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Rates of nitrogen fixation on coral reefs across the continental shelf of the central Great Barrier Reef*

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Abstract

This study was undertaken in 1981 to determine whether there were major variations in potential rates of nitrogen fixation on apparently bare coralline substrata from reefs across the continental shelf of the central Great Barrier Reef. Nitrogen fixation, measured as rates of ethylene production (nmol cm^{-2} h^{-1}), was significantly lower on substrata from two inner-shelf reefs (0.46 and 1.07) than on two middle-shelf reefs (2.10 and 2.97) and on two outer-shelf reefs (3.20 and 3.81). By contrast, algal biomass (mg cm^{-2}) on experimental substrata was significantly higher on inner-shelf reefs (80.8 and 59.4) than on middle-shelf (27.1 and 23.8) and outer-shelf reefs (26.4 and 22.4). The rate of nitrogen fixation was positively correlated with the proportion of "bare" substratum and significantly higher concentrations of dissolved inorganic nitrogen were found in waters over the reefs than in water flowing onto those reefs. The abundance of algal-grazing fishes was reported previously to be significantly lower on inner-shelf reefs. It is suggested that this cross-shelf variation in the activity of algal-grazing fishes may be a determinant of the observed cross-shelf variations in potential nitrogen fixation.

Introduction

The term "coral reef" encompasses a wide variety of reef structures which have formed in tropical environments, ranging from those regions influenced heavily by neighbouring land masses to clear oceanic waters. This wide range of chemical and physical conditions may be reflected in the biological communities occurring on those reefs. For example, on reefs across the continental shelf of the central region of the Great Barrier Reef, there are considerable variations in the communities of *Halimeda* spp. algae (Drew, 1983), scleractinian corals (Done, 1982), alcyonarian corals (Dinesen, 1983), sponges (Wilkinson, unpublished data), crinoids (R. H. Bradbury, unpublished data) and fishes (Williams, 1982; Williams and Hatcher, 1983; Russ, in press) between reefs which are influenced by terrigenous input and reefs bordering the Coral Sea.

Concentration of nutrients in the waters surrounding reefs is a factor which may significantly influence the biological communities on coral reefs. However, the concentrations of dissolved inorganic nitrogen (DIN: NH_4^+, NO_3^-, NO_2^-) in oceanic waters around the study reefs of the central Great Barrier Reef are frequently too low to detect ($NH_4^+ < 0.2$; $NO_3^- < 0.05$; $NO_2^- < 0.02$ μmol l^{-1}) (Andrews and Gentien, 1982; Bellamy *et al.*, 1982; Andrews, 1983). Such low levels of DIN are insufficient to maintain the high productivity observed on coral reefs or to account for the nitrogen lost from reefs (Webb *et al.*, 1975; Hatcher and Hatcher, 1982). The balance of nitrogen is provided through fixation by blue-green algae growing on apparently bare substrata (Mague and Holm-Hansen, 1975; Webb *et al.*, 1975; Wiebe *et al.*, 1975; Wilkinson and Sammarco, 1983). This fixation increases available nitrogen in the immediate vicinity and also downstream from the areas of fixation (Webb *et al.*, 1975; Hatcher and Hatcher, 1982).

Nitrogen fixation depends on the composition of algal communities on reefs, particularly with respect to the proportion of blue-green algae. Recent studies have demonstrated that the activities of grazing fishes have a major effect on the composition of these communities (Miller, 1982; Sammarco, 1983). At the scale of damselfish territories (1 m^2), it has been shown that algal biomass is inversely proportional to fish grazing activities and that the algal community shifts from dominance by red algae to dominance by blue-green algae in areas where grazing is unrestricted (Sammarco, 1983). This shift is accompanied

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by an increase in rates of nitrogen fixation (Wilkinson and Sammarco, 1983).

At the larger, cross-shelf scale, the abundance of algal grazing fishes is significantly greater on middle- and outer-shelf reefs than on inner-shelf reefs (Williams and Hatcher, 1983; Russ, in press).

This study was undertaken to investigate relative rates of potential nitrogen fixation (acetylene reduction) on natural substrata from inner-, middle- and outer-shelf reefs of the central Great Barrier Reef and to determine whether the correlations between nitrogen fixation, algal biomass and fish grazing observed at the small scale (Wilkinson and Sammarco, 1983) also occur at the larger, cross-shelf, scale.

