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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.

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-*

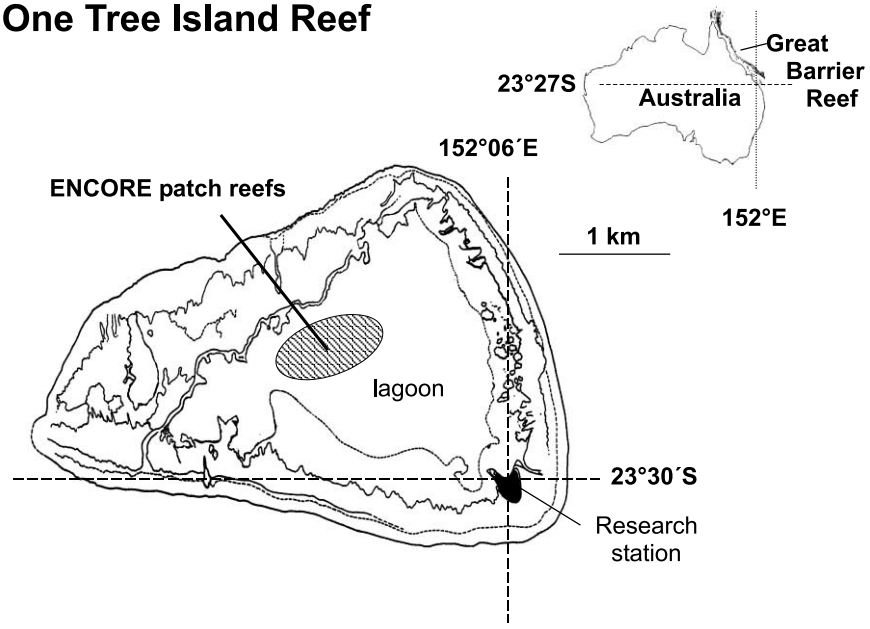

Fig. 1 Map of location of One Tree Island on the southern end of the Great Barrier Reef showing the research station and location of ENCORE experimental patch reefs.

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 $\times 10^{-6}$ 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

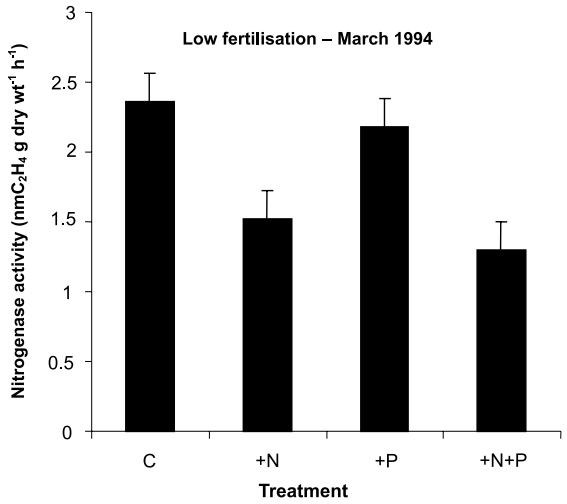

Fig. 2 Rate of nitrogenase activity in experimental patch reefs (nmol ethylene g dry weight sediment^{-1} h^{-1}) during the low-loading phase of the ENCORE study in March 1994 (O'Neil and Capone, unpub. data).

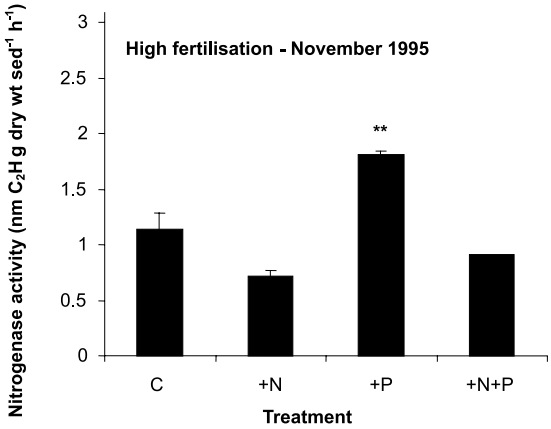

Fig. 3 Rate of nitrogenase activity in experimental patch reefs (nmol ethylene g dry weight sediment^{-1} h^{-1}) during the high-loading phase of the ENCORE study in November 1995. (** indicates significance at $p < 0.05$) (O'Neil and Capone, unpub. data).

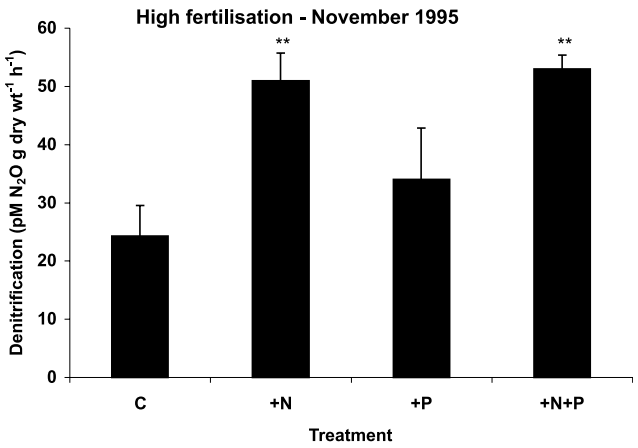

Fig. 4 Rate of denitrification in experimental patch reefs (pmol N_2O g dry wt sediment^{-1} h^{-1}) during the high-loading phase of the ENCORE study in November 1995. (** indicates significance at the $p < 0.05$) (O'Neil and Capone, unpub. data).

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.

TABLE 7

Amino acids, chlorophyll *a*, and tissue nitrogen in *Gracilaria edulis* after 3 days field incubation in One Tree Island ENCORE experimental patch reefs, phase 2 (high nutrient loading period).[A]

Nutrient addition	Amino acids			Tissue nitrogen	Pigment chlorophyll *a*
	Citrulline		Total amino acids		
	(nmol g_{wet}^{-1})	(% total)	(nmol g_{wet}^{-1})	(%)	(mg g_{wet}^{-1})
Control	250[a]	11	2252	1.30	1.03
+P	368[ab]	16	2280	1.26	0.90
+N	588[bc]	24	2380	1.42	1.08
+N+P	716[c]	29	2496	1.52	1.18
F-value	4.8*		0.1	2.2	3.0

[A] [abc] Values in columns for each treatment with the same letter are not significantly different at $p < 0.05$.
* $p < 0.05$.

dependent, with highest rates in summer (Stewart, unpub. data; Drew, unpub. data).

A filamentous macroalgal species common in tropical/sub-tropical waters, *Gracilaria edulis* (Rhodophyta), has been shown to be responsive to elevated nutrients (Horrocks *et al*., 1995; Jones *et al*., 1996). *G. edulis* was collected in Moreton Bay, Queensland (27°13′S, 153°07′*E*), transported to One Tree Island and incubated in the experimental patch reefs for 3 days in clear plastic containers perforated for water exchange. Following the short incubation, plants were analysed for pigment, tissue nutrient and amino acid content (Table 7). The amino acid citrulline was significantly increased under the +N and +N + P treatments. Citrulline, a 3N containing amino acid, has been invoked as a nitrogen storage compound.

Epilithic Algal Community (EAC)

The epilithic algal community is a microscopic algal biofilm, which exists on most dead limestone surfaces of coral reefs (Hatcher and Larkum, 1983). Because such surfaces are common and because the EAC is highly productive (Hatcher and Larkum, 1983), the EAC is an important contributor of food to the herbivores of coral reefs. Also because the EAC is so productive it has generally been thought that the EAC would be responsive to added nutrients (Klumpp and Mackinnon, 1992). Thus the EAC was a major focus of work during the ENCORE project.

Standing crop, growth and primary production of EAC on *Porites* coral plates were examined on 6 occasions during ENCORE. Standing crop was measured as dry weight or as chlorophyll *a*. Growth was measured as increment in dry weight over 7 days. Primary production was measured as oxygen exchange. In all experiments (across all seasons) no significant effect of nutrient enrichment was found in any of the treatments.

To test whether EAC would respond to higher nutrient levels, they were incubated in stirred nutrient-enriched seawater at two levels of nutrient enrichments: 80 μM and 200 μM NH_4Cl or KH_2PO_4 or both combined. EAC from control patch reefs were used in these experiments and showed no significant enhancement of production after 24 h incubation, either to enrichment by N or P or N+P (see Fig. 5). EAC from +N+P patch reefs were also treated in the same way and showed no response at any time.

Analyses of EAC that had been grown for 6 months in the different patch reefs showed no significant difference in the amount of chlorophyll *a* between treatments (Larkum and Koop, 1997), indicating that there was no difference in biomass of the EAC between treatment patch reefs. Based on the rates of uptake from 2 to 20 μM for each treatment from June 1994, there was a trend for uptake of ammonium to be suppressed in the EAC grown in +N patch reefs. At 20 μM, rates for +N patch reefs were 3.7×10^{-3} μM $cm^{-2}\,min^{-1}$, compared with control patch reefs (8.1×10^{-3} μM $cm^{-2}\,min^{-1}$) and +P patch reefs (4.5×10^{-3} μM $cm^{-2}\,min^{-1}$)). This is consistent with the

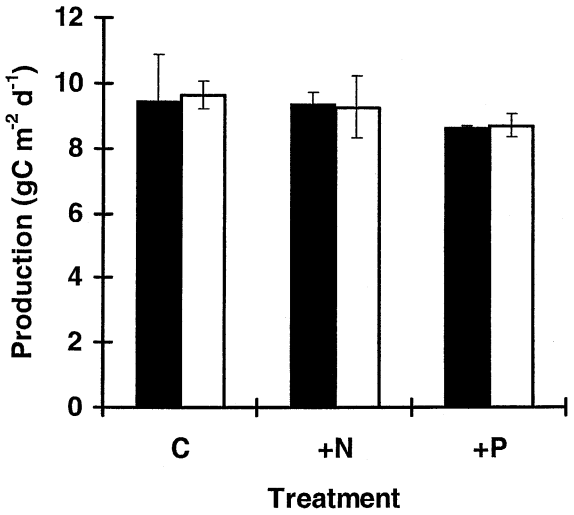

Fig. 5 Effect of short-term enrichment with 80 M NH_4Cl on primary production (g Cm^{-2} day^{-1}) of 12-month-old coral blocks from fertilized patch reefs (+N = 10 μM NH_4Cl; +P = 2 μM KH_2PO_4) and control (C) patch reefs. N = 6SD. Production was measured as oxygen evolution over 30 min. (minus dark treatments) using chambers as described in Larkum and Koop (1997). Dark bars are unenriched EAC, light bars are from 80 μM NH_4Cl incubations.

hypothesis that algae conditioned to higher concentrations of ammonium in the +N patch reefs, would show a lower capacity for ammonium uptake when exposed to episodic increases in concentrations of ammonium (Fujita, 1985).

Rhodoliths

Crustose coralline algae were a conspicuous component of the algae of the experimental patch reefs. These algae are important calcifiers in reef environments contributing to the calcium carbonate reserves and acting as important consolidators of the reef structure (Borowitzka and Larkum, 1986). Phosphate has been suggested to be an inhibitor of calcification in algae (Simkiss, 1964). Kinsey and Davies (1979) reported inhibition of calcification as a result of enrichment of a patch reef at One Tree Reef with urea and phosphate, although the calcifying agent was not identified. There is one report of enhanced levels of phosphate inhibiting the calcification of tropical coralline algae in the field (Björk *et al.*, 1995). These algae are difficult to work with experimentally because they encrust the substratum and other organisms. Rhodoliths (Larkum *et al.* (in press)) were therefore chosen as the experimental organism for this work since they are discrete semi-spherical bodies comprising of a single species (in this case *Lithophyllum kotchyanum*). Replicate rhodoliths were set out on plastic supports and monitored throughout ENCORE. Growth of replicate rhodoliths ($n = 12$) in each experimental patch reef was measured by increase in buoyant weight over periods of 2–4 months throughout ENCORE (Larkum *et al.* (in press)). No effect of enrichment by N or P was found at any time ($p > 0.05$ ANOVA; see Fig. 6 for four seasonal observations).

