Vol. 280: 105–114, 2004 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published October 14

Nutrient-induced perturbations to $\delta^{13}C$ and $\delta^{15}N$ in symbiotic dinoflagellates and their coral hosts

Ove Hoegh-Guldberg[1,*], Leonard Muscatine[2], Claire Goiran[2], Dorthe Siggaard[1], Guy Marion[1]

[1]Centre for Marine Studies, University of Queensland, St Lucia, Queensland 4067, Australia
[2]Department of Biology, University of California at Los Angeles, Los Angeles, California 90095, USA

ABSTRACT: Inorganic nutrients play a critical role in determining benthic community structure in tropical seas. This study examined the impact of adding inorganic nutrients (ammonium and phosphate) on the isotopic composition of 2 reef-building corals, *Pocillopora damicornis* and *Heliofungia actiniformis*, on the southern Great Barrier Reef. The addition of elevated nutrients to patch reefs that pond at low tide did not perturb the C:N ratio of either species or their symbiotic dinoflagellates. The C:N ratios were significantly higher in material extracted from the skeleton (14.8 ± 1.50 and 10.8 ± 1.42) than either host (7.6 ± 0.87 and 6.0 ± 0.71) or symbiotic dinoflagellates (5.7 ± 0.48 and 6.9 ± 0.66) (*P. damicornis* and *H. actiniformis* respectively; ±95 confidence intervals). The ratio of acquired N to background N suggests that the added dissolved inorganic nitrogen (DIN) accounted for 50 to 100% of total nitrogen within the tissues of *P. damicornis* and *H. actiniformis* at the end of the experiment. The addition of the isotopically depleted nutrients ($\delta^{15}N$ = 0‰) to patch reefs significantly decreased $\delta^{15}N$ from control values of between 3 and 4 to values to below 1 in the case of all compartments, while $\delta^{13}C$ values were relatively unresponsive to nutrient treatments. These findings suggest that coral $\delta^{15}N$ has the potential to provide a historical record of the $\delta^{15}N$ of dissolved nitrogen surrounding reef-building corals and their symbiotic dinoflagellates.

KEY WORDS: Nutrients · Corals · $\delta^{15}N$ · Eutrophication · Fertilizer use · ENCORE · *Pocillopora* · *Heliofungia* · Experimental geochemistry

INTRODUCTION

The abundance and distribution of many coral reefs coincide with warm (>18°C), sunlit water characterized by relatively low concentrations of particulate and dissolved inorganic nutrient concentrations (Odum & Odum 1955). Despite low inorganic nutrient concentrations, coral reef ecosystems have rates of primary production that may surpass 5 kg C m^{-2} yr^{-1}, which far exceed the productivity of these ecosystems based on water column nutrient concentrations (Larkum 1981, Muscatine 1990, Sorokin 1990). In spite of their seeming robustness, coral reef ecosystems appear to be particularly vulnerable to changes in water quality (Smith et al. 1981, Lapointe 1997). Recent assessments rate changes to coastal discharge as one of the most serious threats facing coral reef ecosystems worldwide (Tomascik & Sander 1985, 1987a, 1987b, Lapointe 1997, Vitousek et al. 1997a, Edinger et al. 1998, Costa et al. 2000, Heikoop et al. 2000b, McCulloch et al. 2003). The rapid growth of human populations and associated land use transformations in tropical coastal areas has led to an increase in the discharge of sewage, storm water and agricultural run-off onto coral reefs (Gerlach 1981, Vitousek et al. 1997b).

The complex mixtures of inorganic nutrients, sediments, pesticides and heavy metals flowing from many coastal areas have had a series of deleterious influences on coastal ecosystems (Gerlach 1981, Bell 1992, Sebens 1994). Impacts range from ecological phase

*Email: oveh@uq.edu.au

shifts and changes in community structure to the complete loss of reef ecosystems (Hughes 1994, Sebens 1994, Lapointe 1997, McCook 1999). Interpretation of episodic impacts like those associated with the eutrophication in Kaneohe Bay, Hawaii (Maragos et al. 1985) or the mixed anthropogenic stresses on Jamaican reefs (Goreau 1992) is complicated by the fact that these natural 'experiments' are uncontrolled. In order to monitor and address pollution related stress, it is important to identify which of the many possible water quality factors influence benthic ecosystems.

Nutrient isotopic signatures are useful in deciphering pollutant sources in marine communities (Owens 1987, McClelland et al. 1997, Heikoop et al. 1998, Risk et al. 2001). Biological processes preferentially assimilate isotopically light isotopes of a nutrient source. Dissolved inorganic nitrogen (DIN) is an important part of the conditions that determine the state of coral reef ecosystems (Vitousek et al. 1997b). Biological processes which utilize nitrogen sources discriminate against ^{15}N, altering the source nitrogen signature $\delta^{15}N$, or the ratio of $^{15}N/^{14}N$ compared to atmospheric N_2. Enriched $\delta^{15}N$ (8 to 22‰) of coral tissue and other bioindicators have detected the influence of sewage in coastal waters of Indonesia, Jamaica, the Great Barrier Reef and the United States (McClelland et al. 1997, Sammarco et al. 1999, Heikoop et al. 2000a, Risk & Erdmann 2000, Costanzo et al. 2001, Yamamuro et al. 2003). Depleted $\delta^{15}N$ values (–2 to 2‰) generally indicate atmospheric nitrogen fixation or exposure to inorganic fertilizers (Heaton 1986, McClelland et al. 1997, Risk et al. 2001). Inter-site $\delta^{15}N$ comparisons must account, however, for location-specific influences on $\delta^{15}N$. These include bioindicator species, fractionation of source nitrogen during organism uptake, light intensity, ambient DIN concentrations, community baseline $\delta^{15}N$ of producers and consumers, and stepwise enrichment of $\delta^{15}N$ between trophic levels (Minagawa & Wada 1984, Owens 1987, Muscatine & Kaplan 1994). $\delta^{15}N$ analysis from living tissues also only provides an immediate snapshot of nutrient conditions.

Manipulative experiments offer a clear advantage in developing the use of $\delta^{15}N$ in long-term eutrophication studies. Small scale laboratory and raceway experiments (e.g. Hoegh-Guldberg & Smith 1989, Muscatine et al. 1989, Belda et al. 1993, Jokiel et al. 1994, Muller-Parker et al. 1994a, Ferrier-Pages et al. 1998) have provided important information on how aspects of water quality affect the physiology of key reef organisms. Symbiotic corals and clams, for example, exhibit fundamental changes in their physiology when inorganic nutrients are added to the water surrounding them (Hoegh-Guldberg & Smith 1989, Muscatine et al. 1989, Belda et al. 1993, Jokiel et al. 1994, Muller-Parker et al. 1994a, Ferrier-Pages et al. 1998). These laboratory or aquarium experiments, however, may not satisfactorily predict how nutrients affect coral reef organisms in the field. Most of these studies are also restricted to periods of less than 3 mo and consequently may not reveal long-term responses of organisms to changes in water quality. The ideal experiment for isolating the influence of specific anthropogenic changes is a fully replicated, balanced design that varies conditions within natural coral reef ecosystems in known and controlled ways.

