Attachment 7

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REPORTS

ENCORE: The Effect of Nutrient Enrichment on Coral Reefs. Synthesis of Results and Conclusions

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Coral reef degradation resulting from nutrient enrichment of coastal waters is of increasing global concern. Although effects of nutrients on coral reef organisms have been demonstrated in the laboratory, there is little direct evidence of nutrient effects on coral reef biota *in situ*. The ENCORE experiment investigated responses of coral reef organisms and processes to controlled additions of dissolved inorganic nitrogen (N) and/or phosphorus (P) on an offshore reef (One Tree Island) at the southern end of the Great Barrier Reef, Australia. A multi-disciplinary team assessed a variety of factors focusing on nutrient dynamics and biotic responses. A controlled and replicated experiment was conducted over two years using twelve small patch reefs ponded at low tide by a coral rim. Treatments included three control reefs (no nutrient addition) and three + N reefs (NH_4Cl added), three + P reefs (KH_2PO_4 added), and three + N + P reefs. Nutrients were added as pulses at each low tide (*ca* twice per day) by remotely operated units. There were two phases of nutrient additions. During the initial, low-loading phase of the experiment nutrient pulses (mean dose = 11.5 μM NH_4^+; 2.3 μM PO_4^{-3}) rapidly declined, reaching near-background levels (mean = 0.9 μM NH_4^+; 0.5 μM PO_4^{-3}) within 2–3 h. A variety of biotic processes, assessed over a year during this initial nutrient loading phase, were not significantly affected, with the exception of coral reproduction, which was affected in all nutrient treatments. In *Acropora longicyathus* and *A. aspera*, fewer successfully developed embryos were formed, and in *A. longicyathus* fertilization rates and lipid levels decreased. In the second, high-loading, phase of ENCORE an increased nutrient dosage (mean dose = 36.2 μM NH_4^+; 5.1 μM PO_4^{-3} declining to means of 11.3 μM NH_4^+ and 2.4 μM PO_4^{-3} at the end of low tide) was used for a further year, and a variety of significant biotic responses occurred. Encrusting algae incorporated virtually none of the added nutrients.

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Organisms containing endosymbiotic zooxanthellae (corals and giant clams) assimilated dissolved nutrients rapidly and were responsive to added nutrients. Coral mortality, not detected during the initial low-loading phase, became evident with increased nutrient dosage, particularly in *Pocillopora damicornis*. Nitrogen additions stunted coral growth, and phosphorus additions had a variable effect. Coral calcification rate and linear extension increased in the presence of added phosphorus but skeletal density was reduced, making corals more susceptible to breakage. Settlement of all coral larvae was reduced in nitrogen treatments, yet settlement of larvae from brooded species was enhanced in phosphorus treatments. Recruitment of stomatopods, benthic crustaceans living in coral rubble, was reduced in nitrogen and nitrogen plus phosphorus treatments. Grazing rates and reproductive effort of various fish species were not affected by the nutrient treatments. Microbial nitrogen transformations in sediments were responsive to nutrient loading with nitrogen fixation significantly increased in phosphorus treatments and denitrification increased in all treatments to which nitrogen had been added. Rates of bioerosion and grazing showed no significant effects of added nutrients.

ENCORE has shown that reef organisms and processes investigated *in situ* were impacted by elevated nutrients. Impacts were dependent on dose level, whether nitrogen and/or phosphorus were elevated and were often species-specific. The impacts were generally sub-lethal and subtle and the treated reefs at the end of the experiment were visually similar to control reefs. Rapid nutrient uptake indicates that nutrient concentrations alone are not adequate to assess nutrient condition of reefs. Sensitive and quantifiable biological indicators need to be developed for coral reef ecosystems. The potential bioindicators identified in ENCORE should be tested in future research on coral reef/nutrient interactions. Synergistic and cumulative effects of elevated nutrients and other environmental parameters, comparative studies of intact vs. disturbed reefs, offshore vs. inshore reefs, or the ability of a nutrient-stressed reef to respond to natural disturbances require elucidation. An expanded understanding of coral reef responses to anthropogenic impacts is necessary, particularly regarding the subtle, sub-lethal effects detected in the ENCORE studies. **© 2001 Published by Elsevier Science Ltd.**

Introduction

Coral reefs are among the most spectacular marine ecosystems on the planet. They are renowned for their biological diversity and high productivity. In addition to their beauty and biological value, coral reefs contribute to the economies of at least 100 nation states and the livelihoods of over 100 million people. Regions like the Great Barrier Reef and the Caribbean reef systems contribute billions of dollars to their local economies. Despite their beauty and importance, coral reefs have been identified as one of the most threatened marine ecosystems (Goreau, 1992; Sebens, 1994; Wilkinson and Buddemeier, 1994; Bryant and Burke, 1998; Wilkinson, 1998; Hoegh-Guldberg, 1999). The loss of viable reefs would have major consequences for the economies of many small island nations in the Pacific and Indian oceans and the Carribean. Economic impacts would almost certainly be seen in terms of declining fish production, loss of tourism and amenity values. Reefs also protect and stabilize coastlines. Hence, their loss could have drastic consequences in the longer term because of coastal destablization and the loss of other associated habitats like mangroves and seagrasses.

Anthropogenic impacts are the cause of the decline in the 'health' of reefs in many areas of the world (Wilkinson and Buddemeier, 1994). Increasing urbanization of coastal areas, often associated with loss of important coastal habitats (e.g. forests, coastal wetlands) and increased intensive agricultural activities in the nearby catchments have led to increases in the rate of land runoff, which is often loaded with sediment and nutrients from fertilizers which are then discharged into coastal waters after heavy rains. For example, Demouget (1989) estimated that 1000 t of sediment were carried into the lagoon of Tahiti annually where extensive reefs occur. Untreated sewage is also typically discharged into coral reef lagoons in many developing countries. These same reefs may also be subjected to overfishing, and physical removal of the reefs to form marinas or ports, and construction of major tourist complexes. Coral reefs are important tourist attractions and loss or decline in the 'health' of these reefs may have important economic consequences for many countries. All these anthropogenic impacts have the potential to degrade coastal coral reefs.