Calcification was measured by the alkalinity anomaly method at 3 seasons (March and June 94, August 95) and showed no significant effect of nutrient enrichment ($p > 0.05$; ANOVA; Larkum *et al.* (in press)). Growth rates (summer: 0.125 mg g^{-1} day^{-1} and winter 0.076 mg g^{-1} day^{-1}), primary production (6–14 g C m^{-2} day^{-1}), gross calcium carbonate increase (0.36 g g^{-1} yr^{-1} or $\sim$ 1.15 kg m^{-2} yr^{-1}) and calcification rates (70–180 mg $CaCO_3$ g (buoyant weight)$^{-1}$ h^{-1} , with summer rates $\sim$ twice those of winter) were all comparable with other work for tropical crustose coralline algae (Chisholm, 1988; Matsuda, 1989).

Animals

Different aspects of the biology of five major groups of animals were studied as part of the ENCORE project. These were: stomatopods, fish, reef-building corals, soft corals and giant clams. Though not all-inclusive, these groups represent a major proportion of the animal life present in the ENCORE patch reefs. Each of these groups is dealt with as a separate section below. Because of the importance of the symbiotic dinoflagellates (zooxanthellae) associated with many of these animals, one section is devoted to the responses of zooxanthellae within the experiment.

Stomatopods

Gonodactyloid stomatopods are benthic reef crustaceans that typically inhabit shallow reef flats and seagrass communities and live in cavities in hard substrata such as dead coral rubble. Recent studies have demonstrated a sensitive response of gonodactyloid stomatopod assemblages to marine pollution, including eutrophication. They have shown a reduction in abun-

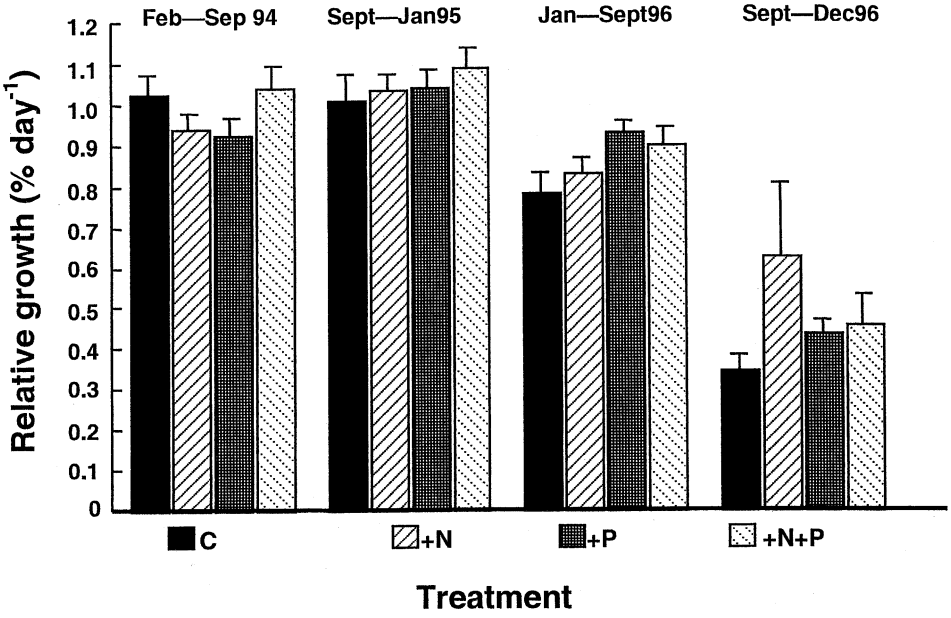

Fig. 6 Effect of nutrient treatments and season on mean relative growth (% day^{-1}) of rhodoliths. Error bars are standard errors of means ($n = 12$).

dance and species richness and apparent recruitment failure with increasing pollution (Steger and Caldwell, 1993; Erdmann and Caldwell, 1997). In this study, the effect of nutrient enrichment on stomatopod recruitment was examined by adding suitable stomatopod habitat (in the form of sun-dried, tagged coral rubble pieces) to the experimental patch reefs, and later collecting the rubble and extracting all animals which had recruited to the rubble during the experiment. A preliminary survey of the surrounding reef flats and patch reefs indicated that the shallow water stomatopod fauna of One Tree Island is dominated by four species (*Gonodactylaceus mutatus, Gonodactylinus viridis, Gonodactylus childi*, and *Haptosquilla glyptocercus*), and only these species were considered in this experiment. Additionally, only those animals which had clearly recruited to the rubble during the experiment (conservatively, those animals $\leqslant 18$ mm total length) were counted. This experiment was conducted during the high-loading phase of ENCORE, from May, 1995 through January 1996.

Results indicated that recruitment of gonodactyloid stomatopods is negatively affected by nutrient enrichment (Fig. 7). One-way ANOVA shows that the mean recruit densities in the 4 nutrient treatments were significantly different ($F = 5.85; df = 3, 8; p = 0.02$). Multiple comparisons of the 4 means using Tukey's studentized range test (controlling procedure-wise Type I error rate at $p < 0.05$) revealed that rubble from the combined $+N+P$ treatment patch reefs had significantly lower stomatopod recruit densities than rubble from the control, $+P$ and $+N$ patch reefs.

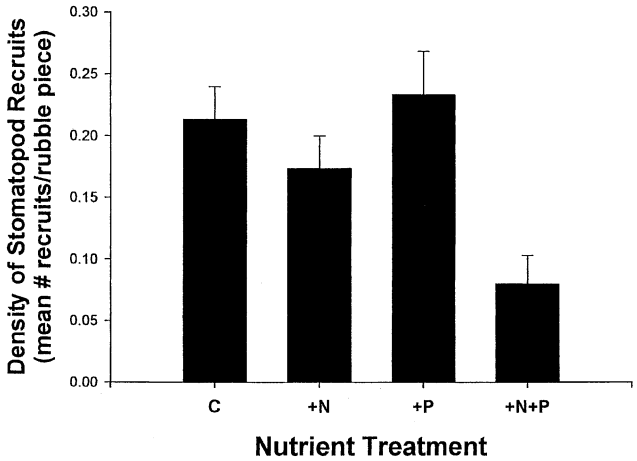

Fig. 7 Mean recruit density of stomatopods (average number of recruits per rubble piece) for each nutrient treatment. Bars indicate standard error.

Fish

Earlier studies at One Tree Island (Hatcher and Larkum, 1983) suggested that grazing fishes remove large quantities of epilithic algae, and so may mask the effects of nutrient enrichment on algal communities. Grazing fish assemblages may respond to nutrient enhancement by changes in: (1) fish density, (2) individual grazing rates, and (3) nesting and egg production.

Fish grazing rates. The majority of roving grazers were small (< 10 cm) parrotfishes, in groups which entered patch reefs with the rising tide (Fig. 8, see also Hawkins, 1995; Booth, 1997, 1998). Densities of fish varied but they could not be related to nutrient treatment (Booth unpub. data). Overall, small scarids removed 1.32 g algae m^{-2} day^{-1} in summer and 0.35 g m^{-2} day^{-1} in winter (Table 8; Booth, 1998). In contrast, territorial damselfish removed 2.0 g algae m^{-2} day^{-1} in summer and 1.0 g m^{-2} day^{-1} in winter. Although the species composition of the EAC differed significantly

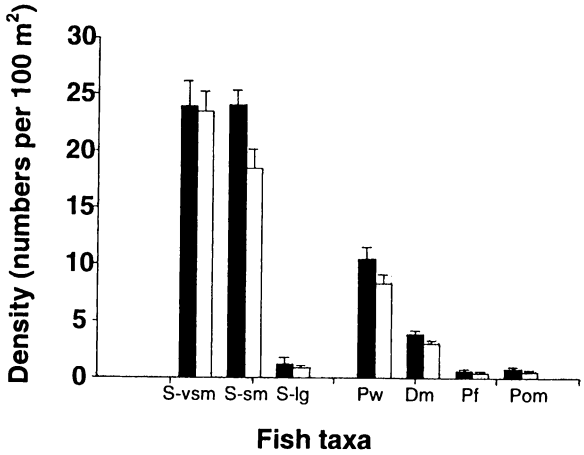

Fig. 8 Density of grazing fish taxa on patch reefs in One Tree Island lagoon in summer (dark bars) and winter (light bars) censuses ($n = 4$ censuses in winter, $n = 5$ censuses in summer, SE shown). S-vsm: *Scarus* spp (< 6 cm TL); S-sm; *Scarus* spp (6–10 cm TL); S-lg: Scarids, acanthurids, siganids (> 10 cm TL), Pw: *Pomacentrus wardi*; Dm; *Dischistodus melanotus*; Pf: *Pomacentrus flavicauda*; Pom; other territorial pomacentrids. (from Booth, 1998).

TABLE 8

Summary of grazing pressure and its components for scarids and pomacentrids (*P. wardi*) on reef tops in One Tree Island lagoon (summer–winter mean values indicated) (from Booth, 1998).

Taxon	Coverage (%)	Feeding rate (bites day^{-1})	Food intake (mg DW $bite^{-1}$)	Density (m^{-2})	Grazing pressure (g m^{-2} day^{-1})
P. wardi	21–24	3514–1366	0.6	0.95–1.25	2.0-1.0
Scarids (< 6 cm TL)	89–86	8400–3168	0.05	0.29–0.30	0.48–0.12
Scarids (6–10 cm TL)	89–86	6600–2100	0.4	0.32–0.27	0.84–0.23
Large scarids	89–86	*ca* 1000	2	*ca* 0.01	*ca* 0.02

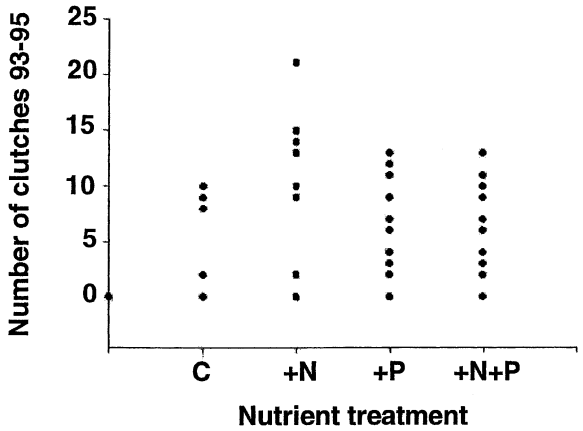

Fig. 9 Total number of clutches (•) received by individual males on patch reefs with different nutrient-enrichment treatments as part of the ENCORE study (from Beretta and Booth, 1998).

inside and outside damsel fish territories (*Pomacentrus wardi*), standing crop was similar (Booth, 1997, 1998).

Fish reproduction. P. wardi males attracted females to lay clutches of eggs between new moon and full moon (peaking at 3/4 moon) in November/December 1993, December/January 1994/95 and December 1995 (Beretta and Booth, 1998). Some males attracted more females and hence guarded significantly more clutches than others, but there were no apparent nutrient-treatment effects (Fig. 9). Lipid analyses from eggs from different nutrient treatments showed no differences (Booth and Beretta unpub. data).