The ENCORE (Enrichment of Nutrients on a Coral Reef Experiment) project is the first attempt at such an experiment (Larkum & Steven 1994, Koop et al. 2001). During this large scale and multidisciplinary study pursued at One Tree Island on the southern Great Barrier Reef, ammonium chloride (NH_4Cl) and sodium phosphate (NaH_2PO_4) were delivered to large replicate patch reefs twice daily for 2 yr. The responses of organisms and processes associated with these reef systems were then studied. The present study measured the effect of long-term eutrophication in the field on $\delta^{13}C$ and $\delta^{15}N$ in 2 species of reef-building corals growing in the ENCORE patch reefs.

MATERIALS AND METHODS

The ENCORE nutrient enrichment experiment. The ENCORE program was established by the Great Barrier Reef Marine Park Authority (GBRMPA) and the Australian Research Council in early 1993. The program involved the addition of nutrients to replicate patch reefs located within One Tree Island's 8 km^2 lagoon (23° 30′ S, 152° 06′ E). Patch reefs were typified by having a thick (>0.5 m) wall around a central pool, which becomes self-contained at low tide. The patch reefs ranged from 16 to 25 m in diameter, 0.5 to 0.8 m in depth, 46 to 152 m^3 in volume and were separated by distances of more than 100 m (Table 1 in Koop et al. 2001). In total, 12 patch reefs were chosen and allocated to 4 nutrient treatments at random (i.e. 3 patch reefs per treatment). The treatments were as follows: N, in which a solution of NH_4Cl was added; P, in which a solution of NaH_2PO_4 was added; N + P, in which a solution of both NH_4Cl and NaH_2PO_4 was added; and C, a set of untreated control patch reefs. Remotely controlled Nutrient Dispersal Units (Larkum & Steven 1994) were used to add nutrients every low tide for 2 yr. Water within the patch reefs was isolated from surrounding water for at least 4 h each low tide, during which time the nutrients were completely depleted (Koop et al. 2001). This, in addition to the enormous dilution ratio between the patch reefs and the sur-

rounding water separating them, meant that patch reefs were effectively isolated and independent of each other.

The ENCORE program began with a pre-enrichment phase (February 1993 to August 1993). The period in which nutrients were added was divided into 2 phases. During the first phase (September 1993 to December 1994), nutrients were added once every low tide to bring the ambient concentration to 10 µM ammonium and/or 2 µM phosphate, depending on the nutrient treatment. At the end of 1994, nutrient concentrations revealed that added nutrients disappeared from the water column of the patch reefs so rapidly that they were often undetectable 2 h after nutrient addition (Koop 1995, Steven et al. 1995). To achieve higher concentrations for longer periods, nutrients were added more regularly (3 times, which were approximately 37 min apart) using twice the original amount of nutrients per addition during the second phase of the experiment (January 1995 to February 1996). During the second phase, ammonium concentrations were 10 to 25 times higher (20 µM) and PO_4^{2-} concentrations were 10 to 100 times higher (4 µM) than concentrations in control patch reefs for several hours each low tide (Koop 1995, Steven et al. 1995, Koop et al. 2001).

Isolation of symbiotic dinoflagellates, host tissue and material isolated from the skeleton. Two species of reef building corals were chosen for the experiment. These species were chosen to represent the range of polyp sizes within corals at One Tree Island: *Heliofungia actiniformis* has single solitary polyps that grow to over 40 cm in diameter while *Pocillopora damicornis* has polyps that are a few millimeters across.

A single *Heliofungia actiniformis* (approximately 15 to 20 cm in greatest diameter) and 2 colonies (>20 cm in diameter) of *Pocillopora damicornis* (brown morphotype, Takabayashi & Hoegh-Guldberg 1995) were collected from each of the 12 ENCORE patch reefs during February 1996. These corals were originally placed in the patch reefs in February 1993. In the case of *H. actiniformis*, individuals were originally 7 to 10 cm in size, while *P. damicornis* colonies were placed in the patch reefs as small nubbins (ca. 5 cm long; described by Takabayashi & Hoegh-Guldberg 1995). Coral colonies from each patch reef were transported to the laboratory at One Tree Island Research Station in February 1996 and maintained briefly in aquaria in running seawater.

Skeleton, animal tissue and dinoflagellates were separated by the method of Muscatine & Cernichiari (1969). Briefly, coral heads were placed in aluminum foil envelopes and were pulverized with a hammer. The resulting skeletal fragments and slurry were transferred to a 500 ml Erlenmeyer flask. Seawater was added to just cover the pulverized coral and the flask subjected to wrist-action shaking for 2 min. The seawater containing symbiotic dinoflagellates and animal tissue was decanted through several layers of surgical gauze and about 150 ml centrifuged (Clements 2000 swinging bucket) for several minutes at a speed sufficient to produce an algal pellet (2000 rpm or $720 \times g$; 5 min). The pellet was washed several times to minimize contamination by animal tissue and saved. The supernatant animal fraction was centrifuged again at higher speed (4000 rpm or $2880 \times g$) to remove residual algae and decanted into plastic containers. Algal pellets and animal tissue fractions were then frozen at –20°C. The pulverized skeleton was frozen overnight, then thawed, rinsed with a jet of fresh water to remove residual tissue and air-dried. Discolored, eroded or contaminated skeletal bits were discarded in favor of clean white skeleton. Skeletal dry weights averaged 337 ± 84 g (mean ± SD). Frozen samples were transported to the University of Sydney and kept frozen until analyzed. Samples of the ammonium chloride (dry pellets) that were added to patch reefs during the ENCORE experiment were also collected for $\delta^{15}N$ analysis.