Increasing nutrient inputs and associated sediment loads have been hypothesized as having the potential to seriously impact coral reefs (Cortes and Risk, 1985). Despite its importance, our understanding of how increasing nutrient loads impact on coral reefs is surprisingly limited. The coral reef literature contains many accounts of coral reef degradation associated with declining water quality (e.g. Banner, 1974; Smith *et al.*, 1981; Walker and Ormond, 1982; Tomascik and Sander, 1985; Hughes, 1994; Sebens, 1994; Hudson *et al.*, 1994). While convincing, the complex nature of the inputs to coastal areas such as industrial and domestic effluents and runoff from land, however, has made it difficult to identify the components (e.g. nutrients, sediment, heavy metals) that are specifically responsible for the reported changes. This has hindered progress towards identifying the factors that are most damaging to coral reefs and hence the development of management strategies that target the sources of important components.

Increased nutrients are considered to be a major factor responsible for deteriorating water quality on coral reefs. In Florida (USA) for example, a multi-agency taskforce has recently announced a major programme of

$7.8 billion over 20 years to improve water quality surrounding the Florida reefs, Florida Bay and the Everglades (Causey, 1999). Similarly in Hong Kong the major decline of reefs within the harbour has been attributed to increased nutrient loads (Scott and Cope, 1990; Morton, 1994). In Jakarta Bay, Indonesia, reefs have been degraded along a gradient away from Jakarta and rivers draining the catchments inland from Jakarta (Tomascik *et al.*, 1997). Reefs close to the coast and Jakarta have become progressively more eutrophic and now include almost no live coral. Further offshore, reefs are in better condition but signs of decline are evident (Tomascik *et al.*, 1997).

While increasing nutrient loads have been recognized as a major threat to reefs, the actual ways in which reefs respond to these increases are poorly understood (Brown and Howard, 1985; Hatcher *et al.*, 1989; Grigg and Dollar, 1990; McCook *et al.*, 1997). A few studies have used existing sewage discharges on the reef, such as those in Kaneohe Bay, Hawaii (Smith *et al.*, 1981; Grigg, 1995) or defined eutrophication and pollution gradients (Tomascik and Sander, 1985, 1987a,b). Monitoring of such natural experiments and documenting effects on the ecology of the systems studied as nutrient levels increased have led to the hypothesis that nutrient levels profoundly affect coral reef ecosystems. Apart from the *in situ* nutrient enrichment experiments of Kinsey (Kinsey and Domm, 1974; Kinsey and Davies, 1979), most studies have been confined to laboratory experiments, which give limited insights into the ways in which reefs respond to elevated nutrients (e.g. Hoegh-Guldberg and Smith, 1989; Hunte and Wittenberg, 1992; Yellowlees *et al.*, 1994; Hoegh-Guldberg, 1994).

There has been concern for some time about increasing nutrient loadings to the Great Barrier Reef (GBR), Australia (e.g. Bennell, 1979; Bell, 1991; Kinsey, 1991) based on: (i) rapid increases in the number of tourists visiting the Great Barrier Reef and associated development of resorts on the reef, (ii) increasing urbanization along the Queensland coast during the 1980s–1990s, (iii) continuing intensive agricultural development and (iv) loss of wetlands. In the period since European settlement (~1850) the coastal catchments adjacent to the GBR have experienced almost complete agricultural and urban development with only 17% of catchments now considered to be in a natural condition (Gilbert, in press). Modelling based on catchment land-use provides estimates that the flux of nitrogen and phosphorus to the Great Barrier Reef lagoon has increased about 4 times since European settlement, from some 2500 tonnes of P in 1850 to about 10 000 tonnes in 1991 and from about 17 000 t of N in 1850 to around 70 000 t in 1991 (Moss *et al.*, 1992; Neil and Yu, 1996). While the inshore reefs of the GBR are most impacted by terrestrial runoff of concentrated nutrient pulses, the river plumes may at times reach parts of the outer GBR reefs (Brodie, 1996).

Water quality, and particularly nutrient pollution, is now considered to be one of the principal 'critical issues' facing the long-term ecological functioning of the GBR (Wachenfeld *et al.*, 1998). Recently published work claims much of the GBR is already in an eutrophic condition (Bell and Elmetri, 1995) while other work identifies nutrient pollution problems as confined to the inshore GBR and not yet affecting the offshore reefs (Brodie *et al.*, 1997; Wachenfeld *et al.*, 1998). As is the case for many reef systems worldwide, the GBR, and particularly the inshore coral reefs of the GBR, is under multiple stresses, for example from fishing pressure (Wachenfeld *et al.*, 1998) and widespread bleaching (Hoegh-Guldberg *et al.*, 1996; Hoegh-Guldberg, 1999; Berkelmans and Oliver, 1999) as well as terrestrially sourced pollution.

The Great Barrier Reef Marine Park Authority (GBRMPA) commenced an integrated research and monitoring programme in 1991 as a result of concerns about the effects of possible eutrophication of the GBR. Research has focused on: (i) the sources of nutrients and other pollutants in the catchment of the GBR, (ii) the transport, dispersion and physical fate of sediments and nutrients in the coastal GBR, (iii) the effects of increased sediments and nutrients on organisms and ecosystems of the GBR, (iv) identifying organism or community response factors which could be used as indicators of ecosystem degradation, and (v) techniques to reduce sediment and nutrient loads or mitigate their effects. The ENCORE (Enrichment of Nutrients on a Coral Reef Experiment) study was initiated in 1991 as a large component of the third and fourth objectives of the research programme. Nutrient enrichment of patch reefs at One Tree Island began in September, 1993 (Steven and Larkum, 1993).