Reef-building corals

For most experiments, reef-building corals were collected and transplanted into the ENCORE patch reefs. Corals were collected as either entire colonies or large portions of colonies, or colonies were broken into small sub-colonies ('nubbins'; Spencer Davies, 1989) that were deployed on plastic racks within the experimental patch reefs. Nubbins were 5–10 cm in diameter, while other colonies ranged in size up to 30–40 cm in diameter.

Coral mortality. Coral mortality was studied by monitoring survivorship among coral colonies or nubbins introduced into the ENCORE patch reefs. No differences in survivorship between treatments were detected during the initial low-loading phase of the ENCORE project. Adding nutrients, however, increased the mortality of some coral species during the second, high-loading phase. Mortality rates of two morphotypes of *P. damicornis* ('brown' and 'pink' = pocilloporin containing), (Takabayashi and Hoegh-Guldberg, 1995; Dove *et al.*, 1995) were significantly higher in patch reefs that received nutrients ($p = 0.007$) and were highest in patch reefs that received a combination of both ammonium and phosphate (271% and 211% of control mortality for brown and pink morphotypes, respectively) (Fig. 10). The mortality of nubbins of the branching coral *A. longicyathus* was not significantly different in the nutrient treatments ($p > 0.05$; Hoegh-Guldberg, unpub. data) although it was generally about 10–20% higher in +N and +P patch reefs.

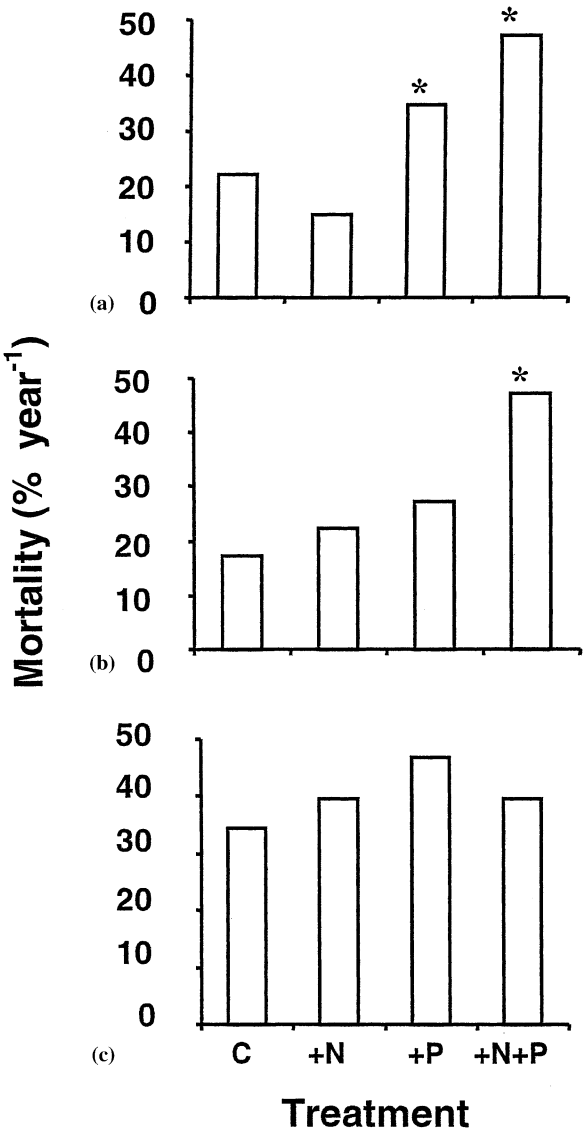

Fig. 10 Mortality rates (percent of colonies dying per year) of (a) *Pocillopora damicornis* (brown morphotype), (b) *P. damicornis* (pink) and (c) *Acropora longicyathus* after nine months in different treatments of the ENCORE project at One Tree Island reef. Shown are means and 95% confidence intervals. Asterisks indicated differences significant at $p = 0.05$. Adapted from Hoegh-Guldberg (1999).

Mortality was lower in larger coral colonies (Ward, 1997; Bucher unpub. data; Steven unpub. data). Aside from cyclone damage, larger portions of *A. longicyathus* and *A. aspera* (up to 5 kg in weight) that were transplanted into patch reefs for reproduction and growth studies suffered little mortality. Some predation by *Drupella* sp, folliculinids (a protist) and mortality from bleaching and disease were limited to single patch reefs and could not be linked with nutrient treatments.

TABLE 9

Summary of coral growth responses to the high-loading phase of the ENCORE nutrient treatments.

Parameter measured	Species used	Response		References
		Ammonium	Phosphate	
Linear extension	*Acropora longicyathus*	Reduced	Increased	Bucher and Harrison (unpub. data)
	A. palifera	No effect	Increased	
	Stylophora pistillata	Increased	Increase with ammonium	Steven (unpub. data) Takabayashi (1996)
Injury repair	*A. longicyathus*	Reduced	No effect	Bucher and Harrison (unpub. data)
Calcification (buoyant weight increments)	*A. longicyathus*	Increased[a]	Increased	Bucher and Harrison (unpub. data)
	A. aspera	No effect	No effect	
	A. palifera	Decreased	Increased	Steven (unpub. data)
	S. pistillata	No effect	Decreased	
	Pocillopora damicornis	Decrease	Decreased	Takabayashi (1996) Hoegh-Guldberg (unpub. data)
	A. longicyathus	No effect	No effect	Hoegh-Guldberg (unpub. data)
Skeletal density				
Bulk density	*A. longicyathus*	Increased	Reduced	Bucher and Harrison (unpub. data)
Micro-density	*A. longicyathus*	Increased	Increased	Bucher and Harrison (unpub. data)
Tissue				
Morphology				
Mucus cell density	*A. longicyathus*	No effect	Reduced	Bucher and Harrison (unpub. data)
Free body wall thickness	*A. longicyathus*	No effect	Increased	Bucher and Harrison (unpub. data)

[a] Increased over year but seasonal (increased in winter and spring but decreased in summer).

Coral growth. A summary of coral growth responses is presented in Table 9. Three teams within the ENCORE project independently measured a number of growth parameters in a range of coral species. The species included massive, columnar, densely branched and open staghorn growth forms. Several trends in growth response were consistent across these studies. Few growth responses were detected in any of the nutrient treatments during the initial, low-loading phase of ENCORE. Marked seasonal and clonal but not treatment variability was noted for *P. damicornis* (Hoegh-Guldberg and Moreno unpub. data; Hoegh-Guldberg *et al*., 1997) and *A. longicyathus* (Bucher and Harrison, unpub. data) during the low-loading phase. In some seasons significant differences in calcification were measured in *A. longicyathus* between nutrient treatments. There were no significant differences, however, when calcification was integrated over a full year (Bucher and Harrison, unpub. data).

During the second, high-loading phase of ENCORE, *A. longicyathus* and *A. palifera* had higher extension rates in the presence of elevated phosphate and reduced extension in ammonium treatments (Fig. 11; Bucher and Harrison, unpub. data; Steven unpub. data). In contrast to these studies, Takabayashi (1996) reported no effect of nutrient treatment on the linear extension rates of small (5–10 cm diameter) colonies of *S. pistillata* during the period of higher nutrient loading.

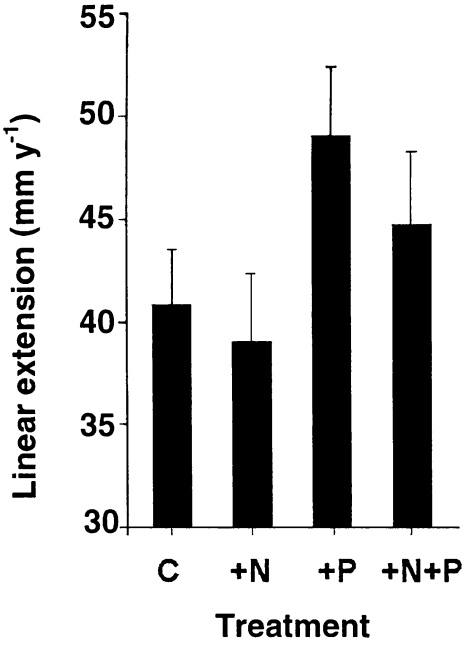

Fig. 11 Mean linear extension rates of *A. longicyathus* branches during the high dose period. Orthogonal analyses of variance showed significantly greater extension ($p < 0.001$) in the presence of phosphate and no significant effect ($p = 0.06$) of ammonium on linear extension ($n = 45$ branches per treatment, error bars are standard errors).

Changes in the weight of calcium carbonate in coral skeletons were measured by changes in the buoyant weight of colonies. Skeletal material represents the majority of any coral's buoyant weight and consequently changes in buoyant weight are primarily due to skeletal growth (Bak, 1973, 1976; Jokiel *et al.*, 1978). This non-destructive method has been used to measure small changes in growth rate in corals during exposure to 'adverse' conditions (Davies, 1989, 1990, 1995). The effect of nutrient addition on calcification, as measured by buoyant weight increments, was both species and nutrient specific. Rates of change in buoyant weight decreased in the presence of N and/or P in small (5–10 cm diameter) colonies of *P. damicornis* but not in *A. longicyathus* after nine months of the high-loading phase (Hoegh-Guldberg unpub. data). Ammonium enrichment also led to a decrease in the rate of calcification of *A. palifera* (Steven and Broadbent, 1997; Fig. 12) but had no effect on *A. aspera* (Bucher and Harrison unpub. data) or *S. pistillata* (Takabayashi, 1996). The effect of ammonium on larger (> 20 cm in diameter) colonies of *A. longicyathus* was dependent on season, with increased calcification in winter and spring but decreased rates in summer (Bucher and Harrison, unpub. data). Integrated over a full year, an overall increase in calcification in this species was found in these larger colonies. This was not the case in the smaller colonies (Hoegh-Guldberg unpub. data). When combined with a reduced rate of linear extension in the larger colonies (Fig. 11), this not only produced higher bulk density than untreated *A. longicyathus* but may also explain the reduced ability of *A. longicyathus* in ammonium treatments to overgrow lesions (Table 10).

The calcification rate of both *A. longicyathus* and *A. palifera* increased in +P treatments (Bucher and Harrison unpub. data; Steven unpub. data). In contrast, +P treatments had no effect on *A. aspera* (Bucher and Harrison, unpub. data) and tended to decrease calcification in *S. pistillata* (Takabayashi, 1996; Fig. 13) and the pink form of *P. damicornis* (Hoegh-Guldberg unpub. data; Fig. 14). In *A. longicyathus*, the changes in linear extension and calcification led to a significant reduction in skeletal bulk density in phosphate treatments (Bucher and Harrison, unpub. data; Fig. 15). Scanning electron microscopy of ENCORE corals found no disruption of the orderly crystal structure in ENCORE corals (Takabayashi, 1996; Bucher unpub. data) but significant increases in micro-density (Fig. 16) suggest that some changes had occurred at the scale of crystal architecture and/or chemical composition.

TABLE 10

Numbers of unhealed wounds on *Acropora longicyathus* nubbins after six months of the high nutrient loading phase of ENCORE.