Measurement of C:N ratios, $\delta^{13}C$ and $\delta^{15}N$. C:N ratios, $\delta^{13}C$ and $\delta^{15}N$ were measured in 3 compartments: symbiotic dinoflagellates, host tissue and coral skeleton. Skeletons were white in color with little visual evidence of endolithic algae. Tissue and skeleton were prepared for stable isotope analysis using the following methods:

(1) Pellets of frozen symbiotic dinoflagellates were lyophilized and then ground to a fine powder. (2) Samples of frozen host tissue were lyophilized, reconstituted in a small volume and then dialyzed (Sigma, benzoylated dialysis tubing cut-off of 1.2 kD) against reverse osmosis (RO) water overnight to remove salt. The water was changed 3 to 4 times during this period. The remaining host tissue was lyophilized again after dialysis. (3) Skeletons were soaked in a solution of commercial sodium hypochlorite (full strength Domestos™) for 4 h or more to remove any residual surface organic matter. After rinsing with RO water, the skeleton was dried overnight at 40°C and then ground into a coarse powder with a mortar and pestle. The ground skeleton was boiled with a 2 M NaOH solution for 15 min to remove any remaining organic material and subsequently rinsed 2 to 3 times with RO water. The samples were dried in an oven at 40 to 50°C for 3 d and then ground in a Tima mill, fitted with a tungsten carbide head that produced a very fine powder. A 10 g subsample of the resulting skeletal powder was acidified with 2 M HCl (Aristar grade 35%) to dissolve the calcium carbonate. The solution was then neutralized using 2 M NaOH and dialyzed (Sigma, benzoylated dialysis tubing, cut-off 1.2 kD) against RO

water overnight (3 to 4 water changes) to separate the aggregate (soluble and insoluble) organic material from the salts. Finally, the dialyzed solution was lyophilized and powdered in a mortar and pestle in preparation for stable isotope analysis.

The stable isotope signatures of the samples and ammonium chloride pellets were analyzed using a Europa Scientific Tracermass spectrometer with an automated in-line sampler (Roboprep). Samples were passed through the spectrometer twice and data on the amounts of each isotope of carbon and nitrogen used to calculate C:N, $\delta^{13}C$ and $\delta^{15}N$. The first run yielded data for carbon and the second run (adjusted to 100 µg N using N measurements from the first run) was used to obtain a more accurate measurement of $\delta^{15}N$. Machine references were run every 6 to 8 samples, with nitrogen being referenced to analytical grade ammonium sulphate and carbon to analytical grade sucrose.

The value of $\delta^{13}C$ and $\delta^{15}N$ (‰) for each sample was calculated as follows:

$$\delta^{13}C \text{ or } \delta^{15}N = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \quad (1)$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$.

Contribution of added nitrogen to overall N budgets of corals and their symbionts. We calculated the proportion of nitrogen atoms originating from the ammonium chloride added during the ENCORE experiment. By comparing $\delta^{15}N$ from colonies from control reefs (no contribution of synthetic ammonium) to those collected from reefs that had synthetic ammonium added to them, the extent to which the added ammonium contributed to the final $\delta^{15}N$ was calculated as follows:

$$\% \text{ contribution} = 100 \times (\delta^{15}N_{control} - \delta^{15}N_{N \text{ or } N+P}) / \delta^{15}N_{control} \quad (2)$$

This calculation was only done for the symbiotic dinoflagellate and host fractions due to the complication of the fact that significant amounts of skeletal organic material originated from before the corals were placed into ENCORE patch reefs. This was not a problem with host or symbiotic tissue which has turn over rates of less than 60 d.

Statistical analysis. Data were analyzed using a 2-factor ANOVA (compartment × nutrient treatment; replicates = single individuals from each patch reef) in the case of *Heliofungia actiniformis* and a 3-factor ANOVA (compartment × nutrient treatment × patch reef nested within nutrient treatment; replicate corals from each patch reef) in the case of *Pocillopora damicornis*. Variances within the data were examined using Cochran's test prior to analysis but were found in all cases to be homogeneous. ANOVA and Cochran's test calculations were done using GMAV5 (Underwood & Chapman 1993, University of Sydney).

RESULTS

C:N ratio

Mean C:N ratios ranged from 5.3 to 12.7 for *Heliofungia actiniformis* and from 5.6 to 15.3 for *Pocillopora damicornis*. A 2-factor ANOVA (compartment × treatment) revealed differences due to compartment ($p < 0.001$, $F = 54.8$, df = 2,24) but not to nutrient treatment. However, trends in both data sets indicated that compartments from corals from the N or N + P patch reefs had the lowest C:N ratios, while those in the P-treated patch reefs had the highest in both species. In this case, the C:N ratios of *H. actiniformis* were significantly higher in the skeletal compartment (Fig. 1A, $p < 0.05$). The next highest compartment was that of the symbiotic dinoflagellates. The lack of significant differences between nutrient treatments meant that data could be pooled across compartments (Fig. 1). In all cases, the material isolated from the skeleton had significantly higher C:N

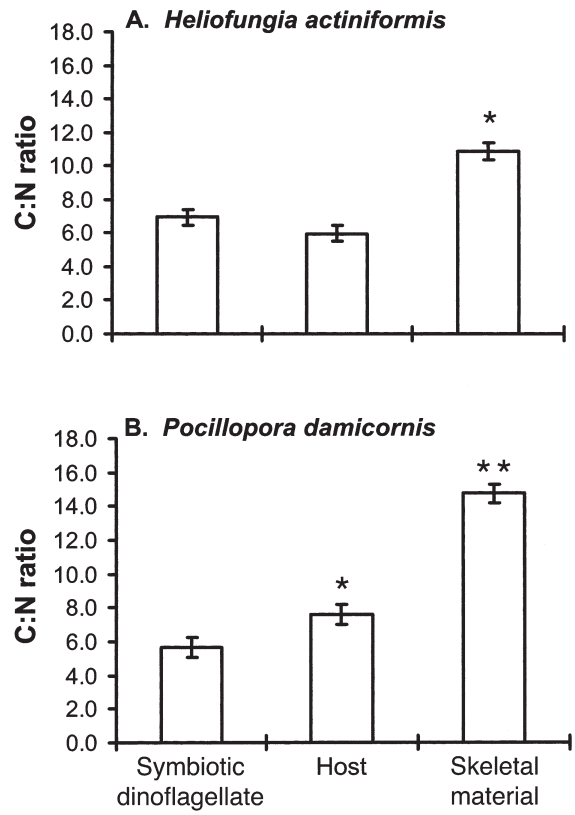

Fig. 1. C:N ratios of symbiotic dinoflagellate, host and organic material isolated from the skeletal compartment in (A) *Heliofungia actiniformis* and (B) *Pocillopora damicornis*. Each bar represents the mean ± SE (all data pooled within compartments; n = 12, *H. actiniformis* and n = 24, *P. damicornis*). Significant differences are indicated by asterisks (*$p < 0.05$, **$p < 0.01$)

ratios than the host or symbiotic dinoflagellates. There was no difference between host and symbiotic dinoflagellate compartments in the case of material from *H. actiniformis* (Fig. 1A). This was contrasted by significantly higher C:N ratios in the host as compared with the symbiotic dinoflagellate compartment in the case of *P. damicornis* (Fig. 1B).

A significant interaction between compartment and experimental patch reef was observed ($p = 0.019$, $F = 2.3$, df = 16,36), indicating that C:N ratios differed between compartments but that this effect was dependent on the patch reef from which the corals were collected. This interaction resulted from the deviation of a single patch reef from a uniform trend observed for the other 11 patch reefs. C:N ratios followed the order of magnitude shown in Fig. 1B, which was specifically symbiotic dinoflagellates < host < skeletal organic material. The exception was data collected from patch reef 8 (N + P) in which the order was host < symbiotic dinoflagellates < skeletal organic material.