Material and methods

Study sites and substrata

Six reefs were sampled across the continental shelf of the Great Barrier Reef at approximately 18°30'S over a 4 wk period in 1981. Two reefs were chosen from each *location* on the inner-shelf (Pandora and Phillips), middle-shelf (Rib and John Brewer) and outer-shelf regions (Dip and Myrmidon) (Fig. 1). A general description of these reefs and their environments has been given by Done (1982). Pieces of substrata (rocks) were collected from 2 reef *zones* on each reef: the reef-slope facing the predominant SE trade winds between 5 and 9 m depth (mean sea level); and the reef-flat zone, 20 to 100 m behind the reef crest at a depth of 1 to 3 m. As there was no well defined reef-flat zone on Pandora and Phillips reefs, substrata were collected from the shallow region adjacent to the reef-slope. In each *zone,* rocks were collected from 3 *sites* approximately 100 m apart and each covering an area of approximately 100 m^2. Between 10 and 15 rocks, such as dead coral heads and plates, *Tridacna* spp. shells and large pieces of rubble, were broken off or selected arbitrarily from within each site. Live corals, and rocks from damselfish territories where algal biomass is enhanced relative to surrounding areas (Sammarco, 1983), were not collected.

Volume of the rocks was measured by water displacement, and surface area was estimated with a Summagraphics TD digitizing board interfaced with a PDP-11/70 computer (Sammarco and Carleton, 1982; Sammarco, 1983). Mean rock volume was 130.8 ml (26 to 280 ml) and mean surface area was 119.6 cm^2 (45 to 260 cm^2).

