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Effects of fish grazing and damselfish territoriality on coral reef algae. II. Nitrogen fixation*

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ABSTRACT: Nitrogen fixation was determined for plates of dead coral substratum placed at 10 m depth behind the reef crest on Britomart Reef, Great Barrier Reef. These plates were maintained for 12 mo under 3 different experimental conditions – exposed to natural levels of fish grazing; within the territories of the damselfish *Hemiglyphidodon plagiometopon* which aggressively excludes most other grazing fish; and within fish exclusion cages. Nitrogen fixation rates (determined by acetylene reduction technique) were highest on plates exposed to fish grazing which had the lowest algal biomass, and were lowest on caged plates with the highest algal biomass. Plates within damselfish territories were intermediate for both parameters. Grazing fish reduced algal biomass and shifted the algal community structure from dominance by red algae to dominance by rapidly colonizing and growing blue-green algae. Thus, nitrogen fixation was directly proportional to the extent of fish grazing and inversely proportional to total algal biomass.

INTRODUCTION

Coral reefs are highly productive ecosystems in tropical and subtropical waters which are low in available nitrogen (Thomas, 1970). A number of efficient physiological mechanisms have evolved in some reef organisms which allow them to collect and recycle nitrogen, e.g. scleractinian corals (Muscatine and D'Elia, 1978). A considerable input of fixed nitrogen, however, is still required to balance the losses derived from physical wash-out from lagoons (Hatcher and Hatcher, 1982), biological export (Johannes et al., 1972), and possible losses through denitrification (Koike and Hattori, 1978).

Nitrogen fixation has been shown to occur on algal-covered substrata of reef crests and reef flats (Mague and Holm-Hansen, 1975; Wiebe et al., 1975; Goldner, 1980). It is believed that water flowing over these algal communities is enriched with fixed nitrogen, thereby contributing to the nutrient requirements of organisms down-current. Nitrogen export of this type has been quantified on the reef flats of Eniwetak Atoll (Webb et al., 1975; Wiebe et al., 1975). Recently, Hatcher and Hatcher (1982) reported increases of fixed nitrogen in water flowing into and eventually out of the lagoon on One Tree Island (southern region, Great Barrier Reef). Demonstrable nitrogen fixation was only evident during daylight hours, and Wiebe (1976) attributed this to blue-green algae such as species of *Calothrix, Hormothamnion,* and *Rivularia.* During the study at Eniwetak Atoll, fish were observed to graze on these blue-green algae, and it was considered that such grazing activities maintained the algal community in an early stage of succession – one with a particularly active growth phase (Tsuda and Kami, 1973; Wiebe et al., 1975). Territorial grazing fish, by contrast, have been shown to enhance algal growth within the territories (e.g. Vine, 1974).

Lobel (1980) hypothesized that nitrogen fixation would be enhanced within damselfish territories under reduced grazing because of larger blue-green algal populations. In contrast, Wilkinson and Sammarco (1982) presented preliminary observations showing that nitrogen fixation was highest on open substratum grazed by fish. We now present experimental evidence to substantiate the hypothesis that fish grazing enhances rates of nitrogen fixation on natural coral substratum. The experimental conditions varied from no fish grazing within cages, through reduced grazing within territories of the damselfish *Hemiglyphidodon plagiometopon* Bleeker (Pomacentridae) to full exposure to natural levels of fish grazing (Sammarco and Carleton, 1982).

