no sewage treatment facility.

Sampling was conducted in July, 1993, and consisted of two transects radiating out from the mouth of each river. Sampling sites along each transect were spaced at intervals of approximately 500 m. Additional sampling sites were added in an attempt to extend coverage throughout the bay, effectively creating a 500 x 500 m sampling grid (Fig. 3). All samples were collected and prepared for transport to Canada over a 4 day period.

Figure 1. A map of southeast asia, locating the study area on the north shore of the island of Java.

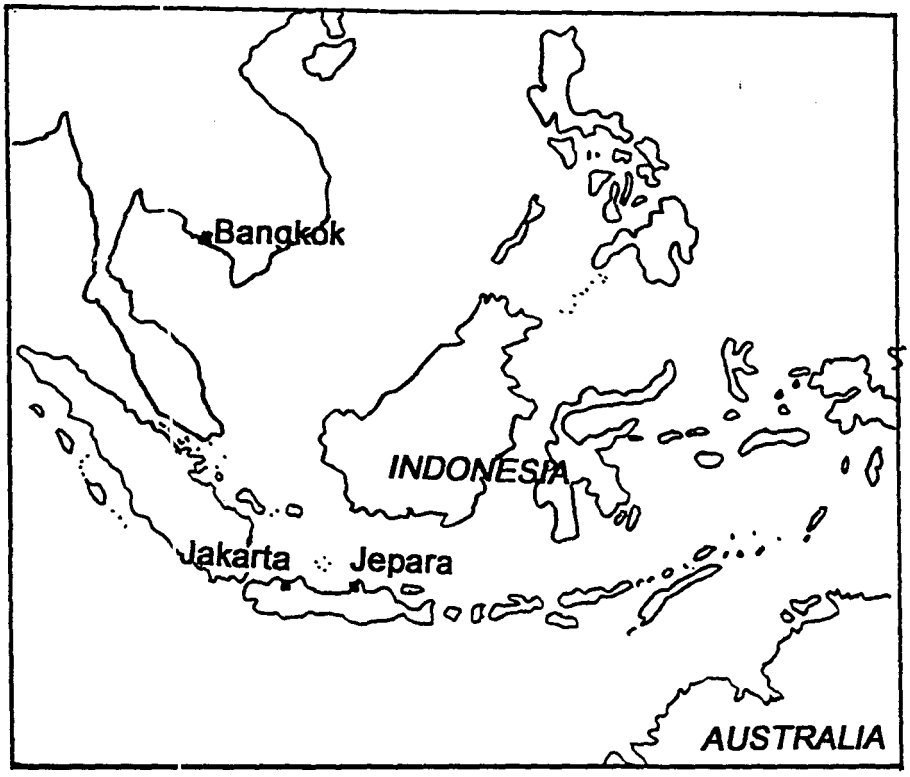


Figure 2. A map of the study area, Jepara Bay, showing local features of interest. The town of Jepara to the southwest is indicated with stipple, and the two rivers into the bay are shown. Areas of coral development are indicated by crosshatching.

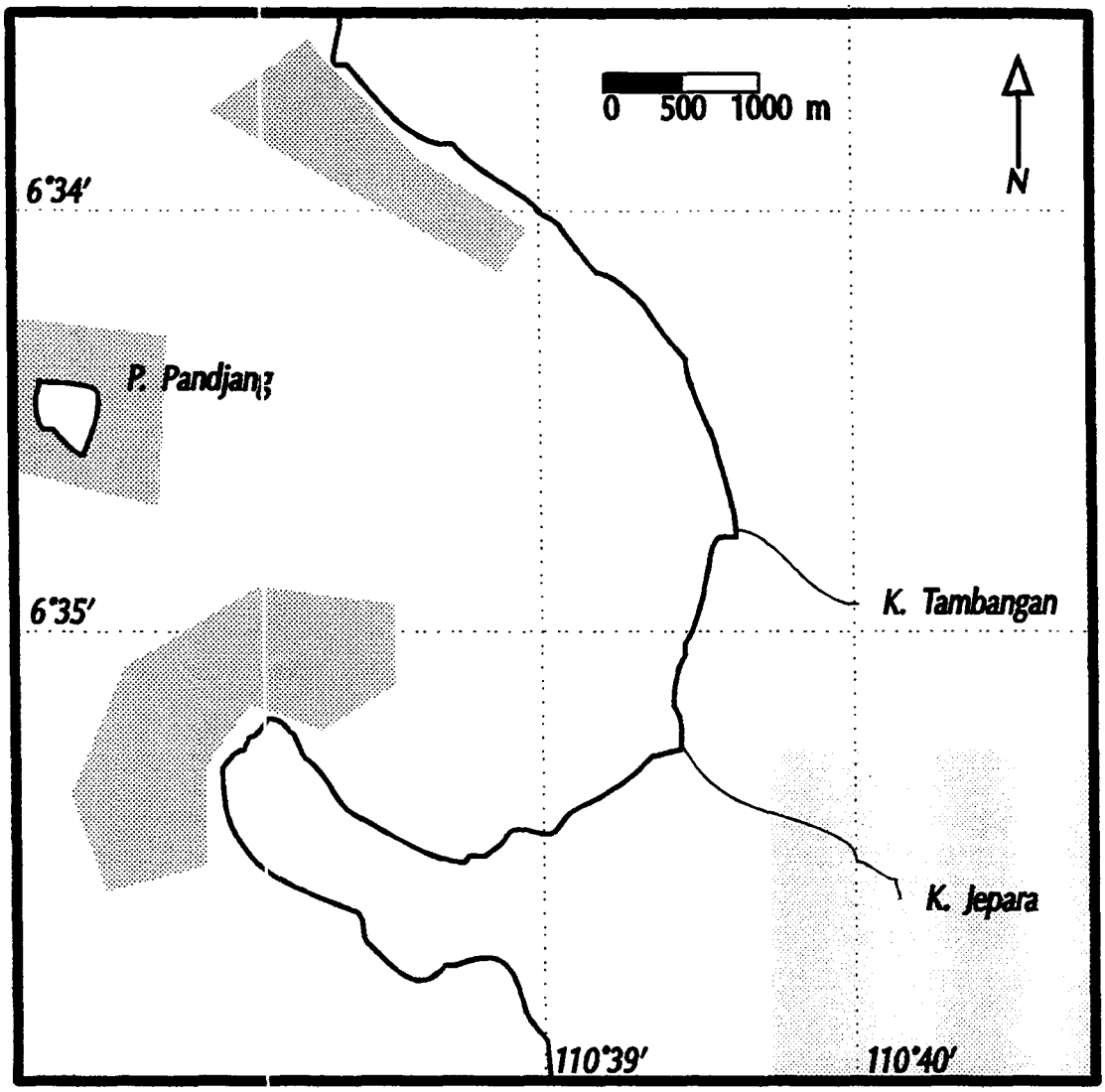
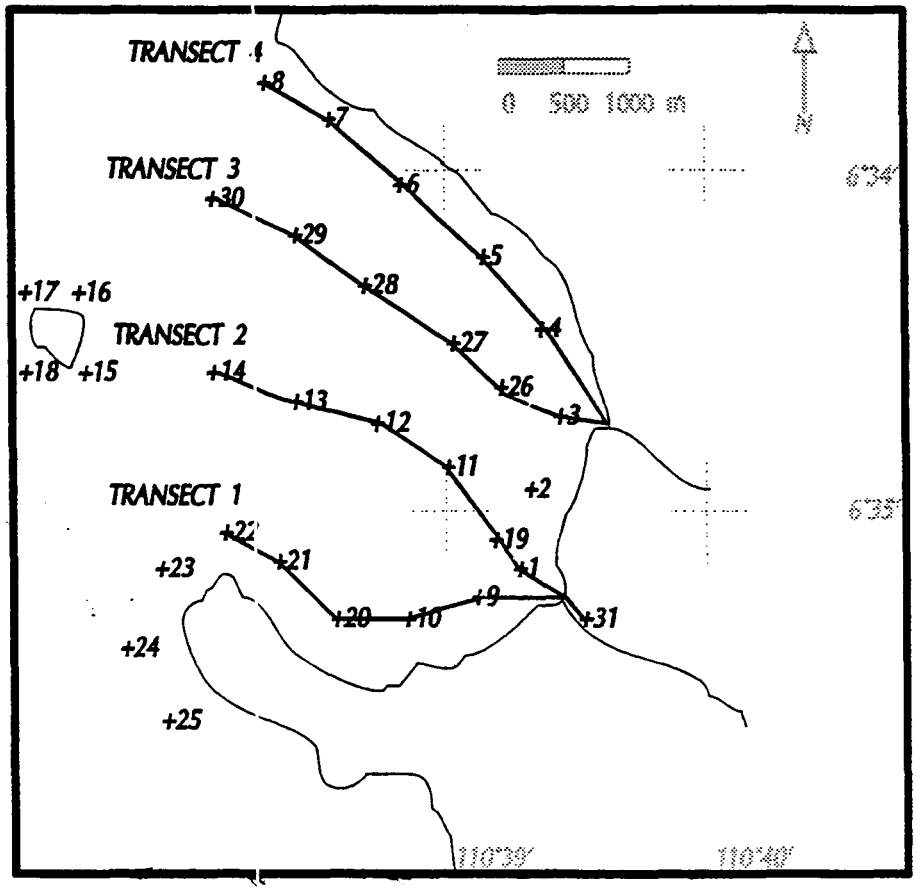


Figure 3. Map showing transects and sampling sites in Jepara Bay. Sampling sites are indicated with a '+' and the corresponding station number, heavy lines are the transects.