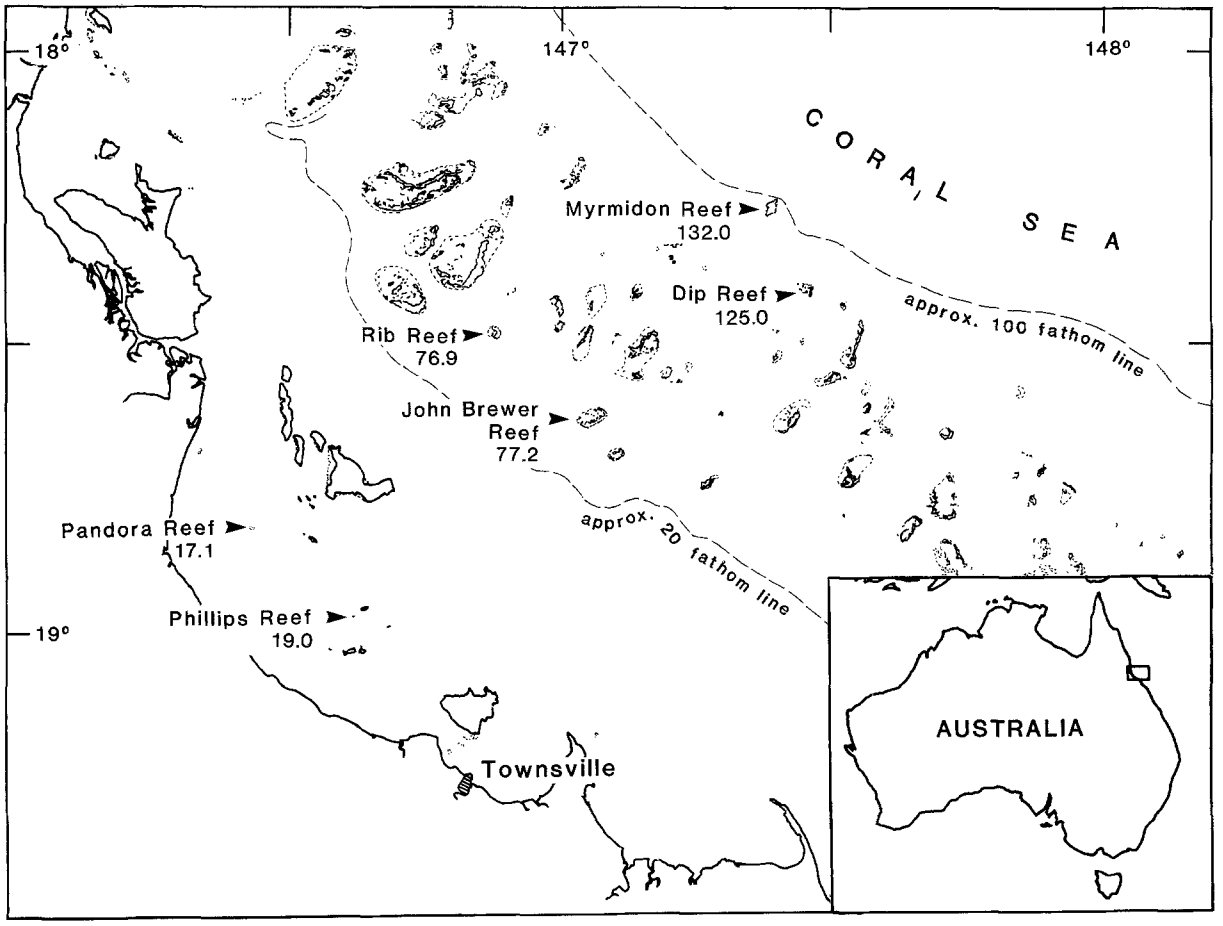

Fig. 1. Central region of the Great Barrier Reef, showing location of the two inner-shelf reefs (Pandora and Phillips), the two middle-shelf reefs (Rib and John Brewer) and the two outer-shelf reefs (Dip and Myrmidon). Distance (km) of each reef from the shore is shown

Nitrogen fixation

The acetylene reduction assay was employed (Stewart *et al.*, 1967). Tests were performed in 18 gas-tight, lyophilization bottles with a mean volume of 1 020 ml (range 520 to 1 420 ml). Five replicate rocks were selected at random from each site collection and preincubated in clean sea water for at least 10 min before being enclosed and evacuated and flushed 3 times with argon. An atmosphere of 20% acetylene (Flett *et al.*, 1976) in argon was introduced, and the rocks were incubated on deck but submerged in flowing water for 4 to 5 h at ambient light within a temperature range of 23° to 26 °C. Two controls were used for each experimental run: duplicate samples were incubated in the dark to estimate non-photosynthetic nitrogen fixation and samples were killed in boiling water to test for phytoplanktonic fixation. Gas samples were collected in 5 ml evacuated tubes at the start of the experiment and at hourly intervals thereafter to control the reaction progress, and were stored under refrigeration. The rocks were frozen at −20 °C after the experiment. Ethylene production was measured in a Tracor 222 gas chromatograph with a Poropak "R" column (100 to 120 mesh) and calibrated against an internal methane standard. Experimental samples were stirred periodically on an electric stirrer and not shaken in the glass vessels during the experimental period; however, the vessels were shaken for approximately 30 s prior to collection of the final gas sample in order to equilibrate ethylene in the gas and liquid phases. Corrections for gas dissolution were made using the formulae of Flett *et al.* (1976).

Algal percent-cover and biomass

An estimate of the percent-cover of algae was determined on thawed rocks using the technique of Sammarco and Carleton (1982) and Sammarco (1983). A 98-square grid was placed over the rocks, and each 1 cm^2 square covered with >50% of a given algal association was recorded as wholly covered. "Bare" substratum, clear of epibenthic algae but still containing cryptic and filamentous algae was also assessed. The presence or absence of blue-green algae on "bare" substratum was confirmed microscopically, but identification to species was not attempted (a species level description has been reported for similar substratum: Sammarco, 1983; Wilkinson and Sammarco, 1983).

Five randomly selected rocks from amongst the 15 of each zone were decalcified in dilute hydrochloric acid to estimate algal biomass. The residue was washed in freshwater to remove salt and $CaCl_2$, oven-dried and weighed after the removal of worms, molluscs etc.

Analysis of nutrients in water samples

Replicate seawater samples were collected in washed, 60 ml, plastic syringes from sheltered positions, approximately 5 cm from the substratum at a depth of 1 to 3 m, from the following zones of each reef: on the fore-reef slope; on the middle of the reef flat; and in open water immediately behind the reef. All samples were filtered immediately through a 20 μm plankton net into darkened reagent bottles and stored at 5 °C. Five of the replicate samples were analysed for NH_4^+ within 2 h of collection by the method of Grasshoff (1976; pp 126–133), using open oceanic or inter-reef seawater, collected at least 100 m upcurrent of the reef as a sample blank. The remainder of the sample was frozen at −20 °C and subsequently analysed for $NO_3^- + NO_2^-$ on a multichannel auto-analyzer (Ryle *et al.*, 1981). The standard deviations for each analytical procedure, determined from the total data set, were: NH_4^+, 0.053 μmol l^{-1}; $NO_3^- + NO_2^-$, 0.022 μmol l^{-1}.