	Treatment			
	Control	+N	+P	+N+P
Number of *nubbins* with unhealed wounds (max. 45 per treatment)	5	15	4	5
Number of *colonies* with unhealed wounds (max. 15 per treatment)	2	8	3	3
Number of *reefs* containing unhealed nubbins (max. 3 per treatment)	2	3	2	2

Coral photophysiology. Photosynthetic performance of the nubbins of two species of corals, *P. damicornis* and *S. pistillata*, subjected to the ENCORE treatments showed no significant difference from control corals during the initial, low-loading phase of ENCORE (Hoegh-Guldberg and Moreno, unpub. data). Nutrient effects were observed in corals during the second, high-loading phase. Takabayashi (1996) measured the maximum gross photosynthetic rate (p_c g max), respiratory rate (r_c), maximum net photosynthetic rate (p_c n max),

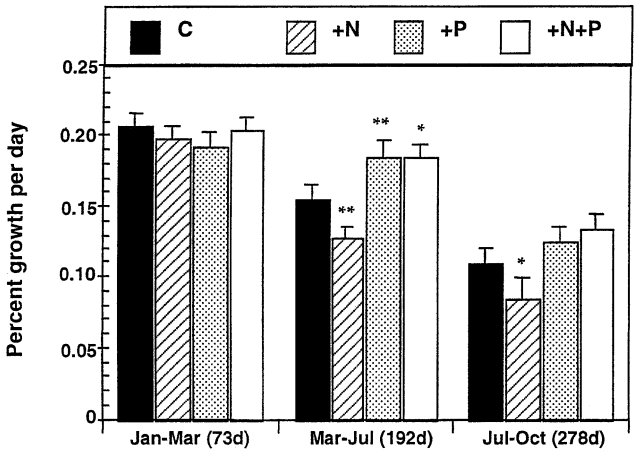

Fig. 12 Adjusted mean percent daily growth (% day^{-1}) of *A. palifera* nubbins grouped by nutrient treatment over three time periods. Errorbars are mean standard errors. Asterisks indicate treatment means (adapted from Steven, 1999); significantly different from controls: * $p < 0.1$, ** $p < 0.05$.

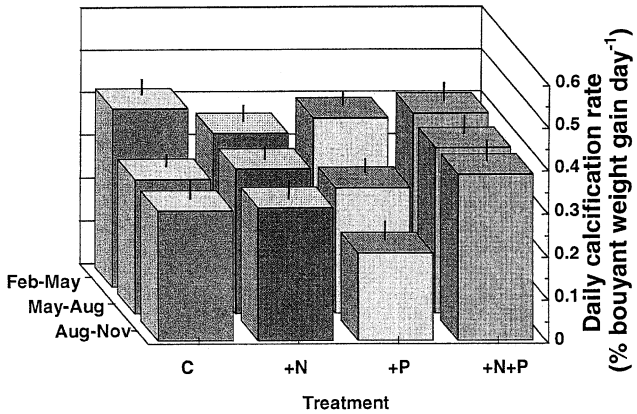

Fig. 13 Daily calcification rate (mean ± SE) of the nubbins of *S. pistillata* during (a) the first three-month period ($n = 15$), (b) the second three-month period ($n = 10$), and (c) the third three-month period ($n = 5$) of the ENCORE nutrient treatment exposure (adapted from Takabayashi, 1996).

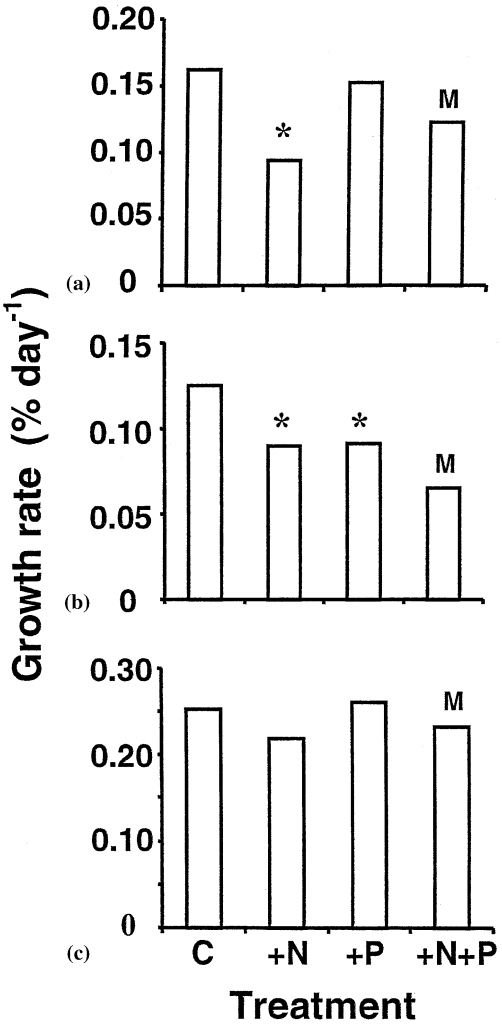

Fig. 14 Growth rates of (a): *P. damicornis* (brown morphotype), (b): *P. damicornis* (pink morphotype) and (c): *A. longicyathus* after nine months in different treatments of the ENCORE project at One Tree Island reef. Shown are means and 95% confidence intervals. Asterisks indicated differences significant at $p = 0.05$. M indicates the fact that the mean is shown for comparison for the N + P treatments but that the loss of nubbins through mortality prevented the data from this treatment being included in the associated ANOVA. The means were calculated from $n = 22$ (*P. damicornis*, brown morphotype), $n = 21$ (*P. damicornis*, pink morphotype) and $n = 21$ (*A. longicyathus*). (Hoegh-Guldberg, unpub. data).

and photosynthetic efficiency (α) in nubbins of *S. pistillata* 3 and 9 months after the start of the high-loading phase. In this study, elevated concentrations of phosphate increased the photosynthetic production and respiration of corals after 3 months of exposure to the high load. The addition of ammonium did not affect these parameters. After 9 months, however, the apparent stimulation of production and consumption by phosphate were replaced by an almost twofold increase in production and consumption per surface area in corals exposed to ammonium (Table 11). This was due to an increase in the number of zooxanthellae (and hence chlorophyll *a*) per surface area, as indicated by a dampening of this difference when rates were standardized to chlorophyll (Table 11).

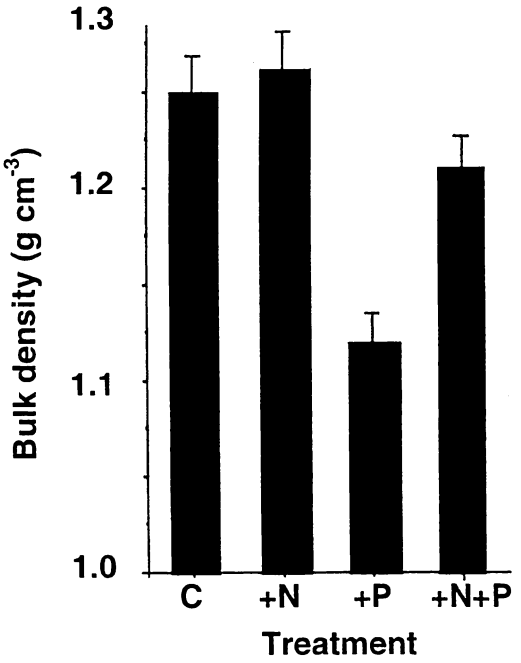

Fig. 15 Mean bulk density of branch tips from *A. longicyathus* grown during the high-loading phase of the ENCORE study. Orthogonal analyses of variance showed significantly lower bulk density (i.e. greater porosity) ($p < 0.001$) in the presence of phosphate and significantly higher bulk density ($p = 0.005$) in the presence of ammonium in *A. longicyathus* branch tips ($n = 45$ branches per treatment, error bars are standard errors).

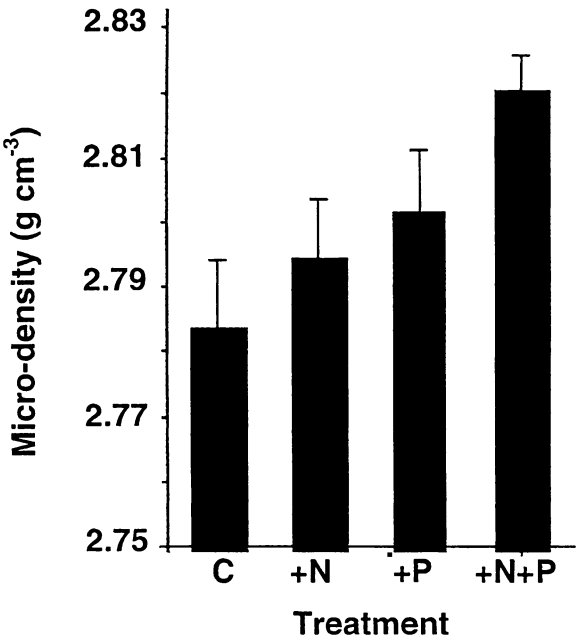

Fig. 16 Mean micro-density of branch tips from *A. longicyathus* grown during the high-loading phase of the ENCORE study. Orthogonal analyses of variance showed significantly greater micro-density ($p < 0.001$) in *A. longicyathus* branch tips in both the ammonium and phosphate treatments ($n = 45$ branches per treatment, error bars are standard errors).

The addition of ammonium also increased the compensation irradiance (I_c) and the intercept irradiance (I_k) after 3 months (Table 12). After 12 months, the

TABLE 11

Maximum gross photosynthetic rate ($p_{c\ g\ max}$), respiratory rate (r_c), maximum net photosynthetic rate ($p_{c\ n\ max}$), and initial slope of $p_{c\ g\ max}$ (α) measured after nine-month incubation in ENCORE treatments during the high-loading phase of the study.[a]

Treatment	$p_{c\ g\ max}$ (μmol O_2 h^{-1} x)	r_c (μmol O_2 h^{-1} x)	$p_{c\ n\ max}$ (μmol O_2 h^{-1} x)	$\alpha(10^{-2}$ μmol O_2 m^2 s μE^{-1} h^{-1} x)
Area				
C	0.95 ± 0.19	0.24 ± 0.05	0.71 ± 0.14	1.26 ± 0.39
+N	1.80 ± 0.92	0.58 ± 0.24	1.23 ± 0.69	3.78 ± 1.44
+P	0.77 ± 0.17	0.24 ± 0.07	0.53 ± 0.11	3.08 ± 1.20
+N+P	0.82 ± 0.24	0.22 ± 0.06	0.60 ± 0.18	0.64 ± 0.13
Chl a				
C	2.37 ± 0.73	0.59 ± 0.02	1.78 ± 0.57	2.84 ± 0.77
+N	2.28 ± 0.99	0.74 ± 0.03	1.54 ± 0.76	7.50 ± 0.41
+P	1.60 ± 0.27	0.47 ± 0.09	1.13 ± 0.20	5.95 ± 0.20
+N+P	1.75 ± 0.78	0.46 ± 0.02	1.29 ± 0.57	1.06 ± 0.25

[a] The figures are expressed as means ± S.E. and are adapted from Takabayashi (1996). Each treatment is calculated per area (cm^{-2}) and also standardized to chlorophyll *a*; thus 'x' in the units is for 'cm^{-2}' in the 'area' table and 'chl a^{-1}' in the 'chl *a*' table.

TABLE 12

The irradiance at which the initial slope of the gross photosynthesis intercepts the horizontal asymptote (I_k), the compensation irradiance (I_c), and the ratio between $p_{c\ g\ max}$ and r_c measured in coral subcolonies after three-month incubation in the ENCORE treatments. The figures are expressed as mean ± S.E. ($n = 2$).