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MATERIALS AND METHODS

Field techniques used in this experiment, performed on Britomart Reef (central region, Great Barrier Reef), are described in detail in Sammarco (1983) and Sammarco and Carleton (1982). To summarize, pieces of foliose plating coral were killed and placed in a lagoonal environment under 3 experimental conditions: (1) fully exposed to natural levels of fish grazing; (2) within territories of the damselfish *Hemiglyphidodon plagiometopon,* which is known to exclude locally most herbivorous fish (Lassuy, 1980); and (3) within cages excluding all fish larger than 1.6 cm. Plates were assessed *in situ* for percent-cover of algae 11 mo after initiation of the experiment (Sammarco, 1983). The plates were retrieved after 12 mo and stored in running seawater. Algae were examined microscopically and identified according to the authorities cited in Sammarco (1983). Subsamples were cut with a seawater-cooled diamond-bladed saw into 2 sizes: 2 × 5 and 4 × 5 cm. Nitrogen fixation by these subsamples was determined using the acetylene reduction technique (Stewart et al., 1967), which is the preferred assay for the nitrogenase enzyme and, hence, for relative rates of nitrogen fixation (Stewart, 1980). The smaller pieces were placed in 25 × 150 mm test tubes closed with Suba seals whilst the larger plates were incubated in 100 ml Alltech vacuum gas sample bottles. Samples were incubated at approximately 10 % surface irradiance (200 to 400 $\mu E\ m^{-2}s^{-1}$) in 25 and 60 ml, respectively, of acetylene-saturated, low nitrogen sea water, previously filtered through 0.45 µm membrane filters. For dark incubation, one test tube containing a smaller plate was wrapped in aluminium foil for each treatment. The gaseous atmosphere within the incubation vessels was evacuated and flushed 3 times with argon and then a final gas phase containing 20 % acetylene in argon was added. Gas samples were taken immediately, and at approximately hourly intervals for 5 h thereafter. Samples were analyzed in a Tracor 222 gas chromatograph with a Poropak 'R' 100 to 120 mesh size column. Due to the nature of the substratum the glass incubation vessels were not shaken or mixed during incubation. The amount of ethylene produced was used as an index of nitrogen fixation activity. Ethylene production was measured against contaminant methane as an internal standard and was calculated using a correction according to Henry's Law (Flett et al., 1976). No attempt was made to convert ethylene production to actual rates of nitrogen fixation during the preliminary study.

The chlorophyll *a* content of the substratum subsamples was measured by successively extracting them in 90 % acetone in water at 0 to 4 °C for 6 and 24 h. Extinction coefficients of the combined extracts at 664, 647, and 630 nm were determined in a Varian 664 series spectrophotometer and chlorophyll *a* content was calculated using the equations of Jeffrey and Humphrey (1975).

Surface areas of plates were determined by covering plates with aluminium foil which was pressed as well as possible into crevices, removing excess foil. The foil was then mounted on a digitizing board (Summagraphics TD) and areas were integrated with the aid of a PDP-11/70 computer. Small plates averaged 27.2 cm^2 total surface area ($s = 1.65$, $n = 9$) and large plates ranged from 41.4 to 45.5 cm^2. Decalcified algal dry weight was determined by removing macroscopic algae from the plates with fine dissecting forceps and placing them in pre-weighed Petri dishes. Samples were decalcified in 5 % HCl then dried in an oven at 65 °C for > 24 h, and weighed. The subsample plates were then decalcified in 5 % HCl. Obvious animal tissue (e.g. worms and molluscs) was removed, and the remnants were examined microscopically for identification of algae. The algal remnants were dried and weighed.

RESULTS

Nitrogen fixation (estimated via acetylene reduction technique) was highest on substratum plates fully exposed to fish grazing ($p < 0.05$). These plates showed obvious signs of recent grazing activity and contained a low algal biomass (Table 1). Conversely, the lowest rates of nitrogen fixation occurred on plates from within cages where the algal biomass was highest ($p < 0.05$). Plates from the damselfish territories were intermediate in both rates of nitrogen fixation and algal biomass. Nitrogen fixation was negligible for plates incubated in the dark in all 3 treatments (Table 1).