A central paradigm for coral reefs is that their primary producers (principally algae) are limited by nutrient supply (principally nitrogen and phosphorus) and, most importantly, that any increase in the nutrient supply to reefs increases the growth and therefore the standing crop of algae. The standing crop would depend on grazing rates of herbivores. The general acceptance of this paradigm has led to the important expectation that with increased nutrient supply, e.g. from urban and agricultural runoffs, algae would out-compete corals, leading to a shift from coral- to algal-dominated reefs. What we still do not know is the levels of nutrient pollution required to elicit a significant growth response from algae.

This paradigm was tested in the ENCORE project using replicated *in situ* experiments at ecologically relevant scales. Coral patch reefs were perturbed in a defined manner, using controlled additions of nitrogen and/or phosphorus, and the responses of a range of biota and abiotic parameters were measured in the experimental patch reefs (Larkum and Steven, 1994). ENCORE is the first replicated experimental study done in the field to measure the impacts of nutrients on coral reefs at ecological relevant scales and will therefore be of great value to reef managers. This paper presents a synthesis of the major results from the ENCORE project.

Methods

Study area

One Tree Island (23°30′S, 152°06′E) is located 70 km off the Queensland coast at the southern end of the Great Barrier Reef (Fig. 1). It is a small platform reef (4.7 × 2.7 km) with an emergent crest and three separate lagoons. The main lagoon is about 10 km^2, and is totally enclosed by a continuous reef. The eastern crest is 0.4 m higher than the other sides, owing to the buildup of ephemeral shingle and rubble banks. The lagoon contains many patch reefs – isolated and roughly circular reefs – dominating the eastern and north-eastern sections, and reticulate reefs that form a complex maze in the central and western sections. Low tide depths in the lagoon vary between 3 and 6 m along the eastern side, and 5 and 7 m along the north-western wall. Tides are semi-diurnal with a mean spring range of 2.1 m. The continuous reef crest isolates the lagoon from swell and tidal inputs for up to 5 h on each tide, when water is ponded. Water is trapped inside the reef as the outside tide falls and remains there during the extended slack water period. Exchange with the ocean is therefore limited to half the tidal cycle.

Estimated residence times of lagoon water are between 0.5 and 5 days (Hatcher and Frith, 1985). Exchange rates are independent of the initial amounts of water entering the lagoon, but vary spatially and temporally according to the point of entry and the wind tide and swell conditions (Frith and Mason, 1986). Overall water movement is windward to leeward.

At 23°S One Tree Reef is near the southern extreme of coral reef formation in the Great Barrier Reef and subject to pronounced seasonal variation (Kinsey, 1979). During the course of ENCORE mean sea surface temperatures (SST) closely followed air temperatures. A minimum of 18.2°C occurred in late July and the highest mean SST of 30.4°C was recorded in late January and February. Temperatures greater than 33°C were recorded in October, 1994 and January 1996, when widespread bleaching (i.e. loss of zooxanthellar pigment) occurred. Cloud cover was greatest from December to March in all years. Winds were predominantly from the south-east although north-easterlies were common in the summer months. Total annual rainfall varied between 1084 mm in 1995 and 2638 mm in 1993. Over 700 mm fell in January 1993, following the passage of Tropical Cyclone Oliver. Salinities within One Tree reef lagoon are 35.6–35.7‰ (Kinsey, 1979).

Structure of experimental patch reefs

Within the patch reefs most of the corals and algae were distributed along the inside wall. Mean cover of live scleractinian corals on the walls ranged from 6% to 26%, the most abundant coral colonies were encrusting (*Porites lichen*, *P. murrayensis*, *Goniopora tenuidens*, *Favites abdita*, *Platygyra sinensis*, *Goniastrea retiformis*) and small branching species (*Acropora bushyensis*, *A. palifera*, *Pocillopora damicornis*, *Stylophora pistillata*, *Seriatopora hystrix*). Coralline algae (*Lithophyllum* spp, *Porolithon* spp) covered up to 12% of the walls. Some calcareous macroalgae formed rhodoliths. Macroalgae, mainly *Laurencia* spp, *Chlorodesmis fastigiata*, *Turbi-*

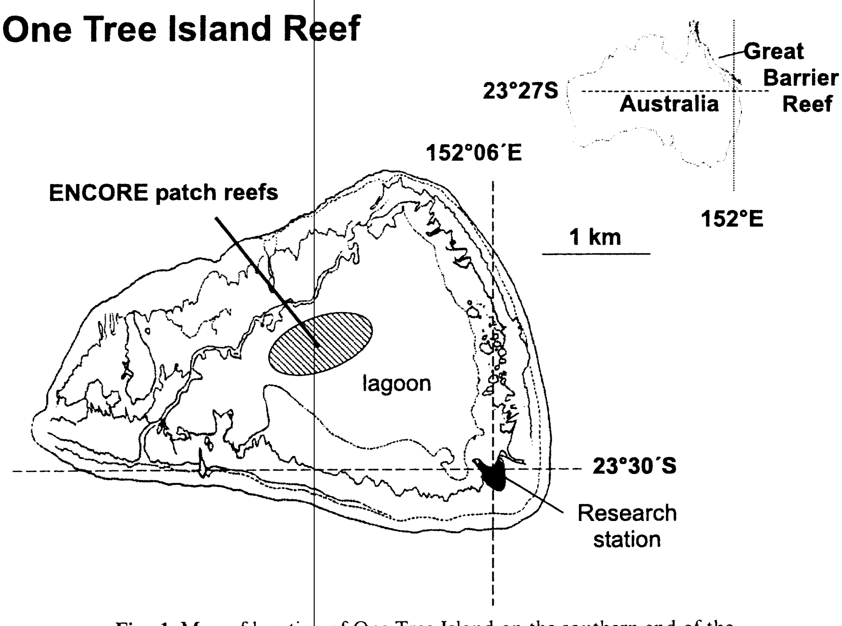

Fig. 1 Map of location of One Tree Island on the southern end of the Great Barrier Reef showing the research station and location of ENCORE experimental patch reefs.

naria ornata and *Caulerpa* spp were seasonal, but low in cover (~2%). The epilithic algal community (EAC) covered all other substrata.