Treatment	I_c (μE m^{-2} s^{-1})	I_k (μE m^{-2} s^{-1})	$p_{c\ g\ max}/r_c$
C	58.9 ± 7.71	141.7 ± 16.0	3.55 ± 0.146
+N	73.0 ± 10.6	206.6 ± 61.7	3.58 ± 0.600
+P	48.1 ± 3.06	121.8 ± 14.0	3.55 ± 0.300
+N+P	71.5 ± 5.95	221.2 ± 25.4	4.07 ± 0.225

stimulation by ammonium had disappeared and phosphate caused a dramatic decrease in I_c and I_k but this did not change the ratio of gross photosynthesis to respiration (Fig. 17). Nutrient treatment significantly ($p > 0.05$) affected I_c and the initial slope (α) only. The Student–Newman–Keuls test revealed that I_c from the +N+P treatment was significantly greater ($p < 0.05$) than from the +P treatment. Also, the α in the +P treatment was significantly greater ($p < 0.05$) than that in the +N+P treatment.

Coral reproduction. Many aspects of sexual reproduction in acroporid species of corals were affected by nutrients; most were inhibited but some were enhanced. Effects were dependent on time, species and the nutrient in question.

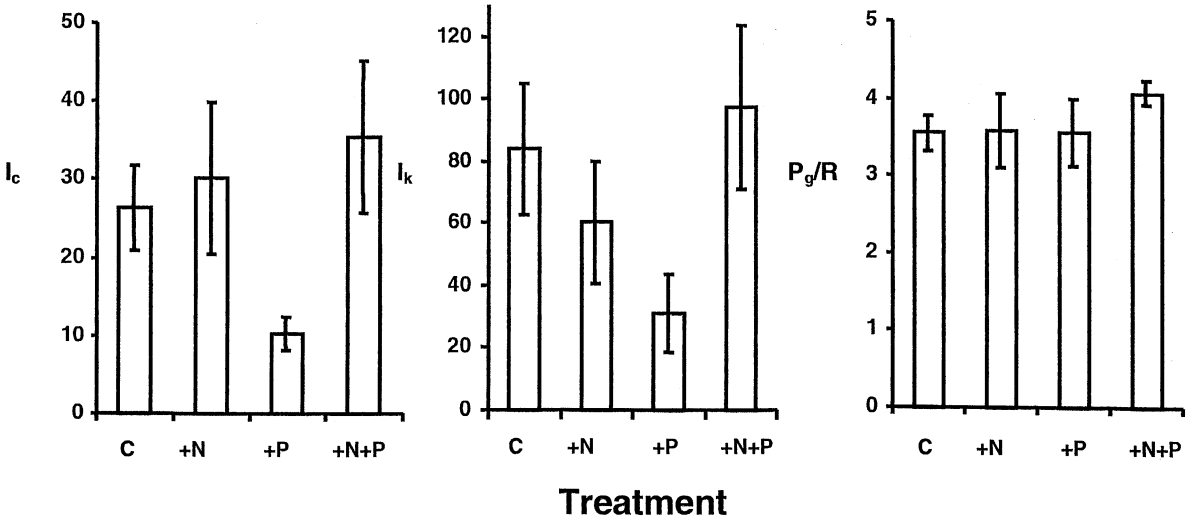

Fig. 17 The irradiance at which the initial slope of the gross photosynthesis intercepts the horizontal asymptote (I_k), the compensation irradiance (I_c), and the ratio between $p_c g$ max and r_c measured in the coral sub-colonies after the nine-month incubation in the second (high-loading) regime of the ENCORE study. The figures show mean ± SE ($n = 2$) and are adapted from Takabayashi (1996).

Ammonium

During the sampling period from 1993 to 1995 corals exposed to elevated nitrogen produced significantly smaller and fewer eggs and contained significantly less testes material than those not exposed to nitrogen (Ward and Harrison, unpub. data). Fertilization rates of *A. longicyathus* eggs were significantly reduced by low concentrations of nitrogen (down to 1M ammonium). Fertilized eggs showed a significant increase in the number of irregular embryos and of embryos that stopped development at the first cleavage stage (Harrison and Ward, unpub. data). Gametes exposed to +N+P in the laboratory had very low fertilization rates (Fig. 18). In similar trials using gametes of the brain coral *G. aspera*, the percentage fertilization was significantly reduced only following exposure to 50 μM + N + P, but there were significantly more deformed embryos developed following exposure of gametes to +N and +N+P treatments (Harrison and Ward, unpub. data).

In settlement trials using larvae of *A. longicyathus* in 1993, settlement rates were reduced by nitrogen treatments with very low settlement in the nitrogen plus phosphorus treatment (Ward and Harrison, 1997). Settlement tiles were mapped and rescored every three months until 1996 to monitor settlement, mortality and spat growth and on these tiles nitrogen reduced settlement of spat of both spawning and brooding species of corals (Ward and Harrison, unpub. data; Fig. 19).

Phosphate. Exposure to phosphorus enrichment affected a variety of reproductive activities in the coral species examined. Contrary to the pattern found in controls and other treatments, corals exposed to phosphorus alone did not have a reduction in the number of eggs per polyp as the gametogenic cycle progressed. The egg numbers prior to spawning were significantly higher than those of corals from controls and other treatments (Ward and Harrison, 2000). Egg size was reduced by phosphorus treatments and these patterns were consistent for both

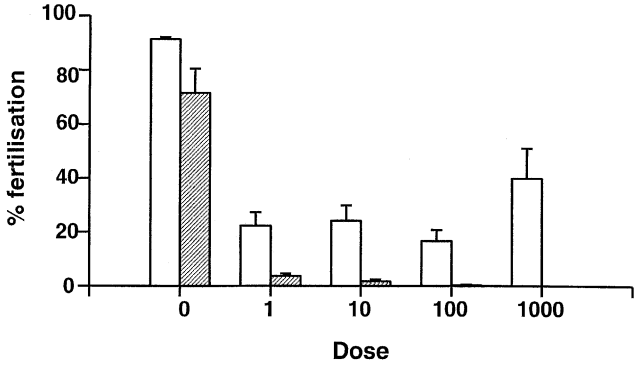

Fig. 18 The percentage fertilization recorded in fertilization trials using eggs and sperm from *A. longicyathus*, which had been exposed to added N and P in the laboratory. Doses were 0, 1, 10, 100 and 1000 M of N and P above background levels. The blank columns represent cross 1 and the shaded columns cross 2. Error bars are standard errors.

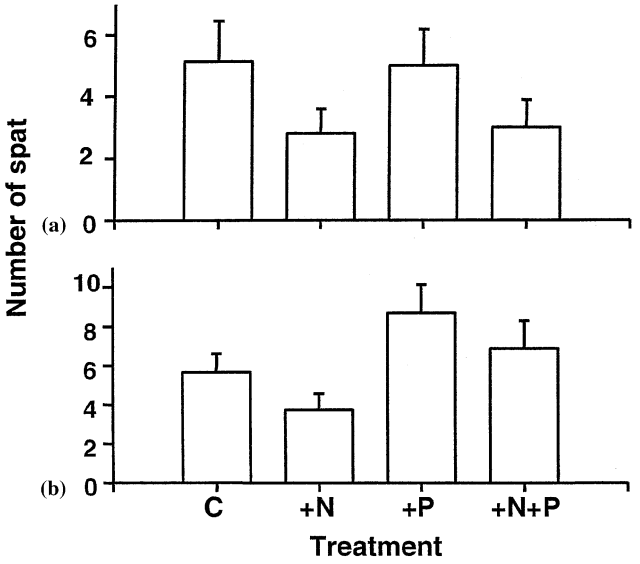

Fig. 19 The number of spat of spawning (a) and brooding coral species, which settled on pairs of terracotta tiles in different nutrient treatments between November 1994 and January 1996. These data do not take account of which spat survived; they are of settlement only. Error bars are standard errors.

A. longicyathus and *A. aspera*. Just prior to spawning, eggs from all colonies exposed to phosphorus alone were very bright red in contrast to eggs in corals from other treatments, which ranged from cream to red with no consistent pattern (Ward, 1997). Phosphorus dramatically reduced fertilization rates of *A. longicyathus* and significantly increased the incidence of irregular embryos and embryos that stopped developing at the first cleavage stage (Harrison and Ward, unpub. data). In fertilization trials with gametes of *G. aspera*, there was a significant increase in the percentage of irregular embryos formed after they were exposed to slightly elevated levels of phosphorus (> 0.5–1 μM) (Harrison and Ward, unpub. data). During the 1993 settlement trials, phosphorus significantly reduced settlement rates and this pattern continued when the tiles were rescored during 1994. When tiles were rescored from November 1994 to January 1996, the phosphorus treatments enhanced the settlement of spat of brooding coral species, but did not affect the settlement of spat of broadcast spawning coral species (Ward and Harrison, unpub. data).

Lipid levels in corals. Lipid levels were monitored in the corals studied for reproduction (*A. longicyathus* and *A. aspera*; see section above). Exposure to elevated nitrogen reduced the amount of lipid in the tissues of the corals, while exposure to phosphorus increased the amount of lipid present at various times during the ENCORE experiment. Reproductive material of corals is rich in lipids and our results followed the general patterns found for the measures of fecundity in these species under similar treatments. Samples were also taken in February 1995 before the gametogenic cycles

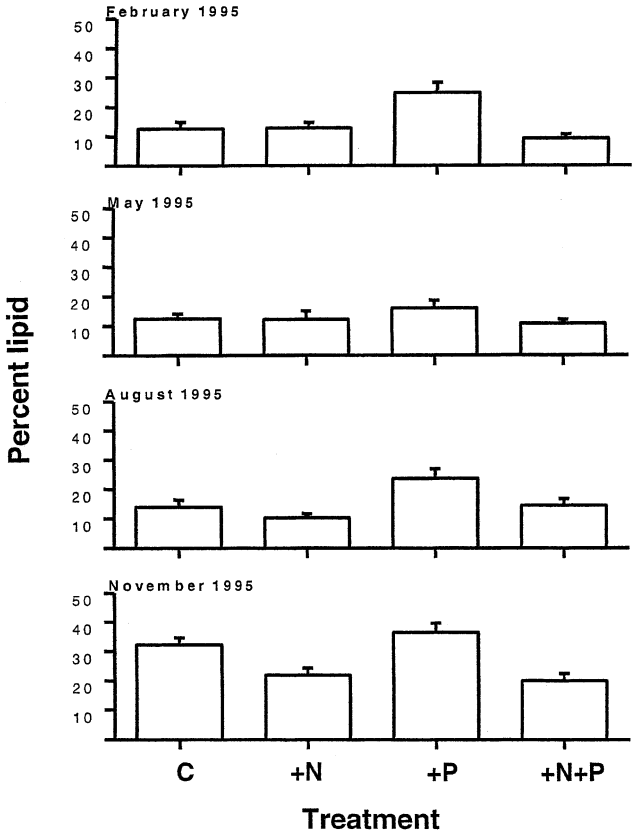

Fig. 20 The percentage of lipid in the tissue of *A. longicyathus* transplanted into experimental ENCORE patch reefs at One Tree Island reef in samples taken in February, May, August and November 1995. Error bars are standard errors.

commenced and the same patterns were observed (Ward, 1997). These results show that even very slight increases in levels of nitrogen and phosphorus can have large effects on lipid levels in coral tissues (Fig. 20).

Soft corals

Major physiological/biochemical indicators were measured in *Sarcophyton ehrenbergi*, a soft coral common on the Great Barrier Reef. In addition, a number of effects not directly related to nutrient enrichment were investigated, e.g. effects of transplantation and competition with a hard coral species, *P. damicornis*. These studies are reported elsewhere (Tentori *et al.*, 1997).