The amount of chlorophyll *a* extracted from these plates varied little between treatments despite the fact that algal biomass (decalcified dry weight – predominantly algae) varied between treatments by up to an order of magnitude. The chlorophyll content was obtained by applying the trichromatic equations of Jeffrey and Humphrey (1975) which were not formulated for mixtures containing large proportions of blue-green algae. Errors in chlorophyll estimation may have arisen because the same equation was used for rocks with 80 % blue-green algal cover as well as for rocks with less than 5 % blue-green algae (Table 2) (Rott, 1980). Biomass was highest inside cages, intermediate within territories, and low on exposed plates (Table 1). Decalcification of experimental plates showed that a considerable proportion of the algal biomass occurred cryptically in the interstices of the coral septa or were endolithic algae (Sammarco, 1983). Cryptic or

Table 1. Ethylene produced from acetylene on coral substratum under 3 experimental conditions: *exposed* to natural levels of fish grazing; within damselfish *territories*; and within *cages*. Exposed plates had significantly higher rates of ethylene production than either territory or caged treatments ($p < 0.05$; one way ANOVA and SNK tests on small plates). Decalcified dry weights are included with the proportion of biomass attributed to cryptic and endolithic algae measured after blocks were decalcified. Chlorophyll *a* content of plates is shown, with no significant differences between experimental conditions ($p > 0.05$). • Data not available

Treatment	Substratum plates Size	Replicate	Ethylene production (nM cm^{-2} h^{-1})	Chlorophyll *a* (μg cm^{-2})	Algal biomass decalcified (mg cm^{-2})	Cryptic algae as % of total algal biomass
Exposed	Small	(a)	42.60	8.15	1.05	78.9
		(b)	42.46	9.63	2.28	78.0
		(c) dark	1.31	11.39	0.76	93.4
	Large		110.22	10.01	•	•
	Mean (light)		*73.37*			
Territory	Small	(a)	22.10	10.41	8.79	5.9
		(b)	5.04	5.52	19.68	1.1
		(c) dark	3.01	11.95	7.26	13.3
	Large		42.95	9.54	2.93	61.6
	Mean (light)		*26.85*			
Caged	Small	(a)	6.56	9.86	23.28	5.5
		(b)	2.14	6.66	12.74	10.2
		(c) dark	0.66	10.83	22.94	3.2
	Large		34.73	8.54	13.13	12.3
	Mean (light)		*16.54*			

Table 2. Distribution of blue-green algal species on coral substratum (see Table 1). Data represent total percent cover of substratum in which each particular species was encountered. (Data expanded from Sammarco, submitted and unpubl. own information)

Algal species	Percentage of substratum containing algae		
	Exposed	Territory	Caged
Oscillatoria amphibia	19.3		
O. nigro-viridis prox.	19.3		
Symploca hydnoides	19.3		
Lyngbya confervoides	19.3		
L. aestuarii	4.8	17.5	
Microcoleus chthonoplastes		3.8	
Phormidium corium		1.1	
L. meneghiniana			2.7
Non blue-green algae	21.4	37.2	82.8

endolithic algae accounted for the majority of algal biomass (78 to 94 %) on plates from the exposed, fully grazed, treatment, but their relative importance decreased as accessibility of the substratum to fish grazing decreased, accounting for a highly variable proportion (1 to 62 %) of the biomass within territories. Only 3 to 12 % of the algal biomass within cages was derived from cryptic algae.

Blue-green algae were particularly common and most diverse on the exposed plates (Table 2). Within damselfish territories, blue-green algae were less prominent, and plates from the cages had very few blue-green algae (Table 2).

DISCUSSION

In our experiments, grazing activities of fish resulted in a reduction of algal biomass on dead coral substratum and a shift in the epibenthic algal community structure from red algae to dominance by blue-green algae (Sammarco, 1983). This shift was accompanied by increased rates of nitrogen fixation. Our results do not support the hypothesis of Lobel (1980) that nitrogen fixation would be enhanced through the activities of damselfish which exclude other grazing fish and appear to 'farm' selected species of algae.

In our preliminary experiments it was apparent that the rate of nitrogen fixation, as measured by the acetylene reduction technique, was inversely proportional to the algal biomass on coral substrata. Coral plates subjected to natural levels of fish grazing had the highest rates of nitrogen fixation (Table 1). Those coral plates had a low algal biomass and fish grazing scars were obvious on the surface. The lowest rates of nitrogen fixation occurred on caged plates protected from fish grazing, where algal biomass was highest. Rates of nitrogen fixation and algal biomass were intermediate on coral plates within territories of the damselfish *Hemiglyphidodon plagiometopon*.