$\delta^{13}C$

ANOVA revealed that $\delta^{13}C$ differed significantly due to compartment but not treatment in both *Heliofungia actiniformis* and in *Pocillopora damicornis* ($p < 0.001$, $F = 22.8$, df = 2,24). The $\delta^{13}C$ of the coral, symbiotic dinoflagellate and skeletal organic material ranged from −13.9 to −9.4‰ in *H. actiniformis*, and from −17.0 to −10.3‰ in *P. damicornis*. In both species, $\delta^{13}C$ of the skeletal organic material was significantly higher than $\delta^{13}C$ of either algal or coral fractions (Fig. 2A).

Analysis revealed that *Pocillopora damicornis* host tissue $\delta^{13}C$ was significantly more depleted ($p < 0.05$) than that of the symbiotic dinoflagellates (Fig. 2B) and that there was a strong influence of patch reef on compartment $\delta^{15}N$. Examination of the effect of compartment within each patch reef revealed, again, that $\delta^{13}C$ values from all patch reefs except one followed the order host > symbiotic dinoflagellates > skeletal organic material. In this case, however, the exception was *P. damicornis* collected from Patch reef 5 (Control) whose $\delta^{13}C$ had the order skeletal organic material > host > symbiotic dinoflagellates. The anomalously high $\delta^{13}C$ for the skeletal organic material in this case was tracked down to a single replicate (Rep 1 = −11.00‰, cf. Rep 2 = −14.01‰).

$\delta^{15}N$

$\delta^{15}N$ for *Heliofungia actiniformis* and its symbiotic dinoflagellates ranged from 0.0 to 5.6‰ and was

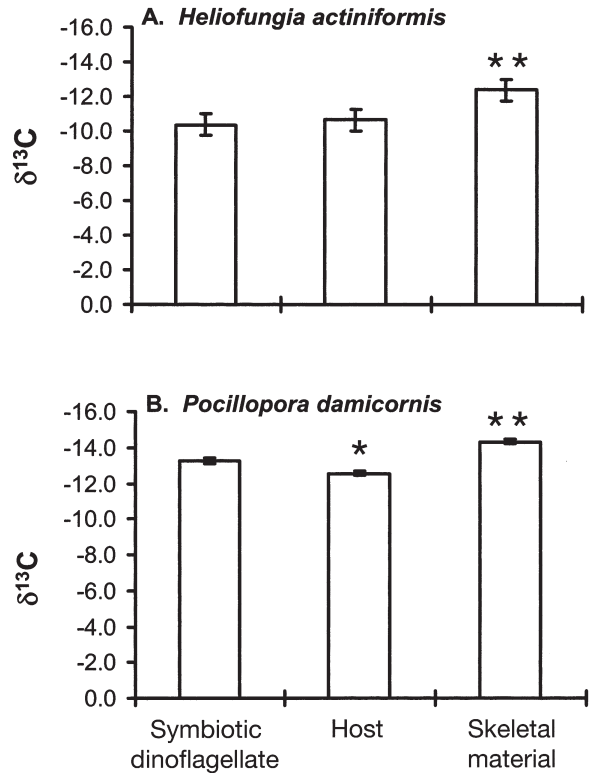

Fig. 2. $\delta^{13}C$ of symbiotic dinoflagellate, host and skeletal organic material from (A) *Heliofungia actiniformis* and (B) *Pocillopora damicornis*. Each bar represents the mean ± SE (all data within compartments pooled; n = 12, *H. actiniformis* and n = 24, *P. damicornis*). Significant differences are indicated by asterisks (*$p < 0.05$, **$p < 0.01$)

strongly influenced by nutrient treatment (2-factor ANOVA, $p = 0.001$, $F = 7.5$, df = 3,24). The effect of nutrient treatment on $\delta^{15}N$ was strongest in the symbiotic dinoflagellates. In this compartment, Control (3.1 ± 0.73‰, 95% confidence interval) and P (3.5 ± 1.46‰) treatments had the most enriched values, while symbiotic dinoflagellates from N (0.4 ± 0.6‰) and N + P (1.4 ± 1.03‰) treatments had the most depleted $\delta^{15}N$ (Fig. 3A). Values tended toward the $\delta^{15}N$ (0.0 + 0.02‰) of the synthetic NH_4Cl that was applied to 2 treatments (N and N + P) over the 2 yr of the study. Similar results were obtained for the host tissue compartment (Fig. 3B). No significant differences in $\delta^{15}N$ of the skeletal organic material were detected between treatments (Fig. 3C).

$\delta^{15}N$ for *Pocillopora damicornis* and its symbiotic dinoflagellates ranged between 0 and 5.1‰. ANOVA revealed a strong interaction between the effects of compartment and treatment (3-factor ANOVA, $p = 0.008$, $F = 4.4$, df = 6,16). $\delta^{15}N$ varied in a similar way to $\delta^{15}N$ from *Heliofungia actiniformis* with colonies from N- and N + P-treated patch reefs aving significantly more depleted values in all 3 compartments ($p < 0.01$, Fig. 4).

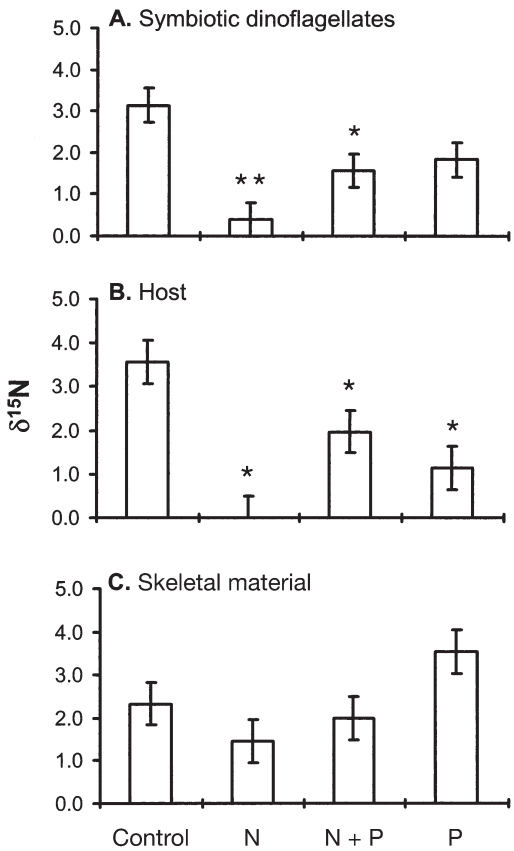

Fig. 3. $\delta^{15}N$ of (A) symbiotic dinoflagellate, (B) host and (C) skeletal organic material from *Heliofungia actiniformis* as a function of nutrient treatment. Each bar represents the mean ± SE (n = 3 replicate patch reefs). Significant differences are indicated by asterisks (*p < 0.05, **p < 0.01)