Methods

Sediment Analysis

A minimum of 1 kg of sediment was collected at each sampling site using snorkel or SCUBA. Samples consisted of the top 3 cm of sediment only. Collected sediment was dried in a drying oven at 60°C for two days. Grain size analysis was conducted on all samples, and was reported using a Folk classification mechanism (after Folk, 1954). That is, grain sizes were broken into sand, silt, clay divisions and considered as a percent of the total amount of sediment per sample. In addition to grain size analysis, heavy metal analyses for Zn, Cu and Cd were conducted, and reported in ppm (equivalent to mg/L) for each sample. Heavy metal analysis was conducted by AAS.

Stable isotope data for C and N were also attempted on all sediment samples. Dried sediments were decalcified, rinsed, and freeze dried, with organic content of aliquots determined using loss on ignition calculations. Appropriate amounts of sediment were then packed into precombusted 9mm Vycor (quartz) tubing with an excess of Cupric Oxide, placed under vacuum and flame sealed. Low organic content ratios did not allow isotopic analysis of N on the sediment samples, however, C data were obtained for some sites.

Chlorophyll-a Analysis

At each site 1 litre water samples were taken from a depth of 1 m. These samples were filtered through GF/F filters using a hand-pump vacuum.

Filters were protected from light and stored in 4 mL Teflon-capped vials, frozen until further analysis could be conducted.

The technique for analysis is a modified Dimethyl Sulfoxide extraction developed by Burnison (1980). The filters were allowed to come to room temperature and their vials filled with dimethyl sulfoxide. The vials were then heated by a block heater to 65°C for 10 minutes. Vials were removed and agitated thoroughly. The DMSO effectively strips the chl-a from the filter. The liquid from the vials was then filtered through a GF/C filter, and the vial and filter rinsed with 90% acetone. Filtrate volume was brought to 10 mL with 90% acetone, and transfered to an acetone-rinsed 5 cm cuvette. Absorbance readings were taken with a Beckman model 25 spectrophotometer at 750, 664, 647, 630 and 448 nm. 10 mL of concentrated HCl is then used to acidify the samples which were reread after 5 mins at 750 and 664 nm. Corrections can then be applied as discusssed in Burnison (1980).

Isotope Data

In addition to the isotope work conducted on the sediments, samples of seaweed and coral were obtained wherever possible. Coral coverage is not equally distributed throughout the bay, but is limited to the shoreline and outer edges (Fig. 2).

Samples were dried for two days at 60°C. At this point, seaweed was powdered with a mortar and pestle and packed for analysis in 6 mm precombusted pyrex with an excess of CuO. These tubes were flame sealed while under vacuum, and a modified Dumas combustion technique was used.

Coral samples were decalcified in dilute acid with the resulting tissue mat rinsed thoroughly in distilled water. The rehydrated mat was then repeatedly centrifuged to remove excess water and freeze dried, prior to further preparation for analysis. Coral samples were also prepared in 6 mm Pyrex tubes. All samples were cyrogenically separated online to yield pure N_2 and CO_2, prior to admission to the mass spectrometer. Analyses were conducted on a VG SIRA mass spectrometer. C was analysed with reference to the PDB standard and N with atmosphere. Precision on analyses was always better than ±0.1. Results are reported in standard delta notation, with units of ‰.. For more detailed explanations of analytical technique see LeBlanc et al. (1989) or Risk et al. (1994).

Results

Data are considered both as linear transects, measured as distance from source, and as computer contoured displays. Multiple y-axis graphs along each transect present all collected data as a function of distance from source (Figs. 5-8). Computer contouring was conducted using GeoSoft software mapping and processing package. Data were randomly gridded at a 600 m grid cell interval and then regridded to smooth the contours. Contoured displays are annotated with suggested threshold values, defined as 0.03 ppm for Zn (30 ppb), 0.005 ppm for Cu, and 0.0002 ppm (0.2 ppb) for Cd. This thresholds are defined as Province of Ontario guidelines, even adjacent to point source discharges such as the rivers (cf. Hamilton Harbour Remedial Action Plan). Chlorophyll-a data are also displayed as a computer contoured data set (Fig. 12) with a threshold

concentration of 0.5 mg/m^3 defined by Bell (1991). The uneven distribution of stable isotope data limits their presentation to the transect profiles (Figs. 5-8). A summary table of all discussed data is also provided (Table 1).

Figure 4. Classification of grain size of sediment samples, numbers represent sample site. Filled triangles correspond to samples collected at less than 5m depth (note the correlation with sand classification), open triangles were collected at greater than 5m depth.

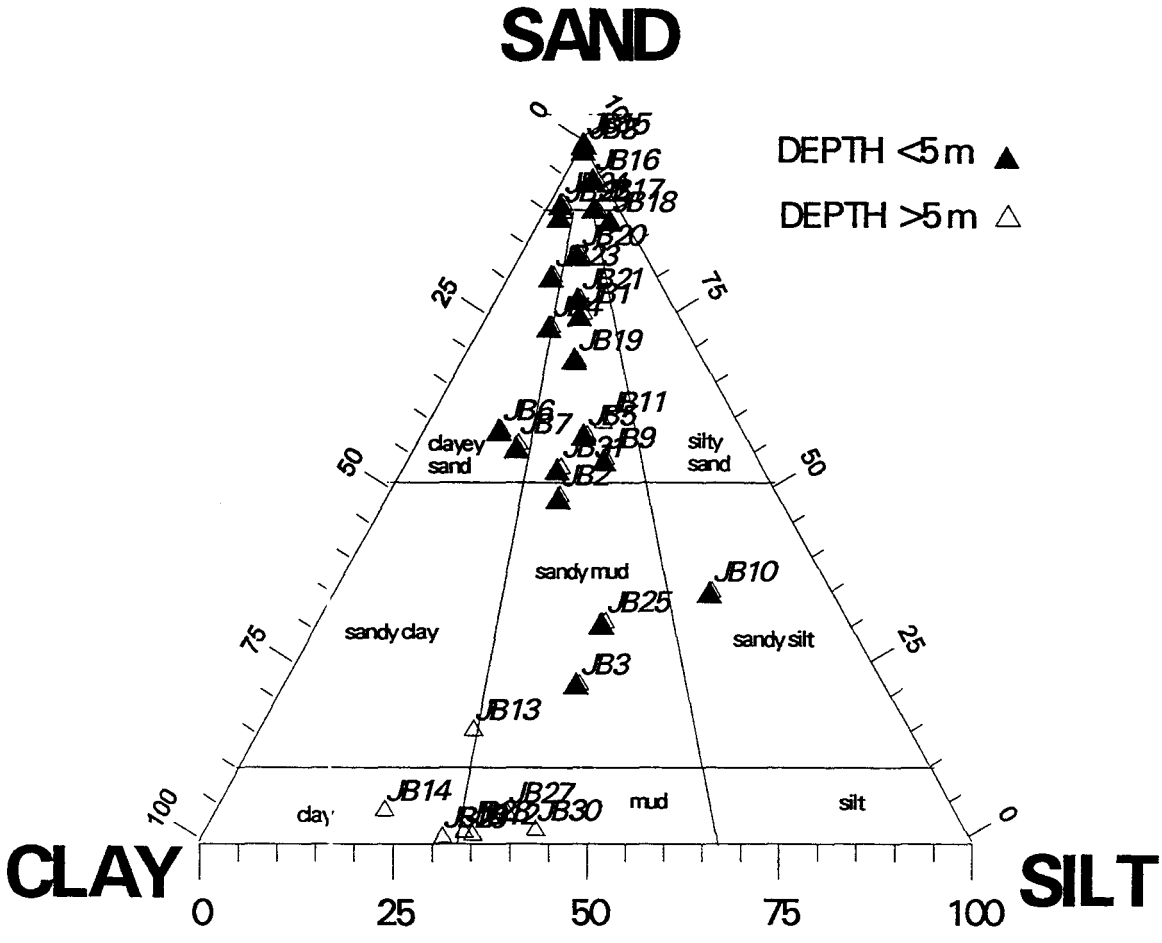


Figure 5. Transect one, inset map of Jepara Bay shows its relative position. Source is Jepara River.