The floor of the patch reefs was predominantly sand (40–60%) with small outcrops of dead coral substrate covered in biota. Coral cover of the floor varied from 5% to 18% and was mainly stands of branching corals such as *A. grandis* and *A. pulchra*. Plastic racks holding a variety of coral, soft coral and algal species transplanted from adjacent areas (see Larkum and Steven, 1994 for project details) were placed on the floor.

The height (h) of the patch reef walls varied from 0.5 to 0.9 m. Projected surface areas of the patch-reef walls and floors varied between 37 and 56 m^2 and 90 and 779 m^2, respectively. The total surface area enclosed within the atolls varied from 107 to 827 m^2. Water volume contained within the patch reefs varied from 27 to 323 m^3 . Volume to total surface area ratios ranged from 0.30 to 0.64 m (Table 1).

Experimental design

The studies summarized in this paper, except the experiments with coral gametes done in the laboratory, were done within the framework of ENCORE conducted in the lagoon of One Tree Reef. Details of the purpose, research programme and experimental design of ENCORE are given in Larkum and Steven (1994). Briefly, 12 patch reefs of similar size, volume and benthic composition were used as natural replicated subsystems (Table 1). During low tide the perimeter of each patch reef isolates a shallow pool (< 1 m) for 2.5–3 h from the surrounding lagoon – thus forming clearly defined boundaries. Twice daily, during each low tide three patch reefs each received one of four treatments:

- no nutrients were added ('control', C),
- inorganic nitrogen was added as NH_4Cl (+N),
- inorganic phosphorus was added as KH_2PO_4 (+P),
- both nitrogen and phosphorus were added (+N + P).

Organisms – either growing naturally within the patch reefs, or transplanted into the patch reef pools – were thus maintained under natural environmental conditions, but subjected to nutrient-enriched waters during low tide in the nine nutrient-enriched patch reefs.

Nutrient additions

The 30-month experiment was divided into a low nutrient loading phase (September 1993–December 1994), followed by a higher loading phase (January 1995–February 1996). During the low-loading phase, concentrated nitrogen and phosphorus were added at the beginning of every low tide as a single pulse to the water body contained within the patch reefs to achieve initial concentrations of 10 μM NH_4–N and 2 μM PO_4–P. During the high-loading phase, nutrients were added 3 times at regular intervals (~37 min apart) every low tide to sustain elevated concentrations of 20 μM NH_4^+–N and 4 μM PO_4^{3-}–P throughout the ponding period. During both phases, the nine patch

TABLE 1

Dimensions of 12 patch reefs used to study the effects of inorganic nitrogen and phosphorus enrichment on patch reef organisms in the ENCORE study at One Tree Island, southern Great Barrier Reef.[a]

Number	Treatment	Patch-reef								Total moles nutrient added			
		Dimensions (m)			Surface Area (SA) (m^2)			Volume (m^3)	Volume/SA	Low loading (670 days)		High loading (430 days)	
		Length	Breadth	Depth	Wall	Bottom	Total			NH_4–N	PO_4–P	NH_4–N	PO_4–P
1	C	15.0	19.3	0.76	36.5	184.0	220.5	60.2	0.33				
2	+N+P	14.5	14.8	0.54	30.5	254.6	285.1	92.5	0.36	145	727	4175	835
3	+N	17.7	8.0	0.60	27.4	165.5	192.9	61.4	0.36		523	2888	
4	+P	17.1	11.0	0.65	45.0	381.3	426.3	135.4	0.36	219			1258
5	C	32.0	25.0	0.50	49.5	779.3	828.7	322.5	0.41				
6	+P	15.8	15.3	0.85	46.5	238.0	284.4	152.4	0.64	255			1380
7	+N	16.0	12.1	0.58	28.0	185.3	213.3	73.6	0.40		629	3470	
8	+P	11.3	8.0	0.51	17.2	90.4	107.6	26.8	0.30	46			245
9	C	16.0	11.5	0.58	29.7	208.8	238.5	76.4	0.37				
10	+N+P	14.5	13.0	0.67	39.0	269.9	308.9	129.7	0.48	215	1075	5977	1195
11	+N+P	13.0	13.5	0.75	35.0	173.8	208.9	89.6	0.52	152	761	4226	845
12	+N	10.7	7.5	0.80	29.3	106.8	136.1	46.1	0.43		378	2097	

[a] The total load of nitrogen and phosphorus added during the low-loading and high-loading phase of the study are also shown.

reefs (+N, +P, +N + P) receiving nutrient additions were near-simultaneously fertilized every low tide by telemetrically controlled nutrient dispensing units (NDUs) – moored adjacent to each patch reef. NDUs discharged concentrated nutrient along several PVC lines with outlets spread throughout the pools of the patch reefs (McGill and Steven, 1994; Koop *et al.*, 2001).

Nutrient loading

Regular monitoring of nutrient levels was done during both low- and high-loading phases of the experiment to validate that desired nutrient levels were being achieved. These results and the mass transfer relationships are detailed in Steven *et al.* (unpub. data) and Steven and Atkinson (unpub. data). We summarize the major findings of this monitoring to demonstrate that the nutrient levels were being achieved and actively assimilated by the patchreef community.

Low-loading phase

Ammonium. In control and +P patch-reefs ambient concentrations of NH_4–N averaged 0.65 ± 0.69 μM (range 0.08–4.04 – Table 2). On all sampling events NH_4–N concentrations in control and +P patch-reefs declined over the low-tide period indicating uptake by the patch-reef community (Steven *et al.* unpub. data). Ammonium uptake rate constants (S_N) varied from 12 to 130×10^{-6} m s^{-1}.