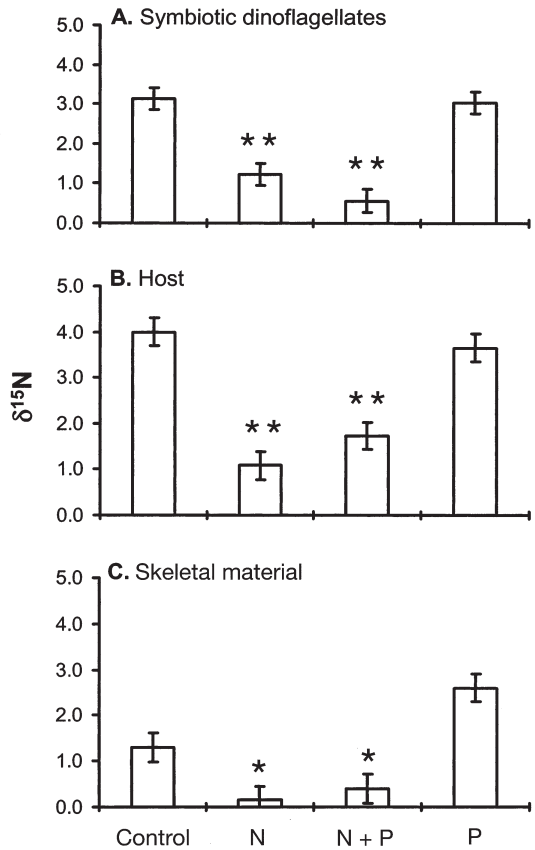

Fig. 4. $\delta^{15}N$ of (A) symbiotic dinoflagellate, (B) host and (C) skeletal organic material of *Pocillopora damicornis* as a function of nutrient treatment. Each bar represents the mean ± SE (n = 2 corals from 3 replicate patch reefs per treatment, i.e. n = 6). Significant differences are indicated by asterisks (*p < 0.05, **p < 0.01)

Percentage contribution of added N to overall N budgets

The contribution of nitrogen atoms to the overall N budget of *Heliofungia actiniformis* in the N-treated patch reefs was 87.2 and 100% for the symbiotic dinoflagellates and host respectively (Table 1). These values were 50.0 and 44.1% for the same compartments in *H. actiniformis* in N + P patch reefs. Values for *Pocillopora damicornis* were similar (60.6 and 72.9% for symbiotic dinoflagellates and host in N-treated atolls; 81.9 and 56.7% for symbiotic dinoflagellates and host in N + P-treated atolls; Table 1).

DISCUSSION

The ENCORE project is the longest (>2 yr), fully replicated experimental framework in which dissolved inorganic nutrients (ammonium and/or phosphate) have been added directly to undisturbed replicate patch reefs. The results indicate that adding inorganic nitrogen with a $\delta^{15}N$ of zero has a profound effect on the natural abundance of stable isotopes of nitrogen

Table 1. Calculated percentage contribution ($100 \times [\delta^{15}N_{control} - \delta^{15}N_{N\ or\ N+P}] / \delta^{15}N_{control}$) of added nitrogen to overall nitrogen budget of symbiotic dinoflagellate and host compartments in the reef-building corals *Pocillopora damicornis* and *Heliofungia actiniformis* in the ENCORE experiment

Host species	Compartment	N	N + P
Pocillopora damicornis	Symbiotic dinoflagellates	60.6	81.9
	Host	72.9	56.7
Heliofungia actiniformis	Symbiotic dinoflagellates	87.2	50.0
	Host	100.0	44.9

within corals, and their symbiotic dinoflagellates and associates. These results are also important for understanding the contribution of external dissolved sources of inorganic nitrogen within algal-invertebrate symbioses and the use of stable isotopes for tracing the origin of natural and anthropogenic nutrients within coral reef ecosystems.

C:N in control and nutrient-enriched corals and symbiotic dinoflagellates

Nutrient treatments did not significantly affect the C:N ratio of either reef-building coral or its symbiotic dinoflagellates. However, the C:N ratio was very high in the material isolated from the skeleton of both species (10.9 ± 1.42 for *Heliofungia actiniformis* and 14.8 ± 1.50 for *Pocillopora damicornis*), and was probably due to the carbon-rich glycoprotein and lipid content of this compartment (Young et al. 1971, Contanz & Weiner 1988, Allemand et al. 1994, Gautret et al. 1997). There were some differences between the distribution of C:N values between species. While symbiotic dinoflagellates in *H. actiniformis* had the next highest C:N ratio, the host fraction was consistently higher than the symbiotic dinoflagellates in *P. damicornis*. These small but significant differences may be a consequence of the apparent differences in trophic mode between these 2 species (that is, slightly different sources of nitrogen), as indicated by the dramatically different polyp sizes. Large polyp species like *H. actiniformis*, for example, are likely to be able to prey on much larger food items than small polyp species such as *P. damicornis*.

Interestingly, the C:N ratio of both corals tended to be lower (though not statistically, $p = 0.059$; 0.071) in N-treated patch reefs as opposed to control or P-treated patch reefs. This differs from the results of previous studies. *Pocillopora damicornis* from Hawaii, for example, responded to the addition of 20 µM ammonium with a decrease of the C:N ratio from approximately 20 to values near 8 over 2 wk (Muller-Parker et al. 1994b). Similar results have been reported for other symbiotic invertebrates (Muller-Parker 1985, Cook et al. 1988). It is interesting to note, however, that the C:N values of corals and symbiotic dinoflagellates from control reefs of the ENCORE project were close to the C:N ratio of organisms from the enriched conditions of these previous experiments (e.g. cf. symbiotic dinoflagellates from *P. damicornis*, control conditions of present experiment: 5.7 ± 0.43 with values of approximately 20, control, and 8, for enriched, symbiotic dinoflagellates from Hawaiian *P. damicornis*, Muller-Parker et al. 1994b). This suggests that *P. damicornis* and their symbiotic dinoflagellates growing in the unenriched control ENCORE patch reefs may have been relatively nutrient replete and hence, relatively unresponsive to additional inorganic nitrogen. This is also consistent with the conclusions of Hoegh-Guldberg & Williamson (1999) who argue that the ambient ammonium concentrations at One Tree Island lagoon are sufficient to supply the nitrogen demand of actively growing *P. damicornis*.