Data analysis

Spatial variation in ethylene production and algal biomass per unit area were analysed using multifactorial analyses of variance (ANOVA) followed by the Student-Newman-Keuls (SNK) procedure for multiple comparisons of the means (Winer, 1971). Homogeneity of variances was determined using Cochran's C-test (Winer, 1971). All analyses were tested for significance at $p < 0.05$. All analyses were orthogonal and balanced by removing replicates at random where appropriate. Sample sizes and details of analysis varied between statistical analyses due to logistic limitations on laboratory analyses. Ethylene production was examined using a 4-factor analysis: *location* (inner-, middle-, outer-shelf) and *zones* (reef-slope and flat) were treated as fixed factors, with reefs (two per location) nested within location and three *sites* nested within each reef, location and zone. Five replicate rocks per site were analysed. Data were transformed by log $(x+1)$ to homogenize variances. Algal biomass per unit area of rock was examined by a 3-factor analysis; location and zones were again treated as fixed factors, with reefs nested within locations. Four replicates (drawn at random from the three sites) were included for the biomass determination. Since raw data values were much less than 1, data were transformed by log $(x10^3)$ rather than log $(x+1)$ to homogenize variances (Underwood, 1981). Unless stated in "Results", interactions in each analysis were not significant.

Results

The potential rates of nitrogen fixation on the experimental rocks measured by the acetylene reduction technique, varied significantly across the central Great Barrier Reef (Fig. 2). Analysis of data from acetylene reduction assays showed:

(a) a significant cross-shelf location effect. Rates of nitrogen fixation were significantly less on substrata from inner-shelf reefs ($p < 0.05$) compared to the other reefs,

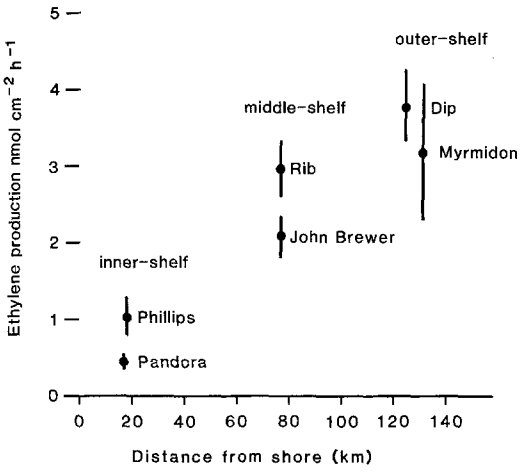

Fig. 2. Nitrogen fixation as ethylene production rates (with standard errors) measured for rocks from reefs across continental shelf of the central Great Barrier Reef. Results for 5 replicate rocks from each of 3 adjacent sites in the fore-reef slope and reef-flat zone are pooled into a single value for each reef. Values for inner-shelf reefs were significantly lower ($p < 0.05$) than values for middle- or outer-shelf reefs

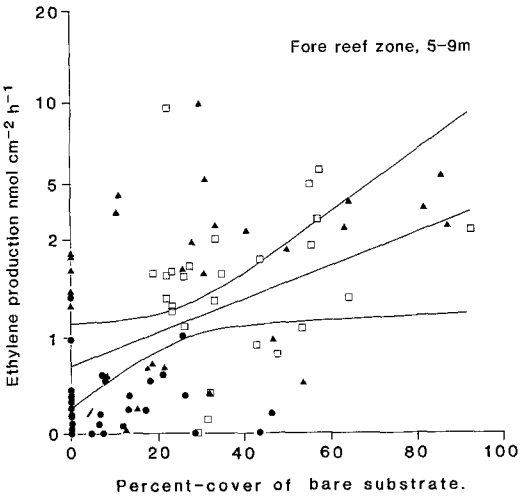

Fig. 4. Correlation analysis of nitrogen fixation as ethylene production with percent cover of cryptic and endolithic algae (measured as "bare" substratum cover) on rocks collected from fore-reef zone (5 to 9 m depth) of 6 reefs across the central Great Barrier Reef. Data pooled for two reefs each from inner- (●), middle-(□) and outer-(▲) shelf rocks. Data also analysed as a Model II regression showing 95% confidence limits for the slope of the line ($p < 0.001$, $r = 0.46$; Sokal and Rohlf, 1981)

but there was no significant difference in fixation rates between middle-shelf and outer-shelf reefs (Fig. 2);

(b) that, although a significant between-reef effect occurred for reefs within the same shelf location (Fig. 2), the means were not sufficiently different for the SNK analysis to distinguish them from one another;

(c) no significant difference in fixation rates between depth zones;

(d) no significant variation in fixation rates on substrata among sites within zones of all reefs.