The total loading to ammonium-enriched patch reefs over the 465 days of the low-loading phase of ENCORE varied from 378 to 1075 moles N (Table 1). This variation in loading resulted primarily from differences in patch-reef volume but also small differences in fertilization success. The initial threshold criteria concentration of 10 μM NH_4–N was achieved, and exceeded except on windy days. Over all sampling events, initial NH_4–N concentrations averaged 11.45 ± 4.85 μM (range 2.03–19.76 – Table 2). Immediately after the nutrient addition (10 min), the concentrations of the three replicates varied greatly as the nutrients discharged from the 4 or 8 outlets had yet to disperse. NH_4–N concentrations were depleted over the low-tide period to concentrations similar to ambient, averaging 0.91 ± 0.79 μM NH_4–N (Table 2).

Both the initial NH_4–N concentration and subsequent depletion depended primarily on prevailing wind speed and to a lesser extent direction. On moderately windy days (2.5–8.2 m s^{-1}), NH_4–N was rapidly mixed – as seen by decreasing variance – throughout the patch-reef within 10 min. Depletion of NH_4–N was rapid and after 1 h concentrations were close to ambient. On very still days (< 2.5 m s^{-1})NH_4–N concentrations were initially patchy, often exceeded desired concentrations, and had low depletion rates. At wind speeds of greater than 10 m s^{-1} initial concentrations of NH_4–N were below 10 μM and rapidly declined to ambient concentrations within 10 min. Under these conditions some, or most of the NH_4–N was probably advected either through or over the patch reef walls and lost. At wind speeds less than 10 m s^{-1}, S_N varied between 22 and 241×10^{-6} m s^{-1} and was positively related to wind speed. S_N differed significantly at wind speeds greater than 10 m s^{-1} suggesting that some or most of the NH_4–N depletion was physical loss rather than biological uptake.

Phosphorus. PO_4–P concentration in +N and control patch reefs averaged 0.2 ± 0.06 μM with a range of 0.1–0.64 μM (Table 2). Over low tide, PO_4–P concentrations often became depleted, but sometimes increased probably resulting from efflux from the sediment (Steven *et al.* unpub. data).

Phosphorus-enriched patch reefs received 46–255 moles P during the low-loading phase of ENCORE (Table 1). Over all sampling events, initial PO_4–P concentrations averaged 2.34 ± 0.98 μM PO_4–P – meeting the 2 μM PO_4–P – criteria and ranged from 0.92 to 4.48 μM. Final PO_4–P concentrations – measured just before the patch reefs were covered by the rising tide – were nearly threefold (0.52 ± 0.32 μM) greater than ambient (0.2 ± 0.06 μM) indicating that not all of the PO_4–P

TABLE 2

Summary statistics of average initial and final nutrient concentrations (μM) of nitrogen and phosphorus in ENCORE patch reefs.[a]

Treatment	Nitrogen				Phosphorus		
	n	Mean NH_4	Mean NO_x	Mean DIN	*n*	Mean PO_4	Diss N:P
Initial concentration							
Control	214	0.65 (0.69)	2.94	3.59	216	0.20 (0.06)	14.70
Low-loading phase	48	11.45 (4.85)	2.94	14.39	47	2.34 (0.98)	6.15
High-loading phase	12	36.20 (21.87)	2.94	39.14	12	5.14 (2.81)	7.61
Final concentration							
Control	214	1.34 (0.57)	2.94	4.28	216	0.16 (0.04)	26.75
Low-loading phase	48	0.91 (0.79)	2.94	3.85	48	0.52 (0.32)	7.40
High-loading phase	12	11.30 (10.20)	2.94	14.24	11	2.40 (1.61)	5.93

[a] Data are calculated from all measurements of nutrients in control patch reefs and from all measurements from patch reefs to which nitrogen (i.e. +N and +N+P) and phosphorus (i.e. +P and +N+P) were added. Relevant nitrogen-to-phosphorus ratios are also shown.

were taken up in the available 2.5–3 h (Table 2). As with NH_4–N, initial PO_4–P concentrations and subsequent depletion depended upon the prevailing wind-speeds. Phosphorus uptake constants (S_P) ranged from 9 to 214×10^{-6} m s^{-1}.

High-loading phase

Ammonium. Ambient concentrations in control and +P patch reefs were 1.34 ± 0.57 μM and ranged from 0.73–5.80 μM NH_4^+–N (Table 2). Ammonium-enriched patch reefs received between 2097 and 5977 moles N over the 430 days of the high-loading phase (Table 1). Initial concentrations of 20 μM NH_4^+–N were met and exceeded (Table 2). Concentrations increased with each nutrient addition, and final concentrations – recorded usually after the third nutrient addition – averaged 36.21 ± 21.87 μM NH_4–N (Table 2). Although significant depletion had occurred by the end of low tide, NH_4–N concentrations were elevated relative to ambient, averaging 11.3 ± 10.20 μM NH_4–N. NH_4 concentrations during the high-loading phase were sustained for the duration of low tide, rather than pulsed as in the low-loading phase. Although NH_4–N concentrations during this phase of ENCORE were threefold those of the low-loading phase, S_N were similar, averaging 127 ± 82 s $\times 10^{-6}$ m s^{-1} and ranging from 26 to 352×10^{-6} m s^{-1} .

Phosphorus. Ambient PO_4–P in control and +N patch reefs averaged 0.16 ± 0.04 and ranged from 0.08 to 0.46 μM (Table 2). Phosphorus-enriched patch reefs received 245 to 1380 moles P (Table 1). PO_4–P concentrations rose with each successive nutrient addition, reaching an average maximum concentration of 5.14 ± 2.81 μM PO_4–P, and subsequently declining to an average 2.40 ± 1.61 μM PO_4 –P at the end of the low tide (Table 2). S_P values during the high-loading phase ranged from 25 to 190×10^{-6} m s^{-1} and averaged $88 \pm 51 \times 10^{-6}$ m s^{-1}.

Daily loads to patch reefs

Total daily loads of nutrients to experimental patch reefs are shown in Table 3. Clearly, the amount of nutrients added during ENCORE increased the loads of both N and P to the reefs considerably over background.

Methods used in individual projects of the ENCORE study are summarized in Table 4.