Perturbations to $\delta^{13}C$ as a result of nutrient treatment

The $\delta^{13}C$ of *Heliofungia actiniformis* and *Pocillopora damicornis* and their symbiotic dinoflagellates are similar to those of other hermatypic corals growing in shallow water (cf. present study: −17.0 to −10.3‰ with corals from same depth in Jamaica: −19.0 to −9.6‰, Muscatine et al. 1989). *P. damicornis* tended to be more depleted in ^{13}C than *H. actiniformis*, which may be due to the thicker tissues and hence, greater tendency for diffusion limitation of CO_2 in the latter species. Diffusion limitation reduces the discrimination against ^{13}C caused by ribulose bisphospate carboxylase/oxygenase (Rubisco) during the photosynthetic fixation of CO_2 and hence, results in reduced depletion of ^{13}C (Goreau 1977, Muscatine et al. 1989). Moreover, the $\delta^{13}C$ of *P. damicornis* reflects its isotopically depleted prey item, zooplankton, which typically falls between −13 and −19‰ (Muscatine et al. 1989).

Differences in $\delta^{13}C$ were detected between dinoflagellate and coral compartments but not between corals from different nutrient treatments. The organic constituents of the skeletal material were always significantly more depleted in ^{13}C than either symbiotic dinoflagellates or host compartments in both species ($p < 0.01$). One explanation is that the calcification pathway sequesters an internal carbon pool which remains depleted compared to carbon products recycled between coral and algae. The differences between symbiotic dinoflagellates and hosts depended on the species investigated (Fig. 2). The $\delta^{13}C$ of symbiotic dinoflagellates and host were similar in *Heliofungia actiniformis*, yet were slightly though significantly different in *Pocillopora damicornis* (host being more depleted in ^{13}C than symbiotic dinoflagellates, $p < 0.05$). Muscatine et al. (1989) observed the greatest differences between dinoflagellates and hosts for corals collected from the shallowest locations, which was interpreted as a consequence of the faster photosynthetic rates and hence, reduced recycling of CO_2 of shallow water corals. The lack of difference between symbiotic dinoflagellates and hosts in *H. actiniformis* may also be a consequence of the reduced exchange of host/symbiont carbon pools with external pools (i.e. greater diffusion limitation, greater re-cycling) expected in corals that have thicker tissues. Recent work

showing that the supply of CO_2 to symbiotic dinoflagellates is limited by boundary layer diffusion around coral colonies and tissues (Lesser et al. 1994) further supports this particular notion.

$\delta^{15}N$ as a tracer of nutrient history

Control values for the $\delta^{15}N$ of the host compartment in both species was 4.0 ± 0.65 (+95% confidence interval; *Pocillopora damicornis*) and 3.6 ± 1.15 (*Heliofungia actiniformis*). Interestingly, these values are indistinguishable from host tissue measured in *Porites lobata* on mid-shelf reefs of the Great Barrier Reef (Fig. 2 in Sammarco et al. 1999). These values are also similar to symbiotic dinoflagellates and their coral hosts growing in shallow water (Muscatine & Kaplan 1994; range from 1 m depth = 0.95 to 4.74‰) and confirm that the $\delta^{15}N$ of corals and their symbiotic dinoflagellates on these reefs are at the lower (more depleted) end of the range reported for marine organisms (–3 to 20‰, Owens 1987). Coral hosts also tended to be more enriched for ^{15}N than their symbiotic dinoflagellates (*H. actiniformis* host 3.6 ± 0.4‰ versus symbiotic dinoflagellates 3.1 ± 0.3‰, not significant; *P. damicornis* host 4.0 ± 0.4‰ versus symbiotic dinoflagellates 3.1 ± 0.3‰, $p < 0.001$). As pointed out by Muscatine & Kaplan (1994), the expected difference of approximately 2.6‰ between an animal and its food source (Rau et al. 1990) is not seen between corals and their symbiotic dinoflagellates, presumably due to the semi-closed recycling of nitrogen atoms between host and symbiont (Muscatine & Porter 1977, Muscatine et al. 1979), which partly counters isotopic discrimination.

Interestingly, the $\delta^{15}N$ of the organic material isolated from within the skeleton of both species tended to be less enriched for ^{15}N than both host tissue or symbiotic dinoflagellates (*Pocillopora damicornis*: 1.3 ± 0.7‰; *Heliofungia actiniformis*: 1.4 ± 0.8‰). The reason for the reduced enrichment of the organic material from skeletal as compared to host tissues is not apparent but may be due to the specific characteristics of either the deposition of the specific coral proteins and/or the influence of microendolithic organisms. Recent work by Allemand et al. (1998) has revealed the direct incorporation of the amino acid aspartate (an amino acid translocated by symbiotic dinoflagellates to hosts, Swanson & Hoegh-Guldberg 1998) into specific proteins that are secreted rapidly as skeletal organic material. One interpretation is that specific proteins of the skeletal matrix are synthesized by pathways that are unique from those of general coral protein metabolism. The authors recognize, however, that the identity of the organic material from within the skeleton remains unresolved and that an alternative explanation of this result is that isotope ratios within the skeleton are due to microendolithic communities within the skeleton of the corals (not obvious visually) studied here.

Highly significant responses in the $\delta^{15}N$ of symbiotic dinoflagellates and host tissue occurred in response to adding chemical ammonium ($\delta^{15}N = 0.0 \pm 0.02$) to the experimental patch reefs ($p < 0.001$). The $\delta^{15}N$ values of corals growing under ammonium-enriched conditions were significantly more depleted than the 2 treatments that did not receive synthetic ammonium. Similar yet slightly weaker trends were also apparent within the organic material isolated from the skeleton. Adding ammonium led to a significant decrease in $\delta^{15}N$ within the skeletal organic material of *Pocillopora damicornis*, though a similar sequence of values was not significant in *Heliofungia actiniformis*. Part of the difference here is probably due to the smaller proportion of the skeleton of *H. actiniformis* (approximately 50%) deposited during the ENCORE experiment as compared to *P. damicornis*. *P. damicornis* was added to the patch reefs as tiny nubbins and almost all (>90%) of its skeleton was deposited after the beginning of the ENCORE experiment. The added ammonium, therefore, would be expected to have a greater impact on the $\delta^{15}N$ of the organic material isolated from the skeletons of *P. damicornis* than on that from *H. actiniformis*.

These results represent the first experimental test of the use of $\delta^{15}N$ to trace nutrient history under fully replicated field conditions. The results are unambiguous. The nutrient history of each patch reef was recorded faithfully in the organic components of both symbiotic dinoflagellates and corals (and their skeletal associates). This outcome would be especially potent given the fact that many anthropogenic sources of change to water quality have drastically different $\delta^{15}N$ signatures (e.g. fertilizer run-off, $\delta^{15}N = 0.0$) to those of marine organisms. Skeletal $\delta^{15}N$ analysis indicates that several time scales of nutrient detection are possible. Tissue turnover within corals and their symbiotic dinoflagellates is relatively short-lived and hence, would dictate that 'recorded' signals would be relatively rapidly lost after cessation of a particular nutrient input event. This may be useful for monitoring short-term changes to the nutrient status of particular reef systems. Nutrient signals from metabolically inactive or dead skeletal organic material, on the other hand, would preserve nutrient signals for far longer periods. This also suggests that analysis of the $\delta^{15}N$ of the organic components of the coral skeleton may allow access to the chronology of nutrient concentrations surrounding a coral during its lifetime. In the case of long-lived *Porites* colonies, this might even allow the reconstruction of nutrient histories that stretch back to periods prior to significant human influence on coral reefs. Use of the $\delta^{15}N$ signature within the tissues of symbiotic dinoflagellates and corals at both these time

scales may, therefore, prove extremely useful for understanding and managing nutrient inputs to coral reef ecosystems.