Reduction of nitrogen fixation does not appear to be

an artifact due to caging, because a significant reduction in nitrogen fixation was also observed within damselfish territories. Damselfish effectively reduce grazing activity by other fish while not producing the side effects such as shading and increased sedimentation, commonly associated with caging. Algal community structure on plates within cages showed some affinities to that within territories, but differed greatly from that on plates exposed to fish grazing.

The majority of nitrogen fixation was apparently due to blue-green algae as nitrogen fixation activity was correlated directly with light. The most important algae on heavily grazed substrata were species of the genera *Symploca, Oscillatoria* and *Lyngbya*. The exposed plates also contained large amounts of *O. nigro-viridis* and *O. amphibia* prox. growing in the interstices between the septa. Species of *Microcoleus, Lyngbya* and *Phormidium* occurred on substrata placed within damselfish territories, conversely only small quantities of *Lyngbya* species were found on the caged plates which had the lowest rates of nitrogen fixation. Of these genera of blue-green algae all but *Symploca* and *Microcoleus* contain species that have been reported to fix nitrogen (Stewart, 1980).

Several hypotheses may be invoked to explain the increase in nitrogen fixation associated with increased levels of fish grazing. Firstly, continuous grazing may maintain the algal community in an early stage of succession, thereby favouring the growth of opportunistic or 'pioneer' species such as blue-green algae. These algae are able to colonize bare substrata rapidly and continue to occupy the interstices of the coral skeletal structure. A second hypothesis, which does not necessarily exclude the first, is that fish grazing removes much of the algal micro-canopy which is composed of red, brown, and green algae, thereby permitting greater penetration of light to the blue-green algae of lower profile. Thirdly, the accumulation of carbonate sediments in dense stands of algae (Sammarco and Carleton, 1982; Sammarco, 1983) reduces light penetration to the blue-green algae, thereby reducing energy available for nitrogen fixation. It is probable that a combination of these mechanisms is involved in the increased nitrogen fixation on heavily grazed substratum. It is also possible that grazing fish selectively remove non-nitrogen fixing algae leaving blue-greens behind, some of which are known to be either distasteful or toxic (Stewart and Daft, 1977).

Two additional observations were made during this study. Firstly, the content of chlorophyll *a* appeared to vary little between treatments, even though there were significantly different amounts of algal biomass on plates from exposed, territory, and caged treatments. Much of the algal biomass on ungrazed substratum consisted of inert structural tissue therefore this implies that productivity may be comparable between the different plates or even higher on grazed plates which have a lower biomass to support. Higher productivity has been demonstrated in lower standing crops of algae in Curacao, Netherlands Antilles (Wanders, 1976, 1977).

The second observation concerned an apparent increase in nitrogen fixation per unit area with increasing sample size; i.e. the larger (45 cm^2) exposed plates had rates almost 3 times higher than the smaller (25 cm^2) plates from the same treatment (Table 1). This anomaly could be due to patchiness in the distribution of blue-green algae on the substratum. On the other hand, a larger proportion of the algae on smaller plates was disturbed or close to cut surfaces than on larger plates and this may have contributed to the observed differences in acetylene reduction activity. Extrapolation of nitrogen fixation rates derived from small samples may be conservative but further work is needed to confirm this.

Damselfish territories containing algal lawns are sites of reduced rates of nitrogen-fixation. Areas defended by damselfish have been estimated to cover from 15 % of lagoonal (Sammarco and Carleton, 1982) to 50 % of back reef (Sammarco and Williams, 1982) habitats on some coral reefs. Attempts to estimate rates of nitrogen fixation over large reef areas should take account of the localized thick algal mats resulting from the activities of damselfish.

It appears clear that fish grazing activities result in an increase in populations of blue-green algae in the epibenthic community on coral substratum and an increase in nitrogen fixation rates. This may lead to an increase in available nitrogen, which, in turn, may locally enhance algal productivity.

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