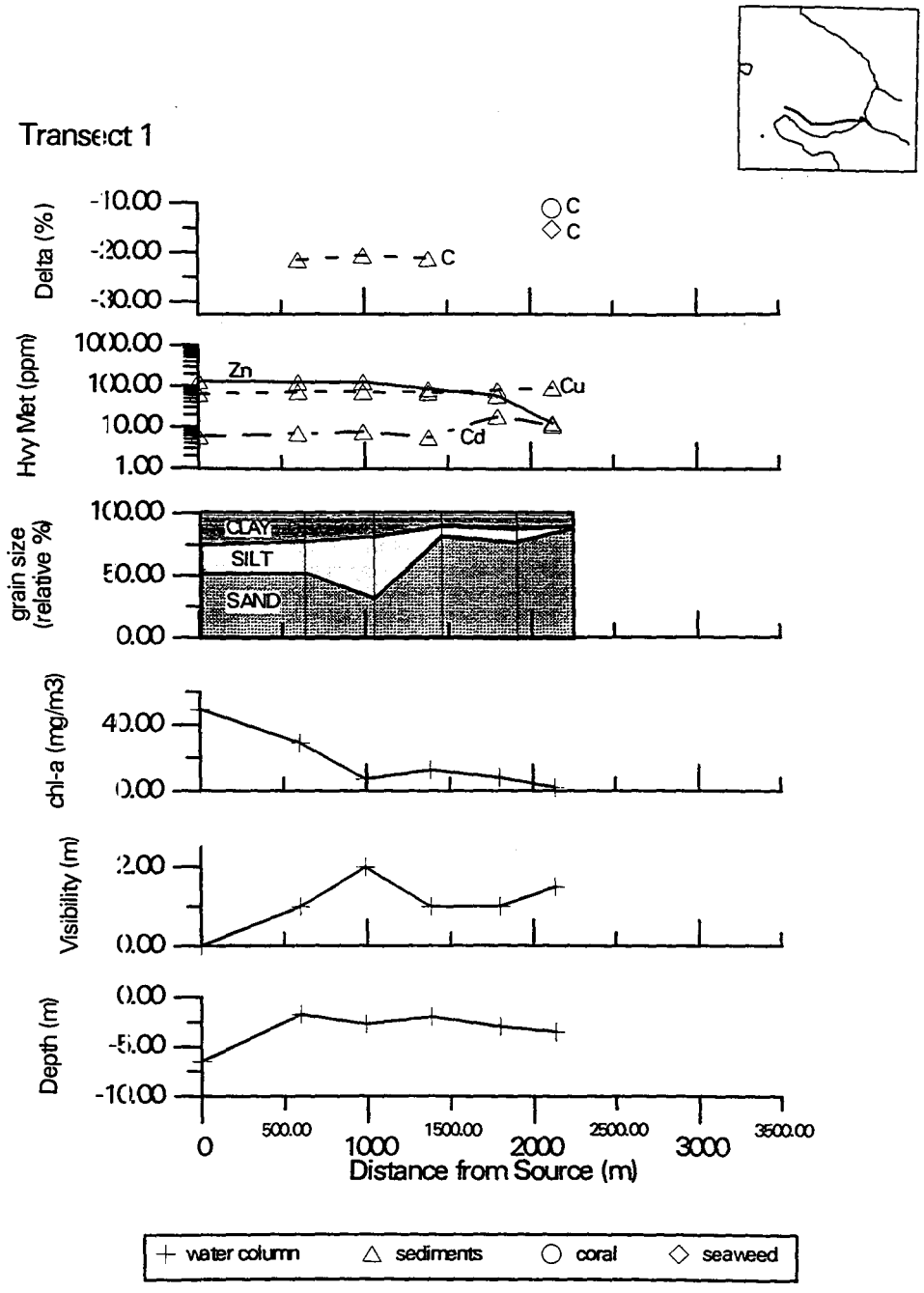


Figure 6. Transect two, inset map of Jepara Bay shows its relative position. Source is Jepara River.

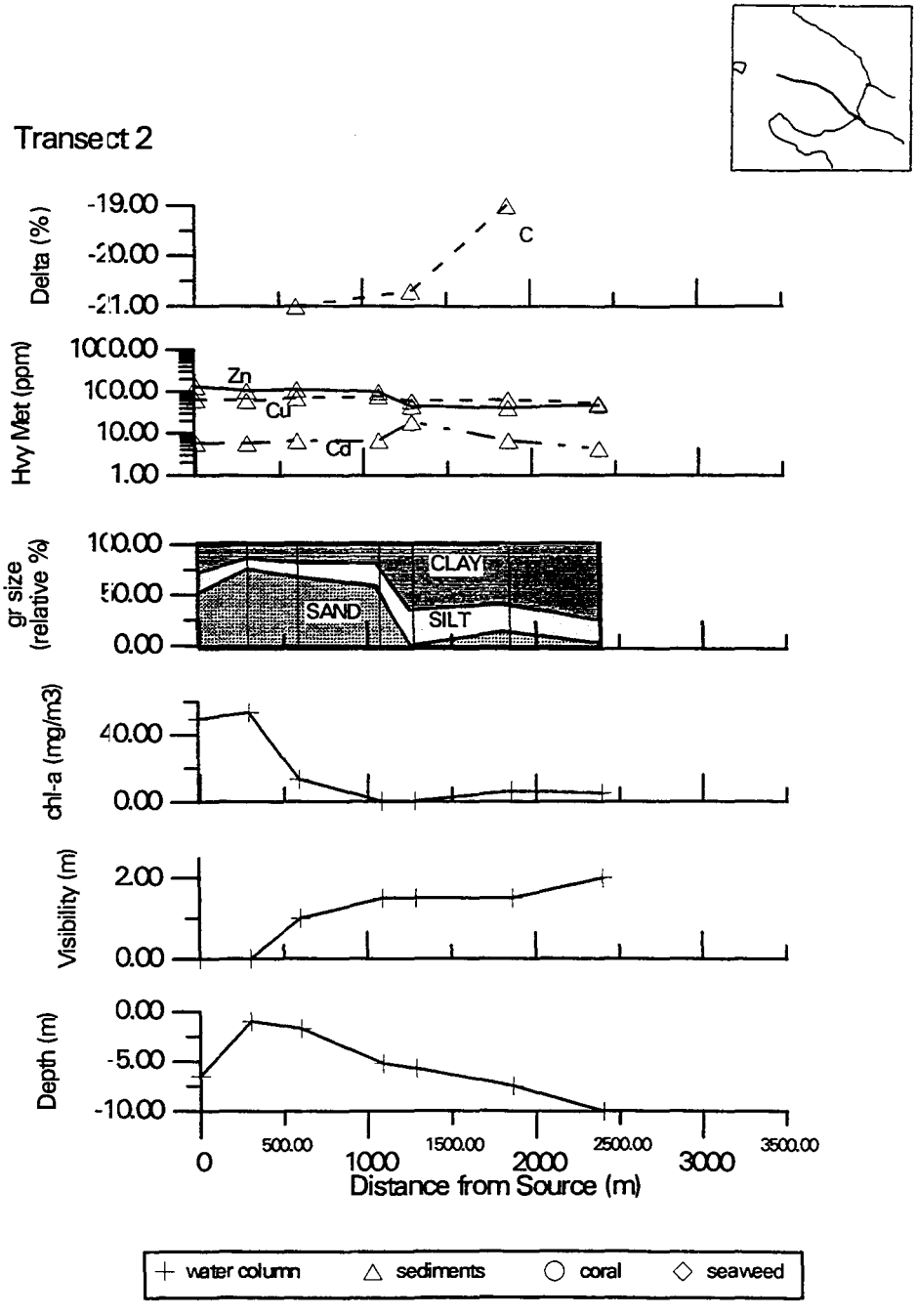


Figure 7. Transect three, inset map of Jepara Bay shows its relative position. Source is Tambangan River.