Results and Discussion

Processes

Nutrient dynamics in patch reefs. The nutrient data indicate that patch reefs showed first-order uptake kinetics. Rate constants are consistent with those calculated by mass transfer and reported in the literature (Bilger and Atkinson, 1985; Steven and Atkinson unpub. data), indicating maximum uptake rates and little loss to the surrounding water. This is supported by the fact that we measured decreases in nutrient concentrations in control patch reefs with final concentrations less than those in surrounding waters (see above; Steven *et al.* unpub. data).

Measurements of ^{15}N uptake. Rapid $^{15}NH_4$ uptake and assimilation were measured in organisms that actively pump water such as the clam *Tridacna maxima* (0.17–1.74 μg ^{15}N cm^{-2} min^{-1}), or those with high surface area/volume morphologies: the red macroalga *Laurencia intricata* (2.5–4.16 μg ^{15}N cm^{-2} min^{-1}), and the branching endosymbiotic corals *Acropora palifera*, *A. pulchra* and *Pocillopora damicornis* (0.1–0.38 μg ^{15}N cm^{-2} min^{-1}). In contrast, low rates of uptake (< 0.3 μg ^{15}N cm^{-2} min^{-1}) were measured in sponges, sediments, epilithic algal plates and red algal rhodoliths. Assimilation of $^{15}NH_4$ by endosymbiotic corals and clams was primarily, but not exclusively, in zooxanthellae. Uptake rates were related to loading: at 120 μM NH_4^+–N uptake rates of biota were 2–4-fold greater than at 40 μM NH_4^+–N (Table 5).

Nitrogen fixation/denitrification. During the initial, low-loading phase of ENCORE nitrogen fixation in treatment patch reefs was not significantly different from control patch reefs, although nitrogenase activity in +N and +N + P patch reefs was consistently lower than in

TABLE 3

Comparison of estimated daily loadings of inorganic N and P for ambient, low-loading phase and high-loading phase of the ENCORE study.[a]

		Nutrient added			
		Nitrogen		Phosphorus	
	Duration (h)	Concentration (mmol m^{-3})	Loading (mmol m^{-2} day^{-1})	Concentration (mmol m^{-2} m^{-1})	Loading (mmol m^{-2} day^{-1})
Ambient	18	0.65	6.2	0.2	0.8
Low load	6	11.45	13.0 (2.1)	2.34	2.1 (2.6)
High load	6	36.2	41.0 (6.6)	5.12	8.0 (10.0)

[a] Numbers in parentheses in loading columns are the number of times ambient loads were exceeded. Ambient conditions were assumed to be 0.65 μM NH_4–N and 0.2 μM PO_4–P with a water velocity of 10 m s^{-1} for a period of 18 h (to take account of an average of 3 h each low tide when the One Tree Island lagoon is separated from the ocean).

TABLE 4

Summary of methods used in the various studies of the ENCORE experiment at One Tree Island, southern Great Barrier Reef.

Parameter	Method	References
Nutrient additions/analyses		
Nutrient addition to patch reefs	Telemetrically controlled doses of nutrients added by Nutrient Dispensing Units	McGill and Steven (1994); Koop *et al.* (2001)
Nutrient sampling in patch reefs	Water samples were taken by pumping from three random locations in each patch reef	
Nutrient concentration measurements	Measurement of NH_4–N, NO_x and PO_4^{3-}–P using standard spectrophotometric techniques	Parsons *et al.* (1984)
Nutrient uptake by patch reefs	Uptake rate constants were converted to transport rates per unit planar surface area of reef	Bilger and Atkinson (1985), Thomas and Atkinson (1997)
^{15}N uptake by organisms	Incubation with added ^{15}N and analysis by mass spectrometry	
Elemental ratios	Samples were dried and analysed on a Perkin–Elmer CHNS 2400 elemental analyser	
Coral growth		
Linear extension	Staining with Alizarin Red S	Lamberts (1978)
Calcification	Buoyant weight increments	Jokiel *et al.* (1978), Maragos (1978)
Injury repair	Re-examination of lesions produced by sampling of branch tips after six months	Meesters (1994)
Skeletal bulk density and micro-density	Displacement methods	Bucher *et al.* (1998)
Tissue morphology	Light microscopy of 0.5–1.0 μm sections of single polyps	Harrison (1980), Harrison *et al.* (1990)
Soft coral metabolism in competition	Secondary metabolites identification by NMR spectroscopy	Vanderah *et al.* (1978); Tursch *et al.* (1978)
Stress level in soft corals	Quantitative estimation of metabolites by NMR	Leone *et al.*, 1995
Soft coral CNP ratios	C & N by Fisons EA1108 elemental analyser P by vanado-molybdo-phosphoric acid colorimetric method	Standard methodology Clesceri *et al.* (1989)
Coral reproduction		
Coral fecundity	Branches decalcified, dissected and eggs and testes counted and measured	Ward (1997), Ward and Harrison (2000)
Coral gamete fertilization trials	Eggs and sperm separated and recombined at known sperm densities. Gametes exposed to elevated doses of nutrients	Ward (1997), Harrison and Ward (unpub. data)
Coral larval settlement trials	Coral larvae reared and allowed to settle on terracotta tiles in settlement cages following larval exposure to elevated nutrients	Ward (1997), Ward and Harrison (unpub. data)
Recruitment studies and spat growth of corals	Terracotta tiles in patch reefs scored for coral spat 3 monthly over 3 years	Ward (1997), Ward and Harrison (2000)
Lipids in coral tissues	Gravimetric extraction using chloroform – methanol	Ward (1995), Ward (1997)
Soft coral metabolism and competition	Secondary metabolites identification by NMR spectroscopy	Vanderah *et al.* (1978), Tursch *et al.* (1978)
	Quantitative estimation of metabolites by NMR	Leone *et al.* (1995)
Soft coral CNP ratios	C & N by Fisons EA1108 elemental analyser P by vanado-molybdo-phosphoric acid colorimetric method	Standard methodology Clesceri *et al.* (1989)
Epilithic algal community		
^{15}N tracer	$^{15}NH_4$ additions to reef water at low tide; isotope analysis on mass spectrometer	Stewart *et al.* (unpub. data)
Nitrogen fixation	Acetylene reduction technique	Capone and O'Neil (unpub. data)
Denitrification	Acetylene blockage technique	Capone (unpub. data)
Biomass measurements	Biomass was scraped from coral blocks, dried and weighed; chlorophyll *a* content was estimated from scrape-samples extracted in acetone and measured spectophotometrically	Parsons *et al.* (1984)
Nutrient uptake rates	Determined from time-series of nutrients in chambers containing EAC on coral blocks samples were analysed with a modification of the phenol-hypochlorite method. Uptake was determined with Michaelis–Menten kinetics	Solorzano (1969), Dugdale (1967)
Carbon production	Estimated from oxygen evolution rates measured in closed incubation chambers (respirometers)	
Macrophytes		
Production of rhodoliths		
Nutrient uptake of fleshy algae		
Chlorophyll ($a+b+c$) analyses for EAC	1. Spectrophotometric analysis	Jeffery and Humphrey (1975), Larkum and Koop (1997)
Giant clams		
Clam biomass, haemolymph & nutrient measurements	N:P analysis, ammonium determination	Belda-Baillie *et al.* (1998)
Amino acid determination	Total amino acids	Magne and Larher (1992)
Bioerosion		
Macro boring, accretion and grazing	Blocks of *Porites lutea* prepared from live coral, washed and dried, attached to grids to control and fertilized patch reefs	Kiene and Hutchings (1994), Pari *et al.* (1998)