None of the nutrient treatments showed any effects on: (1) concentrations of sarcophytoxide (a terpene active in defence and competition), (2) levels of fatty esters (the primary lipid energy storage and membrane component of these corals), and (3) the ratio of terpene to lipid (an indicator of physiological change used to indicate stress in soft corals) in *S. ehrenbergi*. This study has shown that soft corals are not sensitive indicators of nutrient-induced stress in coral reefs (Fleury *et al.*, 2000). *Sarcophyton* species are common on inshore reefs, and so it is not surprising that they are able to accommodate a wide range of nutrient conditions without adverse effects.

Giant clams

One Tree Island is outside the geographic limit of all clam species with the exception of *T. maxima*. This species is present in significant numbers in the lagoon, in the patch reefs and on the reef front. For the ENCORE studies clams in two size classes (65–100 and 200–220 mm) from the One Tree Island reef crest were randomly transplanted into the experimental patch reefs (Ambariyanto and Hoegh-Guldberg, 1997; Belda-Baillie *et al.*, 1998) and then used for a range of biochemical, physiological and ecological measurements.

Clam growth. The dependence of tridacnids on the photosynthetic capacity of their symbiotic zooxanthellae population for much of their energy requirements could be expected to influence the biomass parameters of the whole animal. The nutrients ammonium and phosphate are essential to growth in photosynthetic autotrophs and an increase in availability in what is generally regarded as a low nutrient environment might be expected to cause an increase in biomass of the clam. Simple biomass parameters were measured to determine if any gross changes occurred during the course of the enrichment. A number of growth parameters were measured in the course of the first and second enrichment phases.

Growth of clams measured as changes in shell length and buoyant weight was relatively linear over the period of this study. The percentage daily change in length and buoyant weight of the clams was influenced by season (Ambariyanto and Hoegh-Guldberg, 1997). The highest growth and calcification rates were found during summer and autumn months. These rates were almost double those measured during the winter and spring months (Ambariyanto, 1996; Ambariyanto and Hoegh-Guldberg, 1997; Ambariyanto and Hoegh-Guldberg, 1999a).

There was no effect of nutrient enrichment on the growth (% change in shell length per day, Ambariyanto and Hoegh-Guldberg, 1997) of clams during the initial, low-loading phase of the experiment. In the second phase, however, differences were found after 12 months of nutrient enrichment. +N and +N+P enriched clams exhibited significantly greater growth in shell length than the control and +P treatments (Ambariyanto and Hoegh-Guldberg, 1997). With the exception of the three-month measurement in the first phase there was no significant difference in the % change of the clams' buoyant weight per day. In addition, neither the tissue wet weight, the protein content per gram of clam mantle nor the C:N ratio of the mantle tissue was significantly affected by nutrient enrichment in any of the nutrient treatments. However, changes in the N:P ratio were observed in the larger clams. Addition of P or N, but not N+P, caused corresponding changes in the N:P ratio (Ambariyanto and Hoegh-Guldberg, 1999b).

General host metabolism

Biomass changes. There was no effect of nutrient enrichment on the wet tissue weight of *T. maxima* (g cm^{-1} shell length) during either the first or second phase of nutrient enrichment ($p > 0.05$). The C:N ratio of the mantle of clams from the different treatments ranged from 4.11 to 4.83, and was also not influenced by nutrient enrichment ($p > 0.05$). The protein content per gram mantle of control clams did not change during the first six months of the first phase of the experiment ($p > 0.05$). Significant differences were found in the protein content per gram mantle after 13 months of nutrient enrichment ($p < 0.036$). The mean values revealed a trend whereby the protein content per gram mantle with +N-treated clams had the highest value, followed by +N+P-treated, then +P-treated clams, and finally by clams from control patch reefs. There were no differences in the protein content per gram mantle of the clams during the second phase of nutrient enrichment ($p > 0.05$).

The total number of zooxanthellae per clam (cells $clam^{-1}$) was significantly higher in all nutrient treatments than in controls six months after the beginning of nutrient enrichment during the first phase ($p = 0.044$) and after 13 months ($p = 0.037$).

Haemolymph. The haemolymph is the main conduit for both the supply of nutrients to the animal and zooxanthellae, and also the transfer of photosynthate from the zooxanthellae to the host. Therefore, assuming nutrients are absorbed and they have impact on the symbiosis, haemolymph composition has the potential to be used as a monitor for nutrient perturbations in the water surrounding the clam. The inorganic constituents of the haemolymph approximate those found in seawater and appear to be in equilibrium with that medium (Rees *et al*., 1993). Therefore changes in seawater nitrogen and phosphorus or any response by the clam and its zooxanthellae to that change may be reflected in haemolymph composition.

Monitoring of phosphate, total phosphorus and ammonium levels in haemolymph showed no significant difference after both one and 3 months in the initial, low-loading phase of ENCORE. Ammonium levels were surprisingly high (>30 μM) while phosphate was very low (< 0.1 μM). These results are significantly different from those previously obtained with *T. gigas* in experiments at Orpheus Island (Fitt *et al*., 1995). In contrast to *T. gigas*, *T. maxima* has unexpectedly high levels of ammonium in its haemolymph. Grice (1999) has since confirmed these high levels in *T. maxima*.

With the exception of glycine concentrations increasing with N-enrichment, the free amino acid pool in haemolymph did not vary significantly with nutrient treatment. In *T. gigas* the glutamine:glutamate ratio is dramatically affected by the availability of N (Shepherd *et al*., 1999). However, this ratio was not influenced in *T. maxima*. This, combined with the high ambient concentration of ammonium in the haemolymph of *T. maxima*, indicates there are significant qualitative and quantitative differences between the two clams.

Zooxanthellae

The effect of nutrient enrichment on the mutualistic zooxanthellae population from both corals and clams is a fundamental parameter in determining the impact of nutrient loading on coral reefs.

Population density and mitotic index. A variety of zooxanthellar responses were seen in the ENCORE project. Again, these differences depended on the coral species, colony size and on the nutrient loading. No differences were reported for zooxanthellae from *P. damicornis* (Hoegh-Guldberg and Moreno unpub. data) during the initial, low-loading phase of ENCORE. Similarly, Takabayashi (1996) did not detect differences among nutrient treatments for the population density of zooxanthellae in small colonies of *S. pistillata* during the second high-loading phase. However, in a set of larger coral colonies (approximately 10 × larger) used in a study of the photophysiological responses of *S. pistillata* to nutrient increases, significant differences between treatments were seen (Fig. 21). In this case, the population density of zooxanthellae resident in the coral subcolonies was significantly greater in the +P and the +N treatments compared with corals from control patch reefs ($F3,8 = 7.13$, $p < 0.01$; Fig. 21). In the high-loading phase zooxanthellae densities of large colonies of *A. longicyathus* were significantly higher in +P treatments. In *A. aspera* the highest densities were in the +N+P but +P was also elevated with respect to controls.

The cell density of zooxanthellae in clams in nutrient treatment versus control patch reefs showed trends after three and six months, but these did not become statistically significant until 13 months when significantly

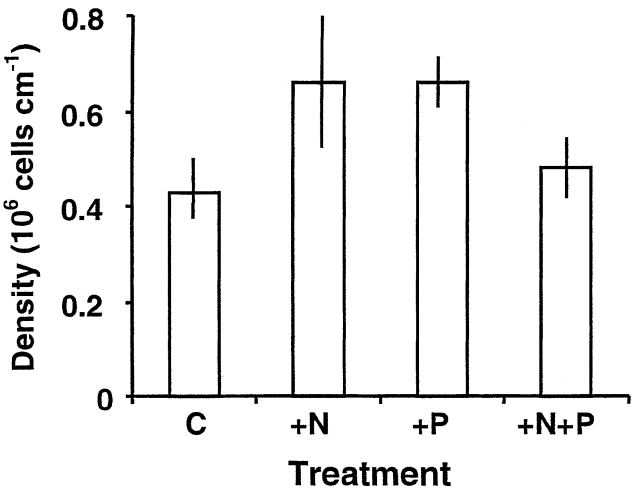

Fig. 21 Population density of zooxanthellae in *S. pistillata* after exposure to +N, +P and +N + P during the second phase of the ENCORE experiment.

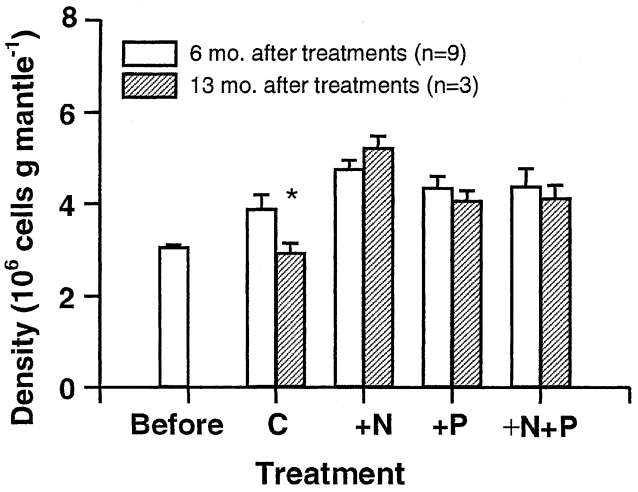

Fig. 22 Population density of zooxanthellae in the clam *T. maxima* as a function of ENCORE nutrient treatments during the first low dose phase.

higher numbers of zooxanthellae were recorded in clams from all three nutrient treatments (Fig. 22). During the second, high-loading phase the increase in cell density was statistically significant after five months in all nutrient treatments.

Changes in density of zooxanthellae have been observed previously in both corals and tridacnids as a result of nutrient enrichment (Hoegh-Guldberg and Smith, 1989; Belda *et al.*, 1993). This can be attributed either to increases in the mitotic index or the fact that the animal can retain and support larger numbers of zooxanthellae.

Experiments to examine the mitotic index (MI) of zooxanthellae of clams showed that maximum division occurred at 03:00 h and minimum at 15:00 h. However it was not until the 13th month that the MI increased statistically over the control. This was in the low-loading phase. Little change was observed in the second, high-loading phase.

Marked decreases in the cell diameter of zooxanthellae in giant clams (*T. maxima*) were seen in nutrient treatments, particularly the +N treatment compared with the control. This may be indicative of a higher division rate and the consequent increase in zooxanthellae density observed in these tridacnids.

Chlorophyll content. A major determinant of productivity within symbiotic organisms like corals and clams is the concentration of the primary photosynthetic pigment chlorophyll. Chlorophyll has been found to be highly responsive to changes in the concentration of nutrients like ammonium (e.g. Hoegh-Guldberg and Smith, 1989). Few significant changes in chlorophyll content of zooxanthellae in any organisms were detected during the initial, low-loading phase (Hoegh-Guldberg unpub. data). The exception was in giant clams. Here the total chlorophyll *a* content of the clams from all nutrient treatments was significantly higher than that of control clams after 6 and 13 months of nutrient enrichment during the low-loading phase ($p < 0.001$ and 0.048 in February, 1994 and November, 1994, respectively) (Ambariyanto and Hoegh-Guldberg, 1996).

During the second phase of the project, however, a number of research teams found that chlorophyll levels did respond to nutrient enrichment. The areal concentrations of chlorophyll *a* in *S. pistillata* were higher in the +N and +N+P treatments than in controls (Fig. 23), (Takabayashi, 1996). The concentrations of chlorophyll *a* per zooxanthella in the +N and +N+P treatments, however, were not significantly different from those of the control (Fig. 24). The increase in the areal concentration of chlorophyll was thus due to an increased population density of zooxanthellae, especially in the +N treatment. These results are consistent with other studies within the project and the scientific literature (Hoegh-Guldberg and Smith, 1989; Muller-Parker *et al.*, 1994; Stambler *et al.*, 1991, 1994). Ambariyanto and Hoegh-Guldberg (1996) also found significantly higher total chlorophyll *a* content in ammonium-treated clams 5 months after the beginning of the high-loading phase of nutrient enrichment ($p < 0.015$). Just as was found in the coral studies, the chlorophyll *a* content per zooxanthella was not affected by nutrient enrichment during the first or second phase of nutrient enrichment.