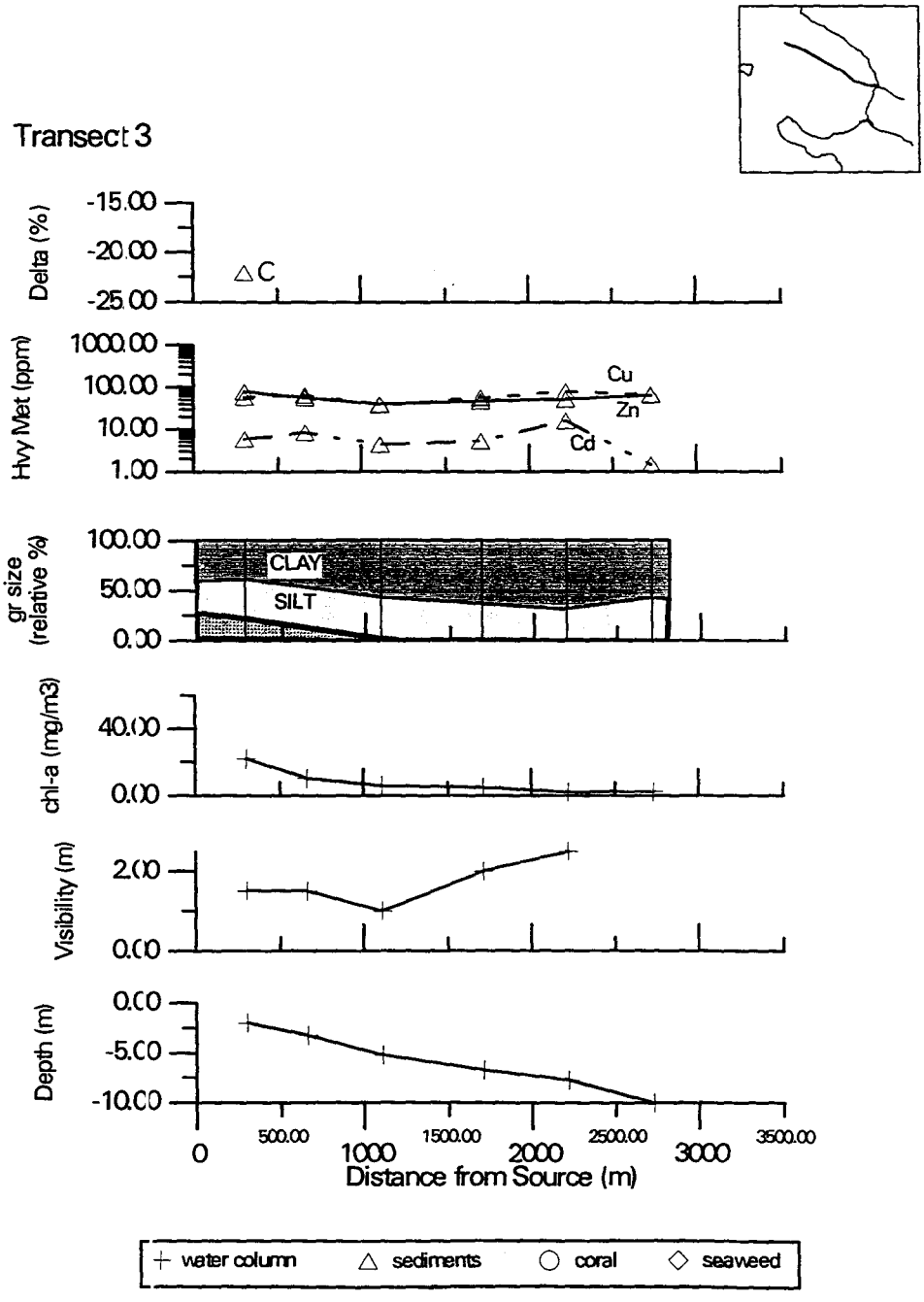


Figure 8. Transect four, inset map of Jepara Bay shows its relative position. Source is Tambangan River.

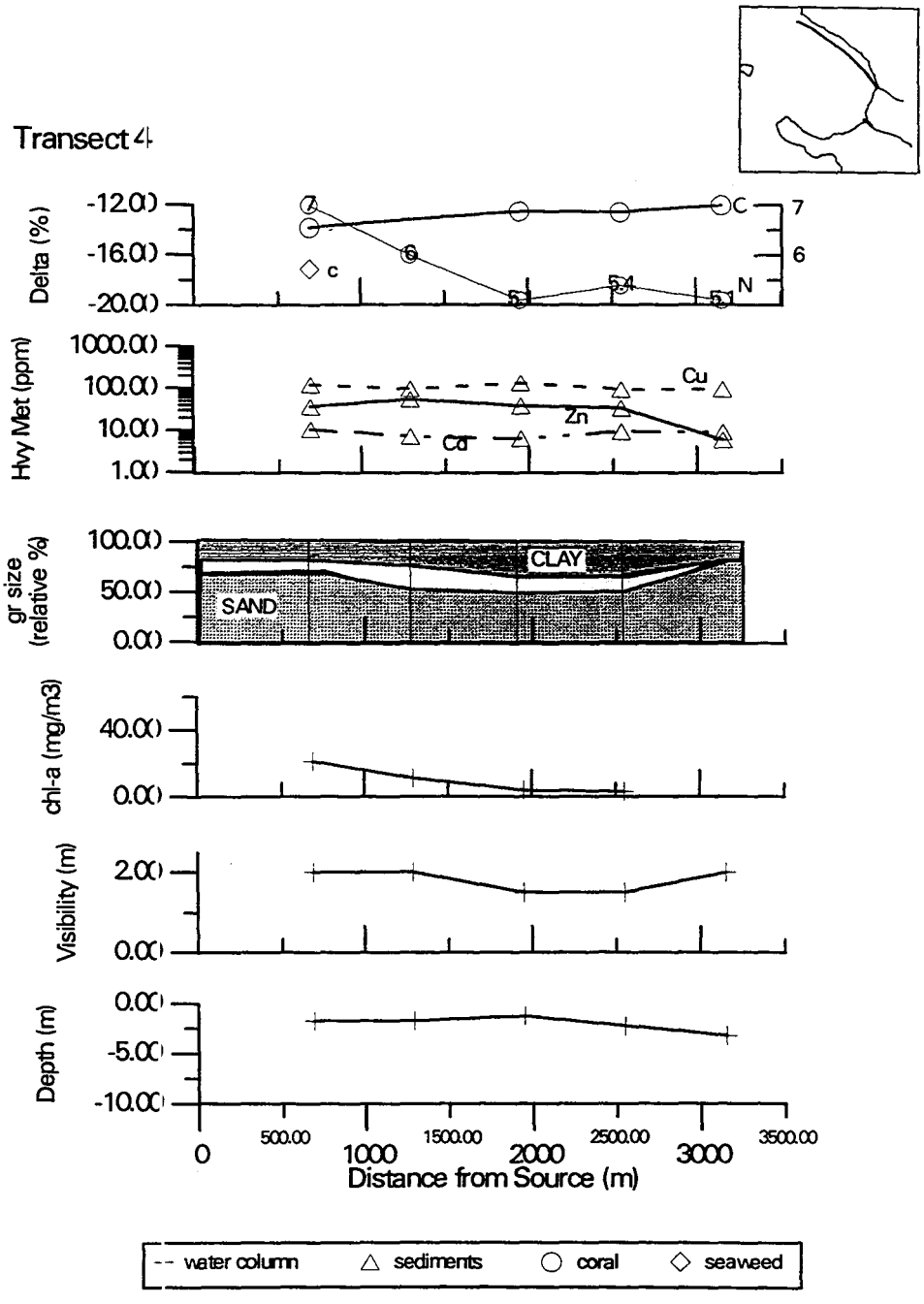


Figure 9. Zn concentrations in sediments from Jepara Bay.

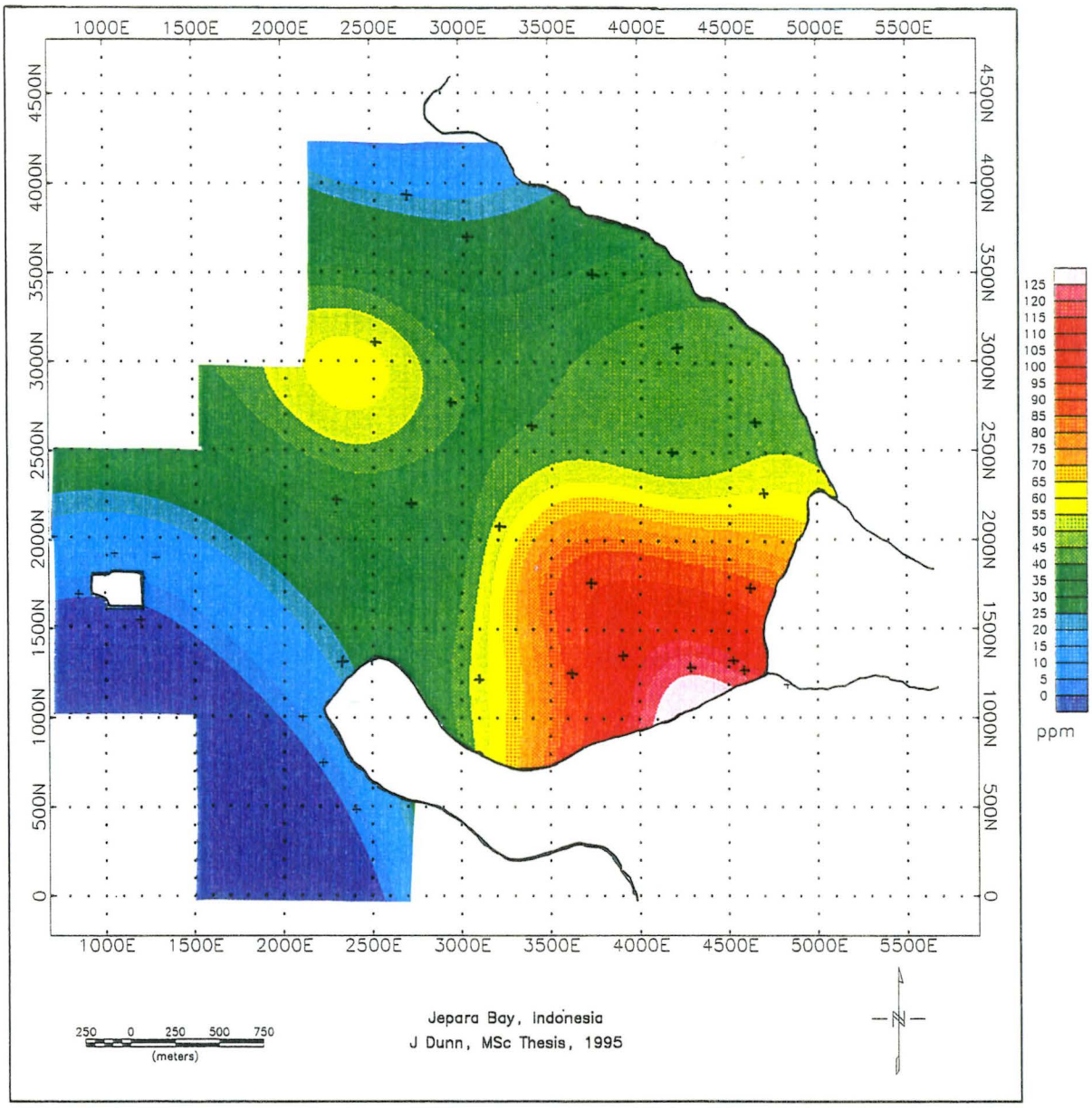


Figure 10. Cu concentrations in sediments from Jepara Bay, measured in ppm.