TABLE 4 (CONTINUED)

Microborings	Cubes of *Tridacna*, calcite and limestone attached to plates on grids in all atolls	Kiene (1994), Perkin and Tseuntas (1976)
Stomatopod recruitment	Collected newly recruited animals from tagged, sun-dried coral rubble pieces placed in patch reef	Erdmann and Caldwell (1997), Steger (1987)

TABLE 5

Summary of ^{15}N uptake (^{15}N cm^{-2} h^{-1}) of corals, clams, macroalgae, soft coral and sediment.[a]

Organism		Control		+N acclimated	
		40 μM	120 μM	40 μM	120 μM
Acropora pulchra	Host	0.17	0.23	0.06	0.1
	Zooxanthellae	0.21	1.85	0.38	1.14
Acropora palifera	Host			0.04	−0.08
	Zooxanthellae			0.95	0.31
Pocillopora damicornis	Host	0.04	0.1	0.01	0.05
	Zooxanthellae	0.25	0.32	0.1	0.38
Tridacna crocea	Whole	1.74	7.22	0.42	1.13
	Host	0.06		0.03	0.02
	Zooxanthellae	0.53		0.17	0.38
Laurencia intricata		2.50	4.16		
Sarcophyton			0.49		
Sediment		0.06	0.1	0.01	0.27

[a] Organisms were subjected to two concentrations of ^{15}N for about 3 h during low tide in the ENCORE study on the southern Great Barrier Reef. +N acclimated organisms came from patch reefs to which inorganic nitrogen had been added twice daily for more than a year; controls were from control patch reefs.

the other patch reefs (Fig. 2). No denitrification experiments were conducted during this phase of the experiment.

Both nitrogen fixation (Fig. 3) and denitrification (Fig. 4) were significantly affected by the nutrient treatments during the high-loading phase of ENCORE. Nitrogenase activity decreased by approximately a factor of 2 from the low-loading phase and exhibited significant ($p < 0.05$) stimulation of nitrogen fixation in the +P treatments (1.76 ± 0.08 nmol C_2H_4 g dry wt sediment^{-1} h^{-1}; Fig. 3) and significant ($p < 0.05$) stimulation of denitrification rates in the +N(51 ± 4.7 pmol N_2O g dry wt sediment^{-1} h^{-1}) and +N + P (53 ± 2.3 pmol N_2O g dry wt sediment^{-1} h^{-1}) treatments, compared with control patch reefs (24.3 ± 5.2 pmol N_2O g dry wt sediment^{-1} h^{-1}; Fig. 4).

Plants

The functional groups of free-living algae in the experimental patch reefs consisted of encrusting algae, macroalgae (filamentous and bushy algae) with erect but

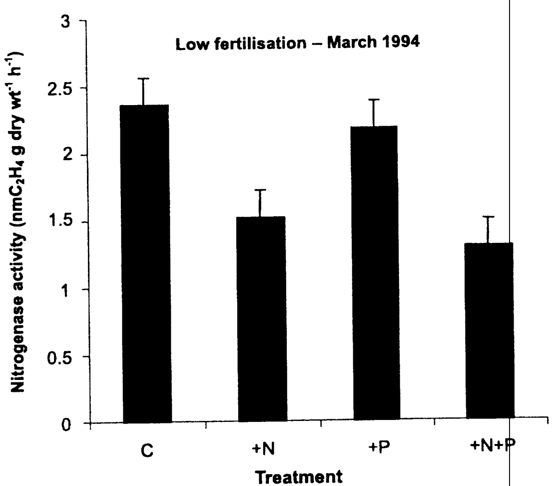

Fig. 2 Rate of nitrogenase activity in experimental patch reefs (nmol ethylene g dry weight sediment^{-1} h^{-1}) during the low-loading phase of the ENCORE study in March 1994 (O'Neil and Capone, unpub. data).