A regression analysis of nitrogen fixation rates against distance of the reef from the mainland showed a positive association (Kendall's rank correlation, $\tau = 0.331$; $p = < 0.001$; $n = 90$; Winer, 1971), with nitrogen fixation rates increasing with distance from the shore (Fig. 2).

No nitrogen fixation was detected for substratum samples which were either boiled or incubated in the dark. Final estimations showed reduced levels due to dissolution of ethylene present in the initial gas sample (Flett *et al.*, 1976).

Total algal biomass on the natural rock substratum also varied significantly with the location of the reef on the continental shelf (Fig. 3). Rocks from inner-shelf reefs (Pandora and Phillips) had approximately three times as much biomass of decalcified algae as those from reefs of the middle- or outer-shelf locations. These latter two locations showed no significant variations in algal biomass between reefs or zones or sites within a zone. However, there were significant differences between zones on the inner-shelf reefs, with approximately 1.4 times as much algal biomass being found on the shallow reef zones than on deeper reef-slope zones of the same reefs.

Analysis of the algal populations on the experimental rocks showed that percent cover of "bare" substratum (equivalent to cryptic and endolithic algal cover: Sammarco, 1983) was significantly higher on middle- and outer-shelf reefs than on inner-shelf reefs (inner, 13.4%; middle, 31.4%; outer, 28.3%: $p < 0.001$). This increase in "bare" substratum was significantly correlated, although weakly, with overall rates of nitrogen fixation ($p < 0.05$, $r = 0.14$, $n = 177$; pooled over all reefs). The strongest correlation was seen between rates of nitrogen fixation and percent "bare" substratum on fore-reef slope rocks ($p < 0.001$, $r = 0.46$, $n = 90$; Fig. 4), while rocks from the shallower reef-flat zones showed no significant correlation

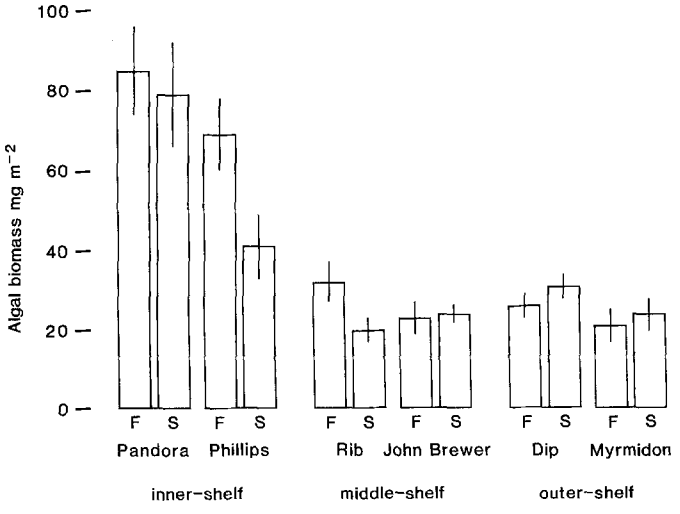

Fig. 3. Algal biomass on and within rocks from reef-flat (F) and fore-reef slope (S) zones of 6 reefs across the central Great Barrier Reef. Biomass is given as weight (with standard errors) of decalcified rocks minus obvious invertebrates. Results for sites within zones are pooled. Biomass on inner-shelf reefs was significantly higher ($p < 0.05$) than biomass on middle- or outer-shelf reefs

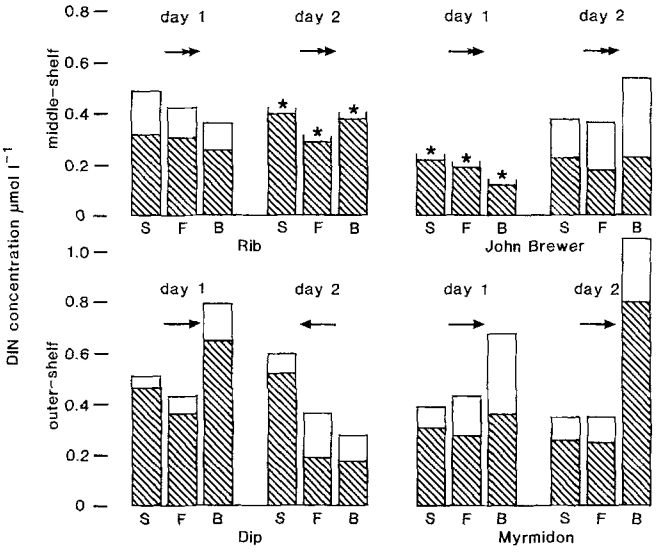

Fig. 5. Concentrations of dissolved inorganic nitrogen (DIN) in seawater samples from middle- and outer-shelf reefs of the central Great Barrier Reef. Data are presented for fore-reef slope (S), reef-flat (F) and back-reef (B) of each reef on each of two separate days. Direction of current flow at the time of sample collection is shown for each day, with a double-headed arrow representing rapid current flow and a single arrow indicating slow current flow. For each sample site, five replicate analyses for NH_4^+, and at least two for $NO_3^- + NO_2^-$, were performed. * Indicates no NH_4^+ data for John Brewer Reef on Day 1 or Rib Reef on Day 2