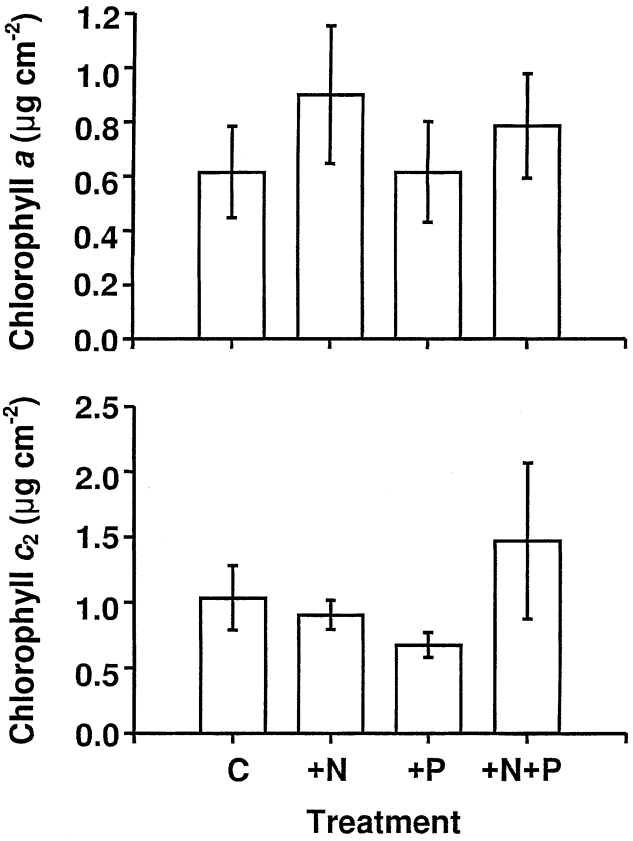

Fig. 23 Concentration of chlorophyll as a function of surface area in the coral *S. pistillata* exposed to the second high dose ENCORE nutrient treatments.

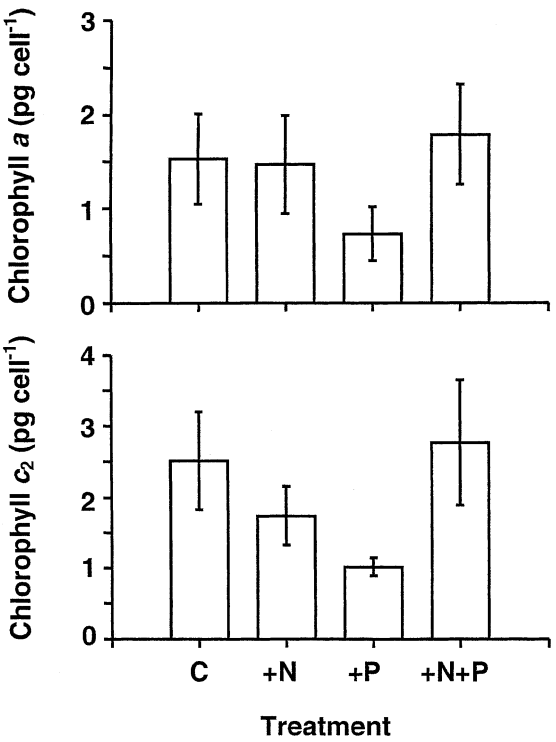

Fig. 24 Concentration of chlorophyll per zooxanthella in the coral *S. pistillata* exposed to the second high dose ENCORE nutrient treatments.

Increases in chlorophyll and concentration per unit area in +N and +N+P treatments were related to the increase in number of zooxanthellae (and hence biomass) rather than increases in chlorophyll per cell. This is consistent with the conclusions of previous laboratory or raceway studies (Hoegh-Guldberg and Smith, 1989; Dubinsky *et al.*, 1990; Stambler *et al.*, 1991; Ambariyanto, 1996).

Ammonium uptake by zooxanthellae. After one month of nutrient enrichment there was no significant change in the capacity for ammonium uptake by zooxanthellae in large giant clams (*T. gigas*) although a trend was evident. After three months exposure to nutrient additions, zooxanthellae from +N-treated clams had a significantly lower ammonium uptake capacity (down-regulated), while zooxanthellae from +P-treated clams had a significantly greater ammonium uptake capacity (up-regulated) than control clams. However, zooxanthellae from +N+P treated clams had N-uptake rates similar to control clams. In the +N+P treatment it is likely that there was an interaction between the two nutrients cancelling out the effect of each in isolation. Results obtained with zooxanthellae freshly isolated from the coral *P. damicornis* showed a similar trend after three months (Fig. 25).

Zooxanthellae ultrastructure. Using electron microscopy, Ambariyanto and Hoegh-Guldberg (1996) demonstrated that in the +N and +N+P treatments there

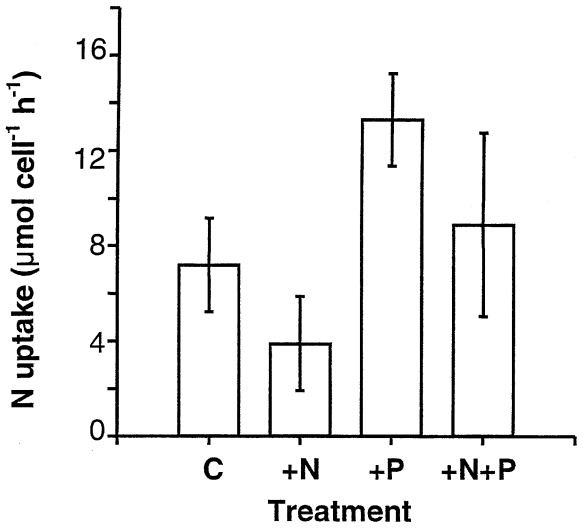

Fig. 25 Effect of nutrient enhancement on the ammonium uptake capacity of freshly isolated zooxanthellae from the coral *P. damicornis*.

was a decrease in cell size and in the amount of starch in the sheath surrounding the pyrenoid of the zooxanthellae chloroplast in giant clams. This was not evident in either the control or phosphate treatment. These results are consistent with the fact that zooxanthellae are nitrogen limited. The zooxanthellae under enriched ammonium conditions are capable of mobilizing starch reserves for the synthesis of amino acids. In a similar study of zooxanthellae from the branching coral *S. pistillata* the zooxanthellae were sectioned and examined using a similar set of methods (Takabayashi, 1996). In contrast to changes observed in clams, zooxanthellae in *S. pistillata* were the same size in all treatments and had similar amounts of starch surrounding their pyrenoid stalks suggesting that, perhaps, the zooxanthellae in *S. pistillata* were not nitrogen limited.

DMSP in zooxanthellae

Studies in the Great Barrier Reef have shown that coral zooxanthellae contain abundant amounts of dimethylsulphoniopropionate (DMSP) (Jones *et al.*, 1994; Broadbent *et al.* unpub. data). The exact role and function of DMSP in algae and coral zooxanthellae is not known, although in algae it has been suggested that DMSP acts as an osmolyte (Kirst, 1989). Recent work suggests that the concentration of DMSP in certain species of algae may be an adaptation to a low nutrient environment (Liss and Galloway, 1993). Nitrogen has been suggested as the most energy efficient preference for the synthesis of osmolytes. During nitrogen limitation, it has been suggested that sulphur can replace some nitrogen-containing osmolytes of similar structure (e.g. glycine betaine), so that nitrogen can be utilized for the more important process of amino acid and protein synthesis. During ENCORE the effect of nutrient enrichment by +N and +P on the synthesis of DMSP was examined in the coral *A. palifera*.

After 65 days DMSP (nmol/polyp) decreased in colonies enriched with +N and +P compared with controls. After 273 days, however, this trend had been reversed, with a significant increase in DMSP in +N+P, and +P enriched colonies, indicating an effect on DMSP from P enrichment. At the cellular level, however, nutrient enrichment showed no clear trend in zooxanthellar DMSP (fmol/cell).

Bioerosion

Bioerosion consists of internal boring by macro- and micro-organisms, and external erosion by grazing organisms such as scarids and molluscs. In many reef situations, dead coral substrata are not only subjected to bioerosion but are added to by calcareous algae and various encrusting fauna including serpulid worms, bryozoans and bivalve molluscs. Thus substrata may experience net gains or net losses of calcium carbonate. Kiene and Hutchings (1994) have discussed the relationships between these two processes.

Gektidis (1997) identified six species of cyanobacteria/cyanophyta, of which three are possibly new species, three genera of green algae each represented by a single species and a rhizoid of a green alga, in his samples of *Tridacna*, calcite and limestone from One Tree Island patch reefs. Differences in the species composition and abundance of these communities could not be related to the various nutrient treatments. While no treatment effect was observed, differences in the structure of these communities varied over time, and hence did rates of bioerosion. Vogel *et al*. (1996) suggest that the position of the patch reefs within the One Tree lagoon is a major factor controlling these communities. Kiene (1994) recorded the highest and lowest average rates of microboring (7.56 and 43.44 g m^{-2} yr^{-1}) on patch reefs with both N and P added. There was a trend for average microboring rates to increase from east to west through the lagoon. In contrast, Kiene (1994) found no differences related to position of the patch reefs in the lagoon with regard to grazing or macroborers after 26 months of exposure and no effects of nutrients. However, he stressed that perhaps a 2-year study period was insufficient to investigate the effects of nutrient addition on macroborers. Complementary studies were done by Hutchings (unpub. data) to investigate rates and agents of macroborers, losses of calcium carbonate due to grazers and gains by accretion, in the control sites, as well as the +N+P sites. To date only substrata exposed for 2 years have been analysed and substrata exposed for a further 2 years are currently being analysed. After 2 years, no significant differences were found between control and nutrient-enriched patch reefs, with all sites experiencing net losses (ie grazing and boring exceeded gains by accretion) of substrata of between 1.57 ± 0.24 and 2.83 ± 1.06 kg $CaCO_3$ m^{-2} y^{-1}. This is in contrast to Kiene (1994) who found that the dry weight of the samples increased at most sites, indicating that net accretion was generally higher than net erosion. The apparent difference in results is probably due to the methods used. Kiene (1994) used dry weight of blocks before and after exposure, whereas Hutchings (unpub. data) measured loss and gains of calcium carbonate from digitized sections of the blocks (see Pari *et al*., 1998). In summary all studies of bioerosion and accretion showed that the addition of nutrients had no significant effects on rates, at least over a 2-year period. No attempt was made to distinguish between the low and high-loading phases. Throughout the 2 years, the infauna boring organisms were characterized by relatively few species, with vermetid molluscs and the polychaete *Dodecaceria* dominant, with increasing exposure time, a more diverse boring community has developed (Hutchings unpub. data).

Conclusions

A summary of all results from the ENCORE study is presented in Table 13.

The study demonstrated a number of important effects of inorganic nutrients on coral reef organisms and biochemical and ecological processes. On the other hand it did not reveal some of the effects generally expected from nutrient impacts.

(1) Nutrients caused considerable effects at the level of the organism (e.g. increased mortality, reduced reproduction of corals) but did not cause coral reefs to convert from coral communities to seaweed-dominated reefs as has been recorded elsewhere (Smith *et al*., 1981). We did not observe a stimulation of primary productivity of epilithic algal communities (EAC), and only saw minor increases in larger macroalgae. In the lagoon of One Tree Island, the fastest growing component of the algal community, the EAC, is not nutrient limited.