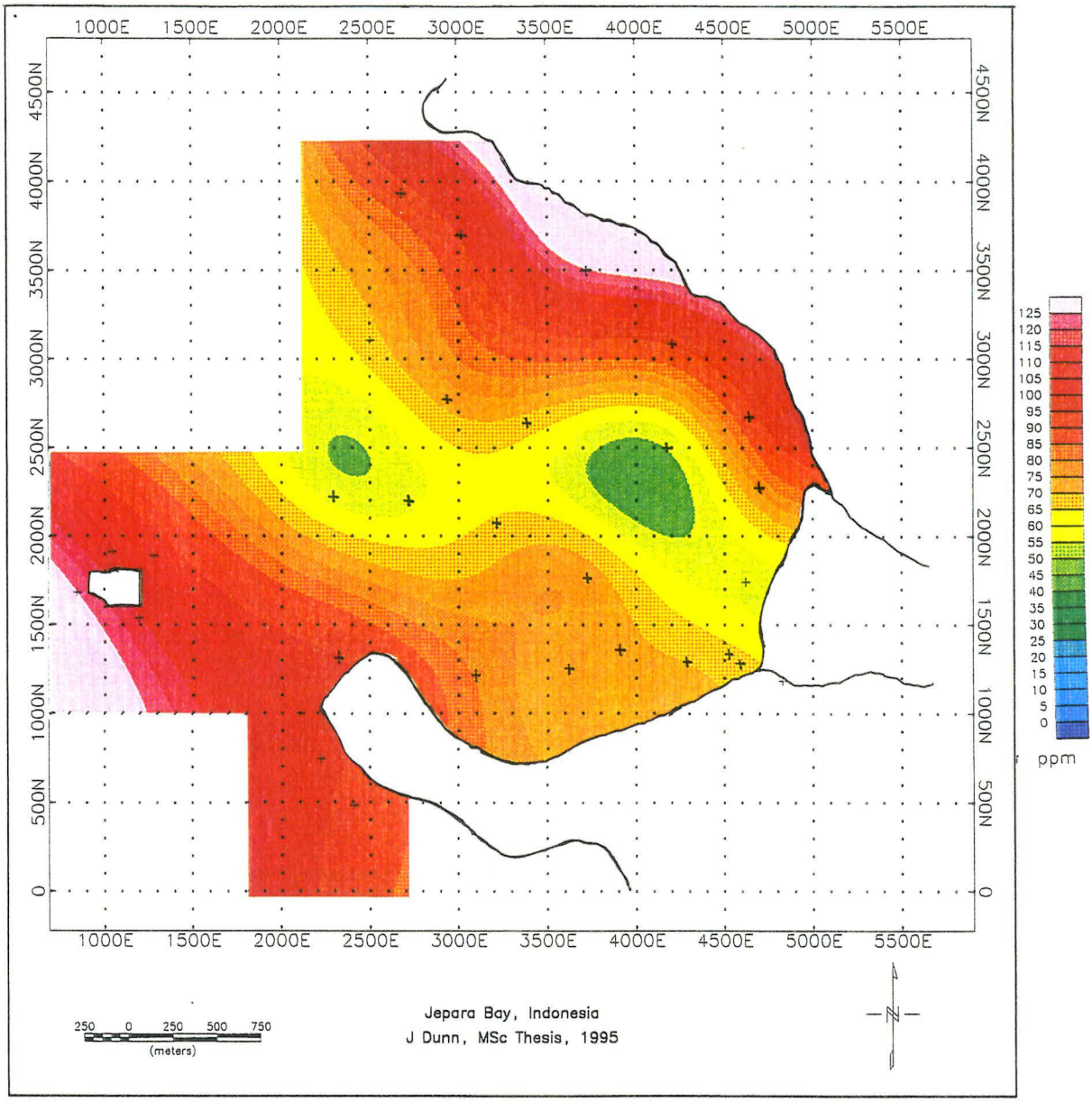


Figure 11. Cd concentrations in sediments from Jepara Bay, measured in ppm.

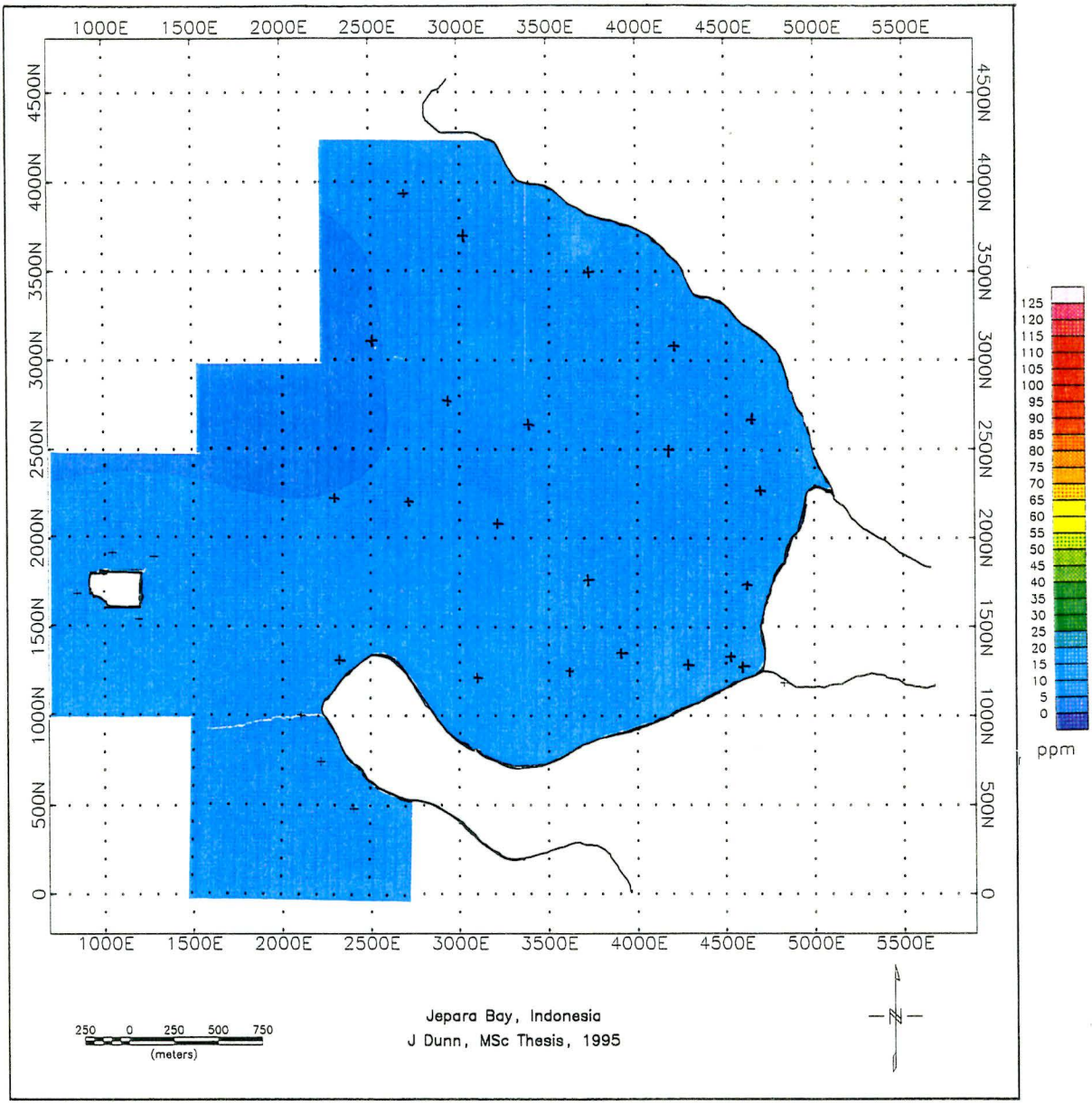
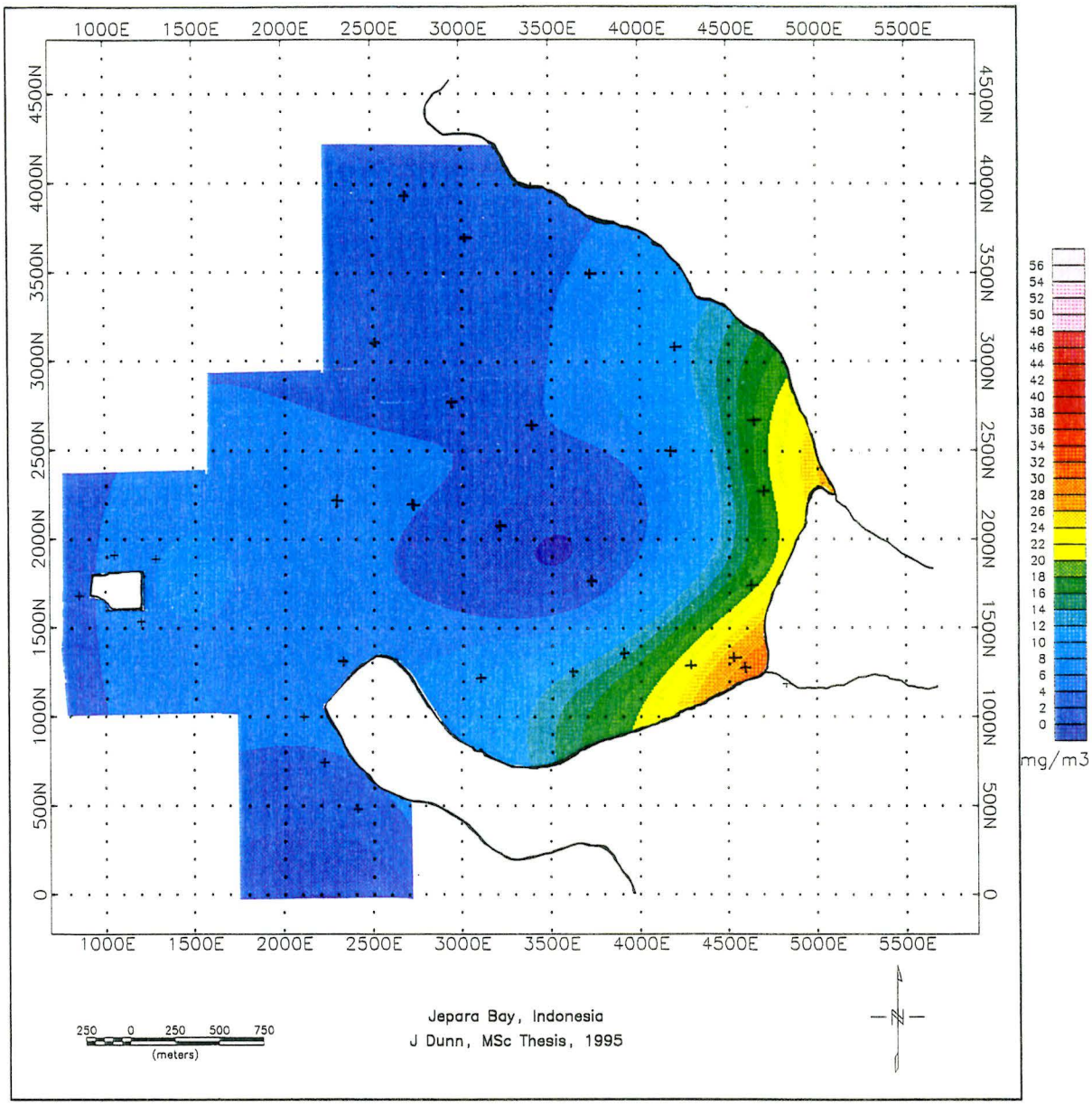