Table 1. Concentration (μmol l^{-1}) of dissolved inorganic nitrogen, as NH_4^+ and $NO_3^- + NO_2^-$ in seawater samples from reefs across the central Great Barrier Reef. Data were pooled for samples taken over 2d from fore-reef slope, reef-flat and back-reef sites, and show sample means ($\bar{x}$), standard errors (SE) and total number of sites sampled (n), with number of replicate analyses (in parentheses) for each reef

Reef	NH_4^+*			$NO_3^- + NO_2^-$			$NO_3 + NO_2^-$ (upstream oceanic water)
	$\bar{x}$	SE	n	$\bar{x}$	SE	n	
Pandora	0.21	0.07	3 (×5)	0.34	0.03	3 (×2)	< 0.05
Phillips	0.08	0.02	3 (×5)	0.42	0.05	5 (×2)	< 0.05
Rib	0.13	0.02	3 (×5)	0.33	0.02	6 (×2)	(< 0.05)[a]
John Brewer	0.21	0.04	3 (×5)	0.20	0.02	6 (×5)	(< 0.05)[a]
Dip	0.10	0.02	6 (×5)	0.39	0.08	6 (×2)	(< 0.05)[a]
Myrmidon	0.17	0.04	6 (×5)	0.38	0.08	6 (×2)	(< 0.05)[a]

* For NH_4^+ measurements the upstream oceanic water was used as a blank
[a] From Bellamy *et al.* (1982)

between bare substratum and nitrogen fixation. Microscopic examination of bare substratum showed a high proportion of cryptic and filamentous blue-green algae. The inner-shelf reefs are dominated by large stands of *Sargassum* spp. and an extensive cover of filamentous red algae, whereas middle- and outer-shelf reefs have considerable coralline algal cover with shorter, sparser algal turfs including filamentous red algae.

Dissolved inorganic nitrogen in water over the reefs (Table 1) showed significantly increased concentrations compared to surrounding seawater. There was a significant increase in DIN along the direction of water flow over Dip and Myrmidon reefs but not over Rib or John Brewer reefs (Fig. 5). This difference is apparently due to the stronger winds (> 10 m s^{-1}) which prevailed when these samples were taken, resulting in considerable mixing, turbulence and a rapid current flow over Rib and John Brewer Reefs. The shallow zones at Pandora and Phillips Reefs were too small (< 450 m wide) to record significant upstream and downstream differences consistently, particularly as the rates of nitrogen fixation were low.

Discussion

Rates of nitrogen fixation were significantly higher on rocks collected from middle- and outer-shelf reefs than on inner-shelf reefs of the central region of the Great Barrier Reef. These rates were positively correlated with variations in the percent-cover of "bare" substratum on the rocks, which also increased from inner- to outer-shelf. Conversely, algal biomass was higher on rocks from inner-shelf reefs than from reefs further from the mainland. The abundance of algal grazing fishes was significantly lower on inner-shelf reefs than on middle- and outer-shelf reefs, where the overall biomass was similar but there were significant differences in the species composition (Williams, 1982; Williams and Hatcher, 1983; Russ, in press). Thus, the algal biomass on the blocks was inversely proportional to the abundance of algal-grazing fishes and rates of nitrogen fixation were directly proportional to the abundance of algal-grazing fishes.