(2) One of the most important observations of this project was the impact of nutrients on coral reproduction. While growth and mortality increased in some species of corals, other species were unaffected. The production of viable gametes and successful fertilization were reduced by the addition of both inorganic nitrogen and phosphorus. This may be a factor contributing to the observed decline of reef-building corals close to developed sections of coastline. Further investigation of these sub-lethal impacts on coral reef organisms is recommended.

The Direct Effect of Increased Nutrient Availability on Coral Reef Organisms

Increasing concentrations of nutrients had a number of direct effects on the organisms living within the ENCORE patch reefs. While many organisms showed subtle responses at biochemical levels (e.g. increased nitrogen storage in macroalgae, decreased starch storage in zooxanthellae, shifts in the activity of assimilation enzymes within the zooxanthellae of clams), some

TABLE 13

Summary of major responses of organisms studied during the low (September 1993–December 1994) and high (January 1995–February 1996) loading phases.[a]

Parameter	Treatment					
	Low			High		
	+N	+P	+N+P	+N	+P	+N+P
Plants						
Phytoplankton	No data	No data	No data	0	0	0
Zooxanthellae (*clam*)						
• Mitotic index	↑	↑	↑	0	0	0
• Cell diameter (clams)	↓	↓*	↓*	No data	No data	No data
• Ultrastructure (size, pyrenoid starch)	↓	0	↓	No data	No data	No data
• Ammonium uptake	↓	↑	0	No data	No data	No data
• Chlorophyll per cell	0	0	0	0	0	0
Zooxanthellae (*coral*)						
• Mitotic index (*Stylophora pistillata*)	0	0	0	0	0	0
• Cell diameter (*S. pistillata*)	No data	No data	No data	0	0	0
• Ultrastructure (size, pyrenoid starch)	No data	No data	No data	0	0	0
• Chlorophyll per cell	0	0	0	0	0	0
• Ammonium uptake	No data	No data	No data	↓	0	0
• DMSP (per zooxanthella)	0	0	0	No data	No data	No data
Incrusting algae and Rhodoliths						
• Buoyant weight	0	0	0	0	0	0
• Growth rates	0	0	0	0	0	0
• Calcification (alkalinity)	0	0	0	0	0	0
• Carbon production	No data	No data	No data	0	0	0
Epilithic algae community						
• Nitrogen uptake	0	0	No data	No data	No data	No data
• Carbon production	0	0	0	0	0	0
• Growth	0	0	0	0	0	0
• Chlorophyll *a*	0	0	0	0	0	0
• Alkaline phosphatase	0	0	0	0	0	0
Filamentous algae						
• Carbon production	0	0	0	No data	No data	No data
• Alkaline phosphatase (*Laurencia intricata*)	0	0	0	0	0	0
• Amino acid content (citrulline)	↑	0	↑	No data	No data	No data
• Total amino acid content	0	0	0	No data	No data	No data
• Total tissue N	0	0	0	No data	No data	No data
• C:N ratio (*Laurencia intricata*)	0	0	0	No data	No data	No data
• Chlorophyll *a* (*Laurencia intricata*)	0	0	0	No data	No data	No data
• Growth (*Laurencia intricata*)	0	0	0	0	0	0
• Nitrogen fixation (community on *Laurencia intricata*)	No data	No data	No data	↓	↓	↓
Animals						
Reef-building corals						
Symbiont population density						
• *Pocillopora damicornis* (nubbins)	0	0	0	No data	No data	No data
• *Stylophora pistillata* (nubbins)	No data	No data	No data	0	0	0
• *Stylophora pistillata* (large)	No data	No data	No data	0	0	No data
Total chlorophyll content						
• *Stylophora pistillata* (nubbins)	No data	No data	No data	↑	0	↑
Mortality						
• *Pocillopora damicornis* (nubbins)	0	0	0	0	↑	↑
• *Acropora longicyathus* (nubbins)	0	0	0	↑*	↑*	0
• *Acropora longicyathus* (large)	No data	No data	No data	0	0	0
• *Acropora aspera*	No data	No data	No data	0	0	0
Growth						
Linear extension						
• *Acropora palifera*	0	0	0	↓	↑	↑
• *A. longicyathus*	0	0	0	↓	↑	↑
Buoyant weight						
• *Acropora longicyathus* (large)	No data	No data	No data	↑	↑	↑
• *Acropora aspera* (large)	No data	No data	No data	0	↓	0
• *Acropora palifera* (large)	No data	No data	No data	↓	↑	0
• *Acropora longicyathus* (nubbins)	0	0	0	↓*	0	0
• *Pocillopora damicornis* (nubbins)	0	0	0	↓	0	↓
• *Stylophora pistillata* (nubbins)	No data	No data	No data	↓*	↓*	0

TABLE 13 (CONTINUED)

Photophysiology						
After 3 months						
• Gross Photosynthetic rate (per cm^2)	No data	No data	No data	0	↑	0
• Net Photosynthetic rate (per cm^2)	No data	No data	No data	0	↑	0
• Photosynthetic Efficiency	No data	No data	No data	0	0	0
• Respiratory rate (per cm^2)	No data	No data	No data	0	↑	0
After 9 months						
•Gross Photosynthetic rate (per cm^2)	No data	No data	No data	↓	0	0
• Net Photosynthetic rate (per cm^2)	No data	No data	No data	0	0	0
• Photosynthetic Efficiency	No data	No data	No data	↓	0	0
• Respiratory rate (per cm^2)	No data	No data	No data	↓	0	0
Acropora longicyathus (*skeletal*)						
• Bulk density	No data	No data	No data	↑	↓	?
• Microdensity	No data	No data	No data	↑	↑	?
• Mucus cell density	No data	No data	No data	0	↓	?
• Free body wall thickness	No data	No data	No data	0	↑	?
Stylophora pistillata (skeletal particle size)	No data	No data	No data	0	↓	0
S. pistillata (Density of symbionts)	No data	No data	No data	0	0	0
• DMSP (per polyp, *Acropora palifera*)	0	↑	↑	No data	No data	No data
Reproduction/Recruitment						
Egg numbers						
• *Acropora longicyathus*	↓	0	↓	↓	0	↓
• *Acropora aspera*	↓	0	↓	↓	0	↓
Egg size						
•*Acropora longicyathus*	↓	↓	↓	↓	↓	↓
• *Acropora aspera*	↓	↓	↓	↓	↓	↓
Testes total						
• *Acropora longicyathus*	↓	↑	↓	0	↑	↓
• *Acropora aspera*	↓	↑	↓	0	↑	↓
Fertilization rates						
• *Acropora longicyathus*	↓	↓	↓	↓	↓	↓
• *Goniastrea aspera*	0	0	↓	0	0	↓
Occurrence of irregular embryos						
• *Acropora longicyathus*	↑	↑	↑	↑	↑	↑
• *Goniastrea aspera*	↑	↑	↑	↑	↑	↑
Settlement success						
• Spawning species	↓	0	↓	↓	0	↓
• Brooding species	↓	0	↓	↓	↑	↑
Lipids						
• *Acropora longicyathus*	↓	↑	↓	↓	↑	↓
• *Acropora aspera*	↓	↑	↓	↓	↑	↓
• *Acropora bushyensis*	↓	↑	↓	↓	↑	↓
Soft corals						
•C:N:P	No data	No data	No data	0	0	0
• Terpene production	No data	No data	No data	0	0	0
• Lipids (fatty ester)	No data	No data	No data	0	0	0
• Stress (terpenes/lipids)	No data	No data	No data	0	↑	0
Giant clams						
Growth						
• Shell length	0	0	0	↑	↑	0
• Buoyant weight	0	0	0	0	0	0
• Wet tissue weight	0	0	0	0	0	0
• Protein content per gram mantle	↑	↑	↑	0	0	0
• C:N (clam only)	0	0	0	0	0	0
• N:P	No data	No data	No data	↑	↓	0
• Haemolymph (N,P)	0	0	0	No data	No data	No data
• Glycine (free amino acid pool)	↑	↑	↑	No data	No data	No data
• Uptake of dissolved free amino acids	No data	No data	No data	0	No data	No data
• Respiration	0	0	0	0	0	0
• Photosynthesis	0	0	0	0	0	0
Density of symbionts	↑	↑	↑	↑	↑	↑
Total chlorophyll content (per clam)	↑	↑	↑	↑	0	0
Stomatopod recruitment	No data	No data	No data	↓*	0	↓
Fish						
• Grazing rate	0	0	0	0	0	0
• *Pomacentrus wardi* fecundity	0	0	0	0	0	0

TABLE 13 (CONTINUED)

Community responses						
• N-fixation	↓	0	↓	↓	↑	↓
• Denitrification (sediments)	No data	No data	No data	↑	↑	↑
• Bioerosion	0	0	No data	No data	No data	No data

[a] Arrows indicate direction of significant changes. Asterisks indicate strong but not significant responses. No data indicate that measurements were not performed. There may have been minor seasonal differences in cases where measurements were repeated over the year – summary indicates the most common type of response in these cases.

organisms showed quite substantial changes. Of these, reef-building corals showed some of the most dramatic changes. The direct effect of nutrients on reef-building corals ranged from slower growth (in many species) to higher mortality rates (up to threefold higher than those growing in control patch reefs). Significant effects on coral reproductive capacity were also observed. In particular, corals exposed to elevated ammonium produced significantly smaller and fewer eggs and contained significantly less testes material than unexposed corals. Gametes exposed to ammonium with or without phosphate had very low fertilization rates. Phosphorus on its own dramatically reduced fertilization rates and also significantly increased the incidence of irregular embryos. In many cases, development was arrested at the first cleavage stage.

These observations suggest that the changes in the abundance of corals associated with eutrophication of tropical coastlines may also be related to more subtle effects such as those on coral reproduction in addition to direct effects on survivorship. It also suggests that a closer study of the reproductive behaviour of corals and their recruitment in areas affected by increased nutrients levels should be done.

Bioindicators of Nutrient Stress

ENCORE research focused on the biochemical, physiological and ecological changes that occur in coral reefs exposed to increased levels of inorganic nutrients, nitrogen and phosphorus. One of the possible outcomes of this type of work is that it can identify organisms and processes that might be useful as biological indicators of nutrient stress. A number of parameters were initially identified as potential bioindicators but did not reveal consistent responses or had responses that were complicated by species-specific behaviour. Notably, the primary production and phosphatase activity of EAC and macroalgae, buoyant weight (= growth) and skeletal structure of reef-building corals and sediment chemistry (e.g. dissolved free amino acid concentrations) did not show the consistent responses necessary for use as bioindicators. Our results did, however, show promise with a number of parameters that had clear and marked responses. These were:

1. Gametogenesis in reef-building corals (Ward and Harrison, 2000).
2. Ultrastructure of zooxanthellae (Ambariyanto and Hoegh-Guldberg, 1997).
3. C:N ratios in coral and zooxanthellae tissue (Hoegh-Guldberg *et al*., unpub. data).
4. Nitrogen fixation in sediments and algal/cyanobacterial communities and sediment denitrification (O'Neil *et al*., unpub. data).

Clearly further development work will be necessary to develop these into useful tools. Specifically, it will be necessary to investigate how a measured change in a particular indicator can be interpreted in ecosystem terms. ENCORE has put a number of parameters on the agenda, which warrant further investigation. With increasing pressure on the world's coral reefs, and the recognized limitations of the much-used physico-chemical parameters as indicators of ecosystem health, developing relevant biological indicators is of supreme importance.

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