Figure 12. Distribution of chl-a concentration in mg/m^3, as mapped by computer contouring. Eutrophic conditions prevail throughout the bay.



Jepara Bay, Indonesia
Sampled data by Site (sites as indicated in chapter 4)

Site no.	Depth (m)	Dist. from K.Jepara (m)	Dist. (m) from K.Tambangan	% sand 2mm>x>1/16mm	%silt 1/16mm>x>1/256mm	%clay 1/256mm>x>1/1024mm	Zn ppm	Cu ppm	Cd ppm	Visibility (m)	δ13C coral	δ13C seaweed	δ13C sediments	δ15N coral	chl-a mg/m3
JB1	-1	300		75.1	12.38	12.52	105.23	61.07	5.81	0					53.686
JB2	-1.5			49.13	22.21	28.66	80.96	59.15	5.47	0.5			-22		22.156
JB3	-2		300	21.97	38.24	39.79	78.74	58.64	5.97	1.5			-22		22.156
JB4	-1.75		690	71.17	8.87	17.01	35.26	118.66	10.22	2	-13.8	-17.1		7	21.304
JB5	-1.75		1290	48.85	18.17	17.66	53.42	93.38	7.03	2				6	11.078
JB6	-1.25		1950	45.05	7.8	24.27	37.06	127.54	6.2	1.5	-12.5			5.1	3.835
JB7	-2.25		2550	54.75	12.89	29.35	32.62	91.56	9.06	1.5	-12.6			5.4	2.983
JB8	-3.25		3150	79.54	0.91	0	5.88	91.44	8.78	2	-12			5.1	
JB9	-1.75	600		54.36	25.49	20.04	124.93	72.96	7.02	1			-21.5		28.973
JB10	-2.75	990		35.11	48.56	15.82	127.34	73.22	7.74	2			-20.6		7.243
JB11	-5.25	1090		59.46	22.68	17.69	97.46	77.14	6.88	1.5					0.426
JB12	-5.75	1290		0.57	34.9	64.47	44.3	59.58	18.57	1.5			-20.7		0.426
JB13	-7.5	1860		15.12	27.11	55.54	40.67	64.87	6.7	1.5			-19		6.391
JB14	-10	2400		4.1	22.18	74.67	46.72	51.92	4.22	2					5.113
JB15	-3			99.14	0.43	0	1.93	98.99	9.3	3	-11.8	-14		5	7.243
JB16	-3			82.12	3.66	1.21	3.31	110.61	9.81	3	-13	-13.5		5	
JB17	-3			67.38	5.05	2.37	8.36	131.94	7.67	2	-13			5	
JB18	-3			75.15	8.05	1.92	14.89	111.04	9.64	4	-13				4.261
JB19	-1.75	600		68.73	15.01	16.79	112.65	70.75	6.78	1			-21		13.634
JB20	-2	1380		83.4	7.88	8.66	85.54	70.01	5.59	1			-21.2		12.782
JB21	-3	1800		77.77	11.13	11.88	58.52	80.93	18.53	1					8.095
JB22	-3.5	2130		89.17	2.51	7.7	12.74	91.98	11.12	1.5	-11	-15.3			2.131
JB23	-4.5			57.22	4.19	9.69	16.33	116.5	6.21	2	-13.6	-15.4		6.2	7.669
JB24	-4.5			85.97	1.79	6.7				2	-13.2	-14.8		6	3.409
JB25	-5			30.96	37.16	31.88	6.6	92.88	8.18	3	-11.9	-13.2		5.5	
JB26	-3.25		660				56.84	66.13	8.42	1.5					10.226
JB27	-5.25		1110	3.63	37.91	58.46	39.24	37.87	4.38	1					5.965
JB28	-6.75		1710	0.98	33.62	65.4	46.93	56.53	5.27	2					4.867
JB29	-7.75		2220	0.2	31.22	68.58	51.58	78.28	15.95	2.5					2.13
JB30	-10		2730	1.22	42.75	56.03	64.57	68.1	1.48	3					2.556
JB31	-0.75	0		52.89	20.3	26.4	131.45	63.43	5.84						49.425
JB32	-6.5										-12.6		-16.2	4.8	

Table 1. A summary of all collected data from Jepara Bay.

Discussion

The bathymetry of Jepara Bay indicates a bowl shaped depression with shallow rims (depth profiles on transects). That is, in general, depth increases with increasing distance from source. Increasing depth, and therefore increasing water column, may create natural gradients within the bay, problematic for resolving an induced anthropogenic gradient. Further, the bowl shape of the harbour, with discharges (rivers) at either enclosed 'corner' creates a focusing effect for finer grained sediments, with the area of least turbidity, the center of the bay, optimising fine settling.

In general, the grain size distribution in the bay shows a sand wedge near each river mouth, with the total % sand decreasing with increasing distance from source. This is particularly evident in transects two and three, which show a pinching out of the sand fraction approximately 1000 m from the shore (Figs. 6 and 7). Transects one and four, along the south and north shore of the bay, respectively, do not show a pinching out of the sand fraction in the sediments, and are more constant in their sand content ratios. Transect one even shows an increase in sand content nearing the point defining the bay's most westerly point (Fig. 5). This is likely a shoreline effect, with local longshore currents (south to north) winnowing fines around the point. This results in a coarse grained sediment near the tip of each point. A similar effect was seen on the north shore, with the sediment predominately a coarse shell hash (coquina), however, since the sediment fractions were recorded on the clastic fractions only, this is not reflected in the sediment profile of transect four (Fig. 8).