The principal agents causing the observed nitrogen fixation were blue-green algae, since nitrogen fixation was

Table 2. Examples of rates of nitrogen fixation on coral reefs obtained from literature and converted to kg N ha^{-1} yr^{-1} (see "Discussion"). Studies marked (w) estimated ethylene production over whole incubation period, whereas those marked (e) estimated ethylene production from linear rates at end of experimental period (see "Discussion"). Dash indicates no data

Reference study	Sample	Nitrogen fixation (kg N ha^{-1} yr^{-1})		Sample size	Incubation vol
		Range	Mean		
Mague and Holm-Hansen (1975)	Scrapings	18.4 – 367.9	–	–	7 ml (w)
Wiebe *et al.* (1975) } Webb *et al.* (1975) }	Scrapings or chips	41.4 – 505.9	492.9	2 cm^2	25 ml (e) 250 – 500 ml (e)
Burris (1976)	Scrapings	3.3 – 14.5	–	0.1 cm^2	7 ml (w)
Potts and Whitton (1977)	Sediment core	2.6 – 384.5	91.3	–	7 ml (w)
Hanson and Gundersen (1977)	Coral rocks	41.0 – 2 503.0	684.0	600 cm^2	2 000 ml (e)
	Sediments	1.4 – 21.1	5.4	–	38 ml (e)
Present study					
Inner-shelf	Coral rock	0 – 20.1	4.4	120 cm^2	1 020 ml (w)
Middle-shelf	Coral rock	0 – 55.1	14.8	120 cm^2	1 020 ml (w)
Outer-shelf	Coral rock	1.9 – 153.9	20.4	120 cm^2	1 020 ml (w)

not evident in dark-incubated samples. Previous studies have shown that filamentous blue-green algae of the genera *Calothrix (Rivularia), Nostoc, Hormothamnion, Scytonema, Hyella, Microcoleus* growing on coral and sandy substrata are the principal causes of nitrogen fixation on coral reefs (Mague and Holm-Hansen, 1975; Burris, 1976; Wiebe, 1976; Potts and Whitton, 1977). In addition to *Microcoleus* spp., species of the genera *Oscillatoria, Symploca* and *Lyngbya,* which Stewart (1980) reported as nitrogen fixers, were found on coral substratum during a companion study at an adjacent reef in the middle-shelf of the central Great Barrier Reef (Sammarco, 1983).

Since all the rocks were collected within a 4 wk period and incubated under similar light conditions in seawater with DIN concentrations below detection levels, variations of the experimental conditions, such as different concentrations of fixed nitrogen, are unlikely causes of the observed variations in nitrogen fixation. The different rates of nitrogen fixation could be due to either variation in abundance and species composition of blue-green algae on the substrata or to variations in the nitrogen-fixing activities of similar populations of blue-green algae.

As there was a higher percent cover of "bare" substratum on rocks from middle- and outer-shelf reefs, and microscopic examination showed a high proportion of cryptic and filamentous blue-green algae on this "bare" substratum, the higher rates of nitrogen fixation on middle- and outer-shelf reefs may be attributable to a greater abundance of blue-green algae on substrata from these reefs than on inner-shelf reefs. A previous study found that the abundance of blue-green algae and rates of nitrogen fixation were proportional to the amount of "bare" substratum present on experimental substrata (Wilkinson and Sammarco, 1983). Furthermore, cryptic algae on the middle-shelf and outer-shelf reefs are more likely to have a higher photosynthetic activity than those on the inner-shelf because of greater light penetration to the substratum. Those blue-green algae on inner-shelf reef substrata experience lower light levels under similar incubation conditions because the dense cover of filamentous red algae with entrapped terrigenous sediment shades the underlying substratum. Thus, the observed cross-shelf variations in experimental nitrogen fixation are attributed to an augmentative interaction between larger populations of blue-green algae plus greater light penetration to these algae on middle- and outer-shelf substrata compared to inner-shelf substrata. Nitrogen fixation has been shown to be directly related to the amount of light received by blue-green algae (Stewart, 1977).

During a study of nitrogen fixation at Enewetak Atoll, Wiebe *et al.* (1975) demonstrated that nitrogen fixation on rocks from 3 to 6 m of the outer-reef slope was an order of magnitude lower than on rocks from the reef flat when incubated under shallow-water conditions. During our study, we could detect no significant differences in rates of nitrogen fixation between substrata from the fore-reef slope (5 to 9 m depth) and the reef flat (1 to 2 m depth). These incubations were carried out under similar conditions of illumination, comparable to that found over the reef flat. They are therefore measures of the potential for nitrogen fixation on substrata from the fore-reef slope rather than rates that exist *in situ* where ambient light is reduced. From our data, it is not possible to compare actual rates of nitrogen fixation from deep and shallow zones nor compare these with those reported by Wiebe *et al.*, (1975). Potential nitrogen fixation rates from deep reef zones (20 to 50 m) of Caribbean reefs have been shown to be negligible (Bunt *et al.*, 1970), suggesting that both actual and potential rates of nitrogen fixation would decrease at depths beyond those chosen for this study.