Contribution of water-borne DIN to the overall nitrogen budget of enriched corals

The contribution of added ammonium for both coral species ranged from 50 to 100%, which revealed that the dissolved nutrients added during the ENCORE experiment dominated the signatures of both animal host and dinoflagellate symbiont. These calculations also support the contention that nitrogen originating from the catabolism of animal prey is a relatively unimportant source of bulk nitrogen for corals. The enriched $\delta^{15}N$ of zooplankton generally lie in the range 6 to 9‰, (Yamamuro et al. 1995) and consequently drive coral $\delta^{15}N$ values in the opposite direction as observed upon addition of synthetic ammonium ($\delta^{15}N = 0$). It is important to add that these calculations do not rule against the qualitative importance of the nitrogen derived from animal prey, which may function as a significant source of essential nutrients (Swanson & Hoegh-Guldberg 1998).

Acknowledgements. The authors are grateful to the managers of One Tree Island Research Station (M. Waugh, R. Sweetapple-Waugh, G. Carter, P. Carter) and the ENCORE program manager A. Steven for assistance with setup and maintenance of the ENCORE project. They would also like to acknowledge the efforts of M. Sutton and S. Bunn at Griffith University who ran the mass spectrometer required for analyzing the samples of this project. This project was funded by a GBRMPA grant (ENCORE program) to O.H.-G.

LITERATURE CITED

Allemand D, Cuif JP, Watabe N, Oishi M, Kawaguchi T (1994) The organic matrix of skeletal structures of Mediterranean red coral, *Corallium rubrum*. Bull Inst Oceanogr Monaco Numero Special 14(1):129–139

Allemand D, Tambutte E, Girard JP, Jaubert J (1998) Organic matrix synthesis in the scleractinian coral *Stylophora pistillata*: role in biomineralization and potential target of the organotin tributyltin. J Exp Biol 201:2001–2009

Belda CA, Lucas JS, Yellowlees D (1993) Nutrient limitation in the giant clam-zooxanthellae symbiosis: effects of nutrient supplements on growth of the symbiotic partners. Mar Biol 117:655–664

Bell P (1992) Eutrophication and coral reefs—some examples in the Great Barrier Reef lagoon. Water Res 26:553–568

Contanz B, Weiner S (1988) Acidic macromolecules associated with the mineral phase of scleractinian coral skeletons. J Exp Zool 248:253–258

Cook CB, D'Elia CF, Muller-Parker G (1988) Host feeding and nutrient sufficiency for symbiotic dinoflagellates in the sea anemone *Aiptasia pallida*. Mar Biol 98:253–262

Costa OS, Leão ZMAN, Nimmo M, Attrill MJ (2000) Nutrification impacts on coral reefs from northern Bahia, Brazil. Hydrobiologia 440:307–315

Costanzo SD, O'Donohue MJ, Dennison WC, Loneragan NR, Thomas M (2001) A new approach for detecting and mapping sewage impacts. Mar Pollut Bull 42:149–156

Edinger EN, Jompa J, Limmon GV, Widjatmoko W, Risk MJ (1998) Reef degradation and coral biodiversity in Indonesia: effects of land-based sources of pollution, destructive fishing practices, and changes over time. Mar Pollut Bull 36:617–630

Ferrier-Pages C, Gattuso JP, Cauwet G, Jaubert J, Allemand D (1998) Release of dissolved organic carbon and nitrogen by the zooxanthellate coral *Galaxea fascicularis*. Mar Ecol Prog Ser 172:265–274

Gautret P, Cuif JP, Freiwald A (1997) Composition of soluble mineralizing matrices in zooxanthellate and non-zooxanthellate scleractinian corals: biochemical assessment of photosynthetic metabolism through the study of a skeletal feature. Facies 36:189–194

Gerlach SA (1981) Marine pollution, diagnosis and therapy. Springer-Verlag, Berlin

Goreau TJ (1977) Carbon metabolism in calcifying and photosynthetic organisms: theoretical models based on stable isotope data. Proc 3rd Int Coral Reef Symp 2:395–401

Goreau TJ (1992) Bleaching and reef community change in Jamaica: 1951–1991. Am Zool 32:683–695

Heaton THE (1986) Isotopic studies of nitrogen in the hydrosphere and atmosphere: a review. Chem Geol 59:87–102

Heikopp JM, Dunn JJ, Risk MJ, Sandeman IM, Schwarcz HP, Waltho N (1998) Relationship between light and the $\delta^{15}N$ of coral tissue: examples from Jamaica and Zanzibar. Limnol Oceanogr 43:909–920

Heikoop JM, Dunn JJ, Risk MJ, Tomascik T, Schwarcz HP, Sandeman IM, Sammarco PW (2000a) $\delta^{15}N$ and $\delta^{13}C$ of coral tissue show significant inter-reef variation. Coral Reefs 19:189–193

Heikoop JM, Risk MJ, Lazierb AV, Edinger EN and 5 others (2000b) Nitrogen-15 signals of anthropogenic nutrient loading in reef corals. Mar Pollut Bull 40:628–636

Hoegh-Guldberg O, Smith GJ (1989) Influence of the population density of symbiotic dinoflagellates and supply of ammonium on the biomass and metabolic characteristics of the reef corals *Seriatopora hysterix* and *Stylophora pistillata*. Mar Ecol Prog Ser 57:173–186

Hoegh-Guldberg O, Williamson J (1999) The relative importance of dissolved nitrogen to the reef-building coral, *Pocillopora damicornis*. Mar Biol 133:561–570

Hughes TP (1994) Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science 265:1547–1551

Jokiel PL, Dubinsky Z, Stambler N (1994) Results of the 1991 United States-Israel Workshop, 'Nutrient limitation in the symbiotic association between symbiotic dinoflagellates and reef-building corals.' Pac Sci 48:215–218

Koop K (1995) Uptake of nitrogen and phosphorus in enriched coral reef ecosystems. Australian Marine Sciences Association Conference. University of Technology, Sydney, Abstract

Koop K, Booth D, Broadbent A, Brodie J and 16 others (2001) ENCORE: the effect of nutrient enrichment on coral reefs: synthesis of results and conclusions. Mar Pollut Bull 42: 91–120

Lapointe BE (1997) Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and Southeast Florida. Limnol Oceanogr 42:1119–1131