Silt and Clay % increases with increasing distance from source in all four transects (Figs. 5-8). Silt reachs a maximum concentration between 1000 and 1500 m from the sources. As mentioned, energy levels allow this sediment focusing in the central bay. This has important ramifications in the interpretation of the heavy metal distributions. Finer grain sizes have larger surface areas per unit volume, thereby allowing more adsorption and adhesion to grains of contaminants (Coakley et al, 1992), including heavy metals. This grain size effect is immediately obvious when considering the heavy metal data from the four transects.

Zn and Cu concentrations seem to be closely related to the silt size fraction of grain size, while Cd is more dependent on Clay size fractions. As a result, the metals do not show a clear gradient from the point source discharge, but rather contain secondary maximums related to the focused fine grained sediments. In the contoured data sets of the heavy metals (Figs. 9-11), The plume of discharge from the two rivers is clearly visible, with secondary highs in the central bay. If these secondary maxima in the bay are solely attributed to a grain size effect, this allows the relative impacts of the two rivers to be seen.

K. Jepara (the Jepara river), is a larger river, with the mouth of the river harbouring the local fishing boats. It flows directly through the town of Jepara, and is the major source of input to the bay. K. Tambangan (the Tambangan river) is too small for boat traffic and flows through the town and surrounding arable land. From the extent of the plumes into the bay, as seen from the heavy metal data (esp. the Zn data, Fig. 9), Jepara River is a major source of contamination to Jepara Bay. While the contamination itself is not surprising, there is little attempt to monitor pollution, the extent of the contamination is distressing. When

compared to Provincial guidelines (Hamilton Harbour Remedial Action Plan), the contamination within the bay is an order of magnitude higher than suggested threshold values (see annotated thresholds on contour diagrams).

The extent of contamination is also puzzling from a source standpoint. Where are the high metal levels coming from? The town of Jepara is built on a coastal plain, so natural mineral content in the surrounding soils is not a possible source, and there is no industry to speak of. The local trade is furniture building, conducted far from the bay itself, using mainly traditional tools. At the current time the only conclusion that can be drawn from the data is that the source is likely anthropogenic. We base this assertion on the other natural tracer which we employed, chlorophyll-a.

Chlorophyll-a measurements indicate the amount of phytoplankton in the water column. This is seen as the most reliable method of indicating a eutrophic envirionment (Smith et al, 1981; Bell, 1989, 1991; Lapointe and Clark, 1992), because it closely approximates the amount of bioavailable nutrients. Also, nutrient measurements of the water column involve complicated chemistry and on-site facilities, not available to us in Jepara Bay, while chlorophyll analysis need not be on-site. The definition of eutrophication often includes 'development of nuisance algae', which is more closely related to amount of bioavailable nutrients than total dissolved nutrients in the system. For these reasons we conducted chlorophyll-a measurements throughout the bay.

The contour diagram of chlorophyll-a distribution in Jepara Bay (Fig. 12) is similar to the heavy metal distribution diagrams. That is, it shows a large plume of discharge from the Jepara river with a smaller discharge from the Tambangan river. There is a minor shoreline effect, likely related to amount of

water column available to the phytoplankton (i.e. in shallow water phytoplankton are more concentrated by necessity). The profiles of chlorophyll-a distribution along the transects (Figs. 5-8) also show an exponential decrease in chlorophyll-a concentration with increasing distance from source.

These data confirm that the two rivers are major sources of contaminant discharge into Jepara Bay, and also allows us to hypothesis a source. With no sewage treatment facilities in a town of 300 000, the major conduit for effluent is the Jepara River. This river supplies a source of nutrients to the bay, allowing a phytoplankton bloom to develop (asserting the river as a source of anthropogenic discharge). The extent of eutrophication in the bay is as alarming as the extent of heavy metal contamination. Chlorophyll concentrations adjacent to the discharges are two orders of magnitude above the recommended threshold value as defined by Bell (1991). This threshold value of 0.5 mg/ m^3 is seen as critical to continued development of coral in a region without filter feeder or algal blooms.

It was the combination of high nutrient values and an anthropogenic gradient which lead us to the use of isotopes to try and pinpoint the source of discharge to the bay. Isotopes have been utilised repeatedly in food web studies (Rau et al, 1981; Mariotti et al, 1984; Cifuentes et al, 1988), and to trace sewage contamination (Sweeney and Kaplan, 1981; Sweeney et al, 1981). This is mainly related to the distinct isotopic signatures from marine and terrestrial environments (Owens, 1987; Michener and Schell, 1994), which may be relatively preserved. At Jepara Bay high nutrient loading and low light levels (Figs. 5-8, 12) combine to suggest corals would function heterotrophically. We base this assertion on studies of coral at depth (and therefore reduced light)

(e.g. Muscatine et al, 1989; Muscatine and Kaplan, 1994; Heikoop et al., this thesis) showing reduced zooxanthellar activity, and zooxanthellae under nutrient enrichment (Jokiel et al., 1994) where translocation to the coral ceased. As heterotrophs, coral may rely on assimilation and absorbtion of particulate and dissolved organic matter. They could also be expected to reflect their dietary source with an enrichment of 3-5‰ related to trophic level effects (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). This would allow us to determine the isotopic composition of the nutrient source to the bay, and pinpoint the contaminant.

Initial attempts to trace sewage contamination on coral reefs met with mixed results (Risk et al, 1989; Allison et al, 1991; Risk et al, 1993), attributed to counteractive effects of concentration and degradation of isotopic signature. More recently, light gradients and gradients in DIN have also been suggested as influencing the signature of $\delta^{15}N$ in coral tissues (Muscatine and Kaplan, 1994; Heikoop et al, this thesis). It was hoped that by collecting over a gradient we would be able to resolve some of these issues, and discern the isotopic signature of the source. However, without accurate light measurements and $\delta^{15}N$ of the water column, correction of these values is problematic at best. Isotopic values show significant change across the bay ($p<0.02$ in sediments $p<0.005$ in corals), (Figs. 5-8, Table 1).This study suggests that the use of isotopes as natural tracers may be suited to corals.

Given the results of the heavy metal and chlorophyll analysis it is not surprising that the coral development in the area is restricted to surrounding the small island and points of the bay (Fig. 2). It is likely that these areas are amply flushed by longshore currents to allow continued coral growth. These corals are

in an obvious state of decline (J. Dunn, pers. observ.) with an abundance of macroborers infesting individual heads, and in some cases up to an inch of flocculent material blanketing the coral. The coral reef which once inhabited Jepara Bay has likely been irrepairably damaged by anthropogenic activities. This may present problems in the future for the local inhabitants, as continued decline of the surrounding reefs will inevitably lead to collapse of the fisheries.

Conclusions

Heavy metal levels of Zn, Cu and Cd are all elevated relative to threshold levels. Chlorophyll-a data, also above threshold levels, indicate high levels of bioavailable nutrients. These data suggest severe anthropogenic impacts to Jepara Bay. Stable isotope data were inconclusive, related to multiple unresolvable gradients affecting $\delta^{15}N$ of the coral tissue (including light and concentration). Multiple tracers are a valid technique for determining anthropogenic input in a coral reef environment.

Acknowledgements

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Chapter 5 - Conclusions

Multiple factors control the $\delta^{15}N$ of coral tissues. These factors were addressed in three manuscripts. Results from Chapter 2 suggest that light can explain up to 90% of the variance in $\delta^{15}N$ of corals. The mechanism by which light affects the $\delta^{15}N$ is related to the symbiotic nature of corals. Symbiosis and conservation of nitrogen between the coral host and its algal symbiont lead to assimilation of all available nitrogen in light-sufficient regimes (i.e. where zooxanthellar activity is unlimited). As a result, there is no fractionation of nitrogen by corals in areas where light requirements are non-limiting. In areas where the coral is light-limited, or dependant upon heterotrophic dietary supplements (e.g. isotopically light plankton), fractionation occurs, making the corals isotopically depleted.

Results from Chapter 3 suggest that concentration and distance from source may play a role in coral $\delta^{15}N$. It is possible that the isotopic signature of sewage is rapidly degraded by cycling in the marine food web. This would result in an increased nutrient flux to the reef, but with an unknown isotopic composition. The effects of the concentration of nutrients on coral isotopic composition are unclear. In increased concentrations of nutrients, corals may be forced to assume a heterotrophic diet, relating to loss of control over the symbiosis. There may be isotopic depletion relating to preferential zooxanthellar assimilation, or isotopic enrichment relating to excretion. Although samples were collected over a concentration gradient in Chapter 4, these ambiguities were not cleared up because of the additional variable of light.

Results suggest that it may be possible to use nitrogen isotopes in coral tissues as a tracer of eutrophication of coral reefs. Light measurements, water chemistry, and samples of all levels of the trophic web would be necessary in order to meet this goal. Research to discern the relative control of environmental variables on $\delta^{15}N$ of coral tissues is also necessary.