Any extrapolation of measurements made during experiments with small samples under conditions which are not completely natural in order to estimate annual rates over large areas is a hazardous procedure. An attempt is made in Table 2 to make a comparison between our data and others reported in the literature. This table lists nitrogen fixation rates per year over a hectare of coral reef surface. Many assumptions are made: a C_2H_4 to N_2 conversion ratio of 4:1 is selected (Peterson and Burris, 1976; R. H. Burris, personal communication); an annual day length of 12 h is assumed; and a correction factor of 1.9 is used to convert measured surface area to actual surface area (Dahl, 1973; Wiebe *et al.*, 1975). The published values fall into two distinct groups: those which have averaged ethylene production rates over the complete incubation period; and those which have used the linear rate of ethylene production during the latter stages of incubation to estimate nitrogen fixation rates. The estimations from linear measurements (Wiebe *et al.*, 1975; Hanson and Gundersen, 1977) are as much as an order of magnitude higher than those using averaged rates (Mague and Holm-Hansen, 1975; Burris, 1976; Potts and Whitton, 1977; and present study). Flett *et al.* (1976) reported that ethylene is quite soluble in water ("at 20 °C, 0.122 volumes of C_2H_4 will dissolve in 1.0 volume of water"). As a result, the gaseous phase from which intermediate samples were taken would be an accurate reflection of the incubation concentration only after either ethylene saturation of the incubation liquid or prolonged agitation. We suggest, therefore, that measurements of ethylene production averaged over the whole incubation period are not comparable with estimations based on final linear measurements when they are used to determine actual rates of nitrogen fixation. Estimations performed over the whole incubation period should include corrections for dissolution of ethylene in water (Flett *et al.*, 1976), whereas estimations of final linear rates are applicable only when the rates are consistently linear, after a lag period of limited duration. The nitrogenase enzyme reacts rapidly with both acetylene and dinitrogen substrates (Stewart *et al.*, 1967; Eady, 1980) and in addition is poisoned by prolonged exposure to acetylene (David and Fay, 1977). Thus, care should be taken using data obtained from the acetylene reduction technique when calculating nitrogen budgets for coral reefs.

The correlation between rates of nitrogen fixation and abundance of algal-grazing fishes and the inverse correlation of both with total algal biomass observed at the small scale (Wilkinson and Sammarco, 1983) were also observed at the larger, cross-shelf scale. The mechanisms that caused variation in nitrogen fixation at this scale may be operative at the larger cross-shelf scale, but remain to be tested. For example, fish remove overshadowing macrophytic algae, thereby producing bare substratum which is available for colonization by the more rapidly growing and opportunistic blue-green algae. Also, more light penetrates to these algae, including the blue-green algae growing within coral skeletons which have been shown to fix considerable amounts of nitrogen (Crossland and Barnes, 1976). Previous studies have shown that algal grazing maintains the algal community in an early stage of succession with enhanced productivity and algal growth rates per unit biomass (Tsuda and Kami, 1973; Wiebe *et al.*, 1975; Wanders, 1976, 1977; Sammarco, 1983). Clearly, however, factors other than fish grazing may affect the distribution, abundance and nitrogen-fixing activity of blue-green algal populations on rocks from reefs across the continental shelf. Hatcher and Larkum (1983) showed that nutrient concentrations and spatial and seasonal variations in species composition were factors, along with fish-grazing, which determined epilithic algal community structure. Detailed experimental studies will be required to elucidate the major factors causing cross-shelf variation in nitrogen fixation.

Previous studies (Johannes *et al.*, 1972; Webb *et al.*, 1975; Wiebe *et al.*, 1975; Hatcher and Hatcher, 1982) have shown that, as a result of nitrogen fixation, there were increased concentrations of DIN in water flowing over and eventually away from the reef. These increases are due to the excretion of DIN by the algae or the nitrification of fixed ammonia by bacteria associated with the blue-green algae (Webb and Wiebe, 1975; Wiebe, 1976). The present study also has shown increased concentrations of DIN on reefs where nitrogen fixation occurs (Table 1) and an increase in DIN along the direction of the current on outer-shelf reefs during calm conditions (Fig. 5). A similar increase was not apparent on middle-shelf reefs, which were examined during rough weather with a more rapid and turbulent current flow.

Thus, the nitrogen fixed by unsubstantial-looking turfs of blue-green algae on the much neglected areas of rubble and reef flat (Johannes *et al.*, 1972) is the major source of coral reef nitrogen (Webb *et al.*, 1975), and is eventually made available to communities downstream.

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