Larkum AWD (1981) Marine primary productivity. In: Clayton MC, King RJ (eds) Marine botany; an Australasian perspective. Longman, Cheshire, p 370–385

Larkum AWD, Steven ADL (1994) ENCORE: The effect of nutrient enrichment on coral reefs. 1. Experimental design and research programme. Mar Pollut Bull 29:112–120

Lesser MP, Weis VM, Patterson MR, Jokiel PL (1994) Effects of morphology and water motion on carbon delivery and productivity in the reef coral, *Pocillopora damicornis* Linnaeus: diffusion barriers, inorganic carbon limitation and biochemical plasticity. J Exp Mar Biol Ecol 178: 153–179

Maragos JE, Evans C, Holthus P (1985) Reef corals in Kaneohe Bay six years before and after termination of sewage discharge (Oahu, Hawaiian Archipelago). Proc 5th Int Coral Reef Symp 4:189–194

McClelland J, Valiela I, Michener R (1997) Nitrogen-stable isotope signatures in estuarine food webs: a record of increasing urbanization in coastal watersheds. Limnol Oceanogr 42:930–937

McCook LJ (1999) Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. Coral Reefs 18:357–367

McCulloch M, Fallon S, Wyndham T, Hendy E, Lough J, Barnes D (2003) Coral record of increased sediment flux to the inner Great Barrier Reef since European settlement. Nature 421:727–730

Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between ^{15}N and animal age. Geochim Cosmochim Acta 48:1135–1140

Muller-Parker G (1985) Effect of feeding regime and irradiance on the photophysiology of the symbiotic sea anemone *Aiptasia pulchella*. Mar Biol 90:65–74

Muller-Parker G, McCloskey LR, Hoegh-Guldberg O, McAuley PJ (1994a) Effect of ammonium enrichment on animal and algal biomass of the coral *Pocillopora damicornis*. Pac Sci 48:273–283

Muller-Parker G, Cook CB, D'Elia CF (1994b) Elemental composition of the coral *Pocillopora damicornis* exposed to elevated seawater ammonium. Pac Sci 48:234–246

Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. In: Dubinsky Z (ed) Coral reefs. Ecosystems of the world, 25. Elsevier, Amsterdam, p 75–87

Muscatine L, Cernichiari E (1969) Assimilation of photosynthetic products of symbiotic dinoflagellates by a reef coral. Biol Bull 137:506–523

Muscatine L, Kaplan IR (1994) Resource partitioning by reef corals as determined from stable isotope composition. II. ^{15}N of symbiotic dinoflagellates and animal tissue versus depth. Pac Sci 48:304–312

Muscatine L, Porter JW (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. BioScience 27:454–460

Muscatine L, Masuda H, Burnap R (1979) Ammonium uptake by symbiotic and aposymbiotic reef corals. Bull Mar Sci 29:572–575

Muscatine L, Porter JW, Kaplan IR (1989) Resource partitioning by reef corals as determined from stable isotope composition. Mar Biol 100:185–193

Odum HT, Odum EP (1955) Trophic structure and productivity of windward coral reef community on Eniwetok Atoll. Ecol Monogr 25:292–320

Owens NJP (1987) Natural variations in ^{15}N in the marine environment. Adv Mar Biol 24:389–451

Rau GH, Teyssie JL, Rassoulzadegan F, Fowler SW (1990) $^{13}C/^{12}C$ and $^{15}N/^{14}N$ variations among size-fractionated marine particles: implications for their origin and trophic relationships. Mar Ecol Prog Ser 59:33–38

Risk MJ, Erdmann M (2000) Isotopic composition of nitrogen in stomatopod (crustacea) tissues as an indicator of human sewage impacts on Indonesian coral reefs. Mar Pollut Bull 40:50–58

Risk MJ, Heikoop JM, Edinger EN, Erdmann MV (2001) The assessment 'toolbox': community-based reef evaluation methods coupled with geochemical techniques to identify sources of stress. Bull Mar Sci 69:443–458

Sammarco PW, Risk MJ, Schwarcz HP, Heikoop JM (1999) Cross-continental shelf trends in coral $\delta^{15}N$ on the Great Barrier Reef: further consideration of the reef nutrient paradox. Mar Ecol Prog Ser 180:131–138

Sebens KP (1994) Biodiversity of coral reefs: What are we losing and why? Am Zool 34:115–133

Smith SV, Kimmerer WJ, Laws EA, Brock RE, Walsh TW (1981) Kaneohe Bay sewage diversion experiment: perspectives on ecosystem responses to nutritional perturbation. Pac Sci 35:279–385

Sorokin YI (1990) Aspects of trophic relations, productivity and energy balance in coral-reef ecosystems. In: Dubinsky Z (ed) Coral reefs. Ecosystems of the world. Elsevier, New York, p 75–87

Steven A, Koop K, Sweetapple R (1995) Nutrient concentrations in the ENCORE microatolls following fertilisation. ENCORE Nutrient Report. Great Barrier Reef Marine Park Authority, Townsville

Swanson R, Hoegh-Guldberg O (1998) The assimilation of ammonium by the symbiotic sea anemone, *Aiptasia pulchella*. Mar Biol 131:83–93

Takabayashi M, Hoegh-Guldberg O (1995) Physiological and ecological differences between pink and brown genotypes of the reef-building coral, *Pocillopora damicornis*. Mar Biol 123:705–714

Tomascik T, Sander F (1985) Effects of eutrophication on reef-building corals. I. Growth rate of the building coral *Montastrea annularis*. Mar Biol 87:143–155

Tomascik T, Sander F (1987a) Effects of eutrophication on reef-building corals. II. Structure of scleractinian coral communities on fringing reefs, Barbados, West Indies. Mar Biol 94:53–75

Tomascik T, Sander F (1987b) Effects of eutrophication on reef-building corals. III. Reproduction of the reef-building coral *Porites porites*. Mar Biol 94:77–94

Vitousek P, Aber JD, Howarth RW, Likens GE and 5 others (1997a) Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7:737–750

Vitousek P, Mooney HA, Lubchenco J, Melillo JM (1997b) Human domination of earth's ecosystems. Science 277: 494–499

Yamamuro M, Kayanne H, Minagawa H (1995) Carbon and nitrogen stable isotopes of primary producers in coral reef ecosystems. Limnol Oceanogr 40:617–621

Yamamuro M, Kayanne H, Yamano H (2003) $\delta^{15}N$ of seagrass leaves for monitoring anthropogenic nutrient increases in coral reef ecosystems. Mar Pollut Bull 46:452–458

Young SD, O'Connor JD, Muscatine L (1971) Organic material from scleractinian coral skeletons. 2. Incorporation of C-14 into protein, chitin and lipid. Comp Biochem Physiol 40:945

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: October 6, 2003; Accepted: June 17, 2004
Proofs received from author(s): September 10, 2004