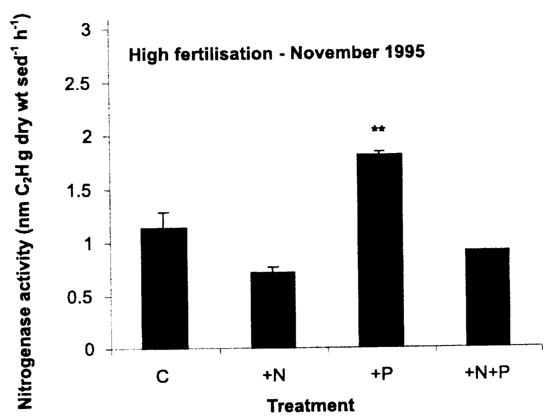

Fig. 3 Rate of nitrogenase activity in experimental patch reefs (nmol ethylene g dry weight sediment^{-1} h^{-1}) during the high-loading phase of the ENCORE study in November 1995. (** indicates significance at $p < 0.05$) (O'Neil and Capone, unpub. data).

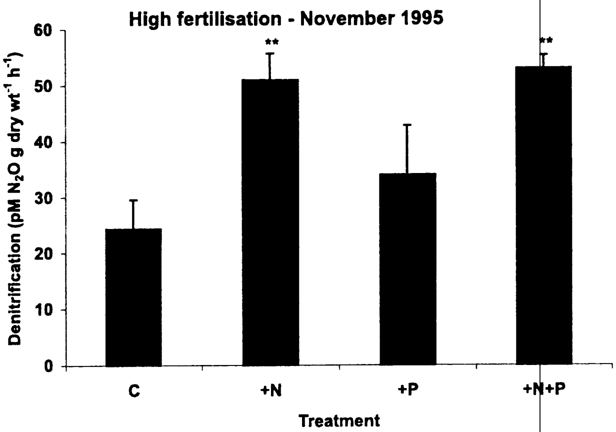

Fig. 4 Rate of denitrification in experimental patch reefs (pmol N_2O g dry wt $sediment^{-1}$ h^{-1}) during the high-loading phase of the ENCORE study in November 1995. (** indicates significance at the $p < 0.05$) (O'Neil and Capone, unpub. data).

flexible thalli, and phytoplankton. The encrusting algae included the epilithic algal community (EAC), crustose coralline algae and a number of less significant algal species which are normally represented in the EAC but occasionally form uni-algal growths. The filamentous and bushy algae were not common in the patch reefs but included, from time to time, *C. fastigiata*, *Laurencia* spp, *Halimeda* spp, *Chnoospora intricata*, *Hydroclathrus* sp and a number of cyanobacteria such as *Lyngbya majuscula*.

Phytoplankton

Phytoplankton primary production was measured in January 1995 only (high-loading phase). Production rates in all treatment patch reefs were not significantly different from controls with levels of chlorophyll ranging from 82 to 261 μg Chl *a* m^{-3} and primary production rates between 1.6 and 4.0 mg C m^{-3} h^{-1} (Table 6). Highest production was measured in the oceanic water 1 km off the One Tree Reef (3.6–4.0 mg C m^{-3} h^{-1}). Using atomic Redfield ratios (C : N = 6.6; C : P = 106) phytoplankton production accounted for the uptake of between one half and one percent of the N added daily to patch reefs during this phase and an even smaller proportion of the P added. The phytoplankton could thus not have been responsible for the rapid loss of nutrients added to the enriched patch reefs.

Macroalgae

Macroalgae had variable responses to elevated nutrients. Some of the filamentous algae had rapid nutrient uptake and assimilation with significant ecophysiological effects. Other macroalgae, however, particularly encrusting forms, had little enhanced nutrient uptake and assimilation with no detectable ecophysiological effects. Filamentous macroalgal biomass was low in the patch reefs and did not visibly respond to elevated nutrients.

The filamentous macroalga with the most rapid nitrogen uptake, *L. intricata* (Rhodophyta), was analysed in some detail (Stewart, unpub. data). Uptake rates of NH_4^+ exceeded NO_3^- uptake and these rates were not affected by phosphorus concentration. NH_4^+ assimilation in both light and dark conditions was observed, with storage as glutamine in the dark and conversion into serine, threonine and glycine in the light. Inhibitor and ^{15}N tracer studies are consistent with NH_4^+ assimilation by the glutamate synthase cycle, rather than the glutamate dehydrogenase cycle. The rapid uptake and assimilation of NH_4^+ by *L. intricata* as well as the ability to assimilate NH_4^+ in the dark are indications that this species has adapted to utilize irregular pulses of nutrients.

The activity of the enzyme alkaline phosphatase was assayed to provide an indication of the degree of phosphorus limitation. High phosphatase activity, providing a mechanism for cleaving PO_4^{-3} from organic compounds, is indicative of P limitation. No significant effect was observed in *L. intricata* during the initial nutrient enrichment phase, but significant reductions in alkaline phosphatase activity were observed in the +P and +N + P treatments in the higher nutrient enrichment phase. Enzyme activity was highly temperature

TABLE 6

Phytoplankton biomass and production 1 km outside One Tree Reef (OS1, OS2) and in 8 of the experimental patch reefs at 11.00–1500 h on 20 January 1995.[a]

Site	Vol (m^3)	Biomass (μg Chl m^{-3})				Production (mg C m^{-3} h^{-1})			
		3 μm	3–1 μm	< 1 μm	Total	3 μm	3–1 μm	< 1 μm	Total
OS(1)	–	49	19	88	156	1.87	0.89	1.25	4.02
OS(2)	–	87	33	85	205	1.13	1.07	1.42	3.62
C(1)	143.8	59	33	169	261	2.24	0.48	0.52	3.24
C(5)	256.5	42	19	49	111	1.14	0.16	0.30	1.59
+N(3)	73.6	39	18	41	97	1.00	0.36	0.46	1.83
+N(7)	84.5	33	18	31	82	1.47	0.32	0.47	2.26
+P(4)	176.5	42	18	28	88	1.02	0.32	0.47	1.81
+P(6)	29.5	36	26	69	131	1.10	0.48	0.56	2.14
+N+P(2)	117.6	30	20	56	106	1.68	0.48	0.47	2.63
+N+P(10)	148.6	41	11	56	108	1.19	0.42	0.64	2.26

[a] C = control, +N = enriched in N; +P = enriched in P; +N + P = enriched in both N and P. Numbers refer to ENCORE patch reef numbers.