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Appendix One - Data Tables

Table 1. Isotopic Data from Zanzibar (Chapter 3) in per mille units. Far right column indicates year of collection.

	δ 15N	δ13C	
Chumbe	4.6	-9.4	1992
	4.7	-10.1	1992
	3.2	-9.6	1992
	4.4	-10.4	1992
	3.6	-12.7	1992
	4.4	-10.2	1992
	4.4	-11.7	1992
	4.3	-10.8	1992
	4.0	-11.0	1992
Mean	4.2	-10.7	
Std dev	0.5	1.0	
Chapani	5.2		1993
	4.9		1993
	5.1		1993
	5.0		1993
	4.8		1993
	4.7		1993
	4.7		1993
	4.6		1993
	4.7		1993
	4.5		1993
	4.4		1993
	4.4		1993
	4.2		1992
	3.6	-10.1	1992
	4.1	-8.9	1992
	4.5	-11.0	1992
	4.5	-10.1	1992
	4.1	-11.0	1992
	4.4	-9.5	1992
	4.3	-10.1	1992
	4.2	-9.4	1992
	4.1	-8.7	1992
	4.3	-10.8	1992
Mean	4.5	-10.0	
Std dev	0.4	0.8	

Table 2. Data collected in Indonesia during the summer of 1993. Site, sample type, and distance from source of contamination are all indicated. Isotopic values for $\delta^{15}N$ and $\delta^{13}C$ are reported in units of ‰ (Chapter 4)

Site Number	Sample Name (prefix/description)	Analysed For: C	N	Distance from Source (m)
1				300
2				
3				300
4	JB H1 GONIA?	-13.8	7.0	690
	JB #2 GONIA	-13.8	7.5	
	JB SW -1	-17.1		
	JB SW -2	-7.7		
	JB SW -3	-14.6		
5	JB	*	6.0	1290
6	JB PORITES	-12.5	5.2	1950
7	JB 7B PORITES	-12.9	5.5	2550
	JB 7A PORITES	-12.7	5.5	
8	JB	-12.1	5.2	3150
	JB	-10.5		
9				600
10				990
11				1090
12				1290
13				1860
14				2400
15	JB	-11.8	5.1	3500
	JB NS	-11.9	5.4	
	JBH1#2	-12.2	5.0	
	JB SW -1	-12.4		
	JB SW -2	-12.4		
	JB SW	-14.0		
16	JB	-12.5	4.4	3500
	JBH1	-14.6	4.3	
	JB 2L	-11.9	5.1	
	JBH2	-13.3	5.1	
	JB SW	-13.5		
	JB SW -2	-10.0		
17	JB	-13.0	4.9	3500
18	H1 LIGHT	-11.7	*	3500
	H1	-13.2	5.1	
19				600
20				1380
21				1800
22	JB PORITES	-15.3	6.4	2130
	JB SW	-11.0		
23	JB1L	-11.5	5.3	2500
	JB	-13.6	6.2	
	JB SW	-9.8		
	JB SW -2	-15.5		
24	JBH2	-13.2	6.1	around pt

Table 2. Data collected in Indonesia during the summer of 1993. Site, sample type, and distance from source of contamination are all indicated. Isotopic values for $\delta^{15}N$ and $\delta^{13}C$ are reported in units of ‰ (Chapter 4)

Site Number	Sample Name (prefix/description)	Analysed For: C	N	Distance from Source (m)
1				300
2				
3				300
4	JB H1 GONIA?	-13.8	7.0	690
	JB #2 GONIA	-13.8	7.5	
	JB SW -1	-17.1		
	JB SW -2	-7.7		
	JB SW -3	-14.6		
5	JB	*	6.0	1290
6	JB PORITES	-12.5	5.2	1950
7	JB 7B PORITES	-12.9	5.5	2550
	JB 7A PORITES	-12.7	5.5	
8	JB	-12.1	5.2	3150
	JB	-10.5		
9				600
10				990
11				1090
12				1290
13				1860
14				2400
15	JB	-11.8	5.1	3500
	JB NS	-11.9	5.4	
	JBH1#2	-12.2	5.0	
	JB SW -1	-12.4		
	JB SW -2	-12.4		
	JB SW	-14.0		
16	JB	-12.5	4.4	3500
	JBH1	-14.6	4.3	
	JB 2L	-11.9	5.1	
	JBH2	-13.3	5.1	
	JB SW	-13.5		
	JB SW -2	-10.0		
17	JB	-13.0	4.9	3500
18	H1 LIGHT	-11.7	*	3500
	H1	-13.2	5.1	
19				600
20				1380
21				1800
22	JB PORITES	-15.3	6.4	2130
	JB SW	-11.0		
23	JB1L	-11.5	5.3	2500
	JB	-13.6	6.2	
	JB SW	-9.8		
	JB SW -2	-15.5		
24	JBH2	-13.2	6.1	around pt

	JB SW	-14.8		
25	JB	*	5.5	around pt
	JB H2	-11.9	5.5	
	JB SW	-13.2		
26				660
27				1110
28				1710
29				2220
30				2730
31		-12.2		0
32	JBH1	-12.6	4.6	5000
	JB H1 (A)	*	4.8	
PI	1	-13.0	5.0	infinite
	2	-12.1	4.7	
	3	-13.2	5.0	
			5.4	
1956		-11.7	5.7	infinite
K1	H1	-15.2	9.4	1000
	H2	*	8.2	
		-14.5	*	
K2				2000
K3	H2	-15.4	7.745, 9.046	3000
	A	-15.4	7.0	
		-13.6	8.0	
K4				
K5	?C	-13.7		5000
	PLATY	-9.6	*	
	GONIA?	-15.4	3.2	
K6		-13.3	3.4	6000
K7		-13.0	*	7000
	H3	-14.8	4.5	
	JB SW	-13.5		
K8				8000
K9		*	4.5	9000
K10		*	*	10000

**key to prefixes: JB = Jepara Bay, Central Java, Indonesia
PI = Para Island, North of Sulawesi
1956 = Ruang Island, North of Sulawesi where there was an eruption i
leaving a huge lava flow down the Island and onto the reef
K = the thousand islands, north of Jakarta

h = head
sw = seaweed

Table 3. Measurements of organic contents of sediments from Jepara Bay Using a loss on ignition technique. These data were used to estimate packing weights for sediment isotopic analysis. (Chapter 4)

sediments were combusted for 2.5 hours at 550°C
combustion vesicle was a porcelain boat precombusted at 550°C for 15 mins.
(to remove possible contaminants)

					mg sed to be packed		
Boat #	Sample #	Initial wt. (g)	Post Combust	% Org.	C	N	gr. size
18	9	11.866	11.826	0.33	900	4501	br. med. sand
12	19	12.103	12.018	0.71	424	2122	f-med br. sand
13	27	10.845	10.744	0.93	321	1606	br. silt
16	12	12.622	12.515	0.85	353	1767	br. silt
17	1	12.721	12.645	0.60	498	2489	br med-coarse sand
20	20	11.863	11.783	0.67	447	2235	br. med sand
15	26	11.374	11.291	0.73	411	2053	br. fine sand
19	3	13.411	13.310	0.75	398	1990	br. fine sand
20	22	11.441	11.410	0.27	1093	5467	lt br silt
19	23	12.700	12.684	0.13	2381	11906	br silt
15	4	11.364	11.286	0.69	437	2183	v. f. sand
18	21	12.817	12.696	0.94	318	1592	br f sand
13	25	11.138	11.002	1.22	245	1226	br v f sand
17	24	12.487	12.447	0.32	927	4634	gr silt
16	7	12.228	12.180	0.39	767	3836	silt
12	6	11.653	11.608	0.39	775	3873	br silt
12	18	11.368	11.357	0.09	3242	16209	br silt
19	8	12.698	12.684	0.11	2631	13155	br f sand
16	16	12.173	12.129	0.36	831	4154	br silt/sand
20	5	11.395	11.366	0.25	1199	5997	grey sand
15	14	11.296	11.236	0.53	566	2831	br silt/mud
0	28	11.745	11.620	1.06	284	1418	br silt
17	13	13.156	13.019	1.04	289	1445	br/gr silt
13	4	10.504	10.453	0.48	624	3119	br v f sand
18	30	12.714	12.579	1.06	283	1413	br f sand
9	29	11.046	11.016	0.28	1090	5449	br v f sand
15	21	11.336	11.253	0.74	407	2035	gr v f sand
17	21	13.173	13.029	1.10	273	1367	br sand
13	10	10.681	10.625	0.53	567	2835	br sand
12	31	11.629	11.580	0.43	704	3518	br sand
16	2	12.939	12.802	1.06	283	1414	br sand
20	11	11.624	11.579	0.38	783	3914	br sand
19	24	13.342	13.227	0.86	348	1739	gr silt

LEGEND:

INITIAL WEIGHT:

the weight of a precombusted porcelain crucible plus the weight of decalcified, rinsed, dried sed.

POSTCOMBUSTION WEIGHT

after two and one half hours of ashing at 550°C, the weight of the crucible and afore mentioned seds. are taken again. The difference in weight is attributed to loss of organics during ashing.