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Nitrogen Fixation (Aceteylene Reduction) in a Temperate Coastal Lagoon off Perth, Western Australia

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Abstract Nitrogen fixation (acetylene reduction) was determined in representative samples from the dominant marine plant associations and sediments of the Marmion Lagoon, north of Perth, Western Australia. Nitrogenase activity was low but ubiquitous. Cyanobacteria are probably the dominant causal agents associated with plant materials, while heterotrophic bacteria dominate the sediments. Biological nitrogen fixation can meet less than 0.4% of the nitrogen required annually for macrophyte productivity. This is discussed in relation to other processes that contribute nitrogen to the benthic communities and waters of the Marmion Lagoon.

INTRODUCTION

Extensive seagrass beds and algal communities, including seagrass epiphytes, characterise the shallow coastal waters of the southern half of Western Australia. Phytoplankton densities are typically low, except near discharge areas of the few rivers. Detached macrophytes are often abundant, drifting with the currents, aggregated in the swash zone, in littoral beach wrack accumulations, or as fragmented particulates in the water column. Annual benthic primary productivity is high (Kirkman 1981, 1984; Rosser, in press ; Borowitzka & Lethbridge, pers. comm.), despite the apparent lack of nutrient enrichment from oceanic sources, such as shelf upwelling processes (Rochford, 1980).

Submarine groundwater discharged from the coastal plain is a major source of nitrate for marine macrophytes in nearshore habitats (Johannes, 1980; Johannes and Hearn, 1985). Near Perth, groundwater can supply nearly half of the nitrogen required annually for marine macrophyte production in the immediate coastal waters. Nitrogen is also reintroduced to these habitats from nutrient recycling processes. Recycled nutrients from decomposing macrophytes in accumulations on the shore and in the swash zone (Robertson & Hansen, 1982; Hansen, 1984) and remineralisation processes in intertidal beach sediments (McLachlan *et al.*, 1985) can both contribute significantly to the total annual nitrogen requirements of the marine macrophyte communities. Advection and mixing processes associated with the circulation of shelf and coastal waters may provide additional nutrients for nearshore macrophyte communities. However, data so far available (Hearn & Hunter, 1985; Pearce *et al.*, 1985) do not allow estimates of nitrogen supplied by these processes.

Biological nitrogen fixation is also a potential source of nitrogen (see Capone, 1983). Small blooms of *Trichodesmium* are infrequent episodic events in the shelf waters and probably contribute some fixed nitrogen to the nearshore benthic communities. However, benthic plant communities contain a variety of habitats which might sustain significant biological nitrogen fixation. These communities are most fully developed inshore of the coastal limestone reef system, which extends for about 700 km along the temperate west coast of Western Australia. The nitrogen fixation (acetylene reduction) capability of samples from epilithic and sediment habitats was determined to assess the significance of the process - its magnitude and spatial distribution - as a nitrogen source for the shallow temperate coastal communities near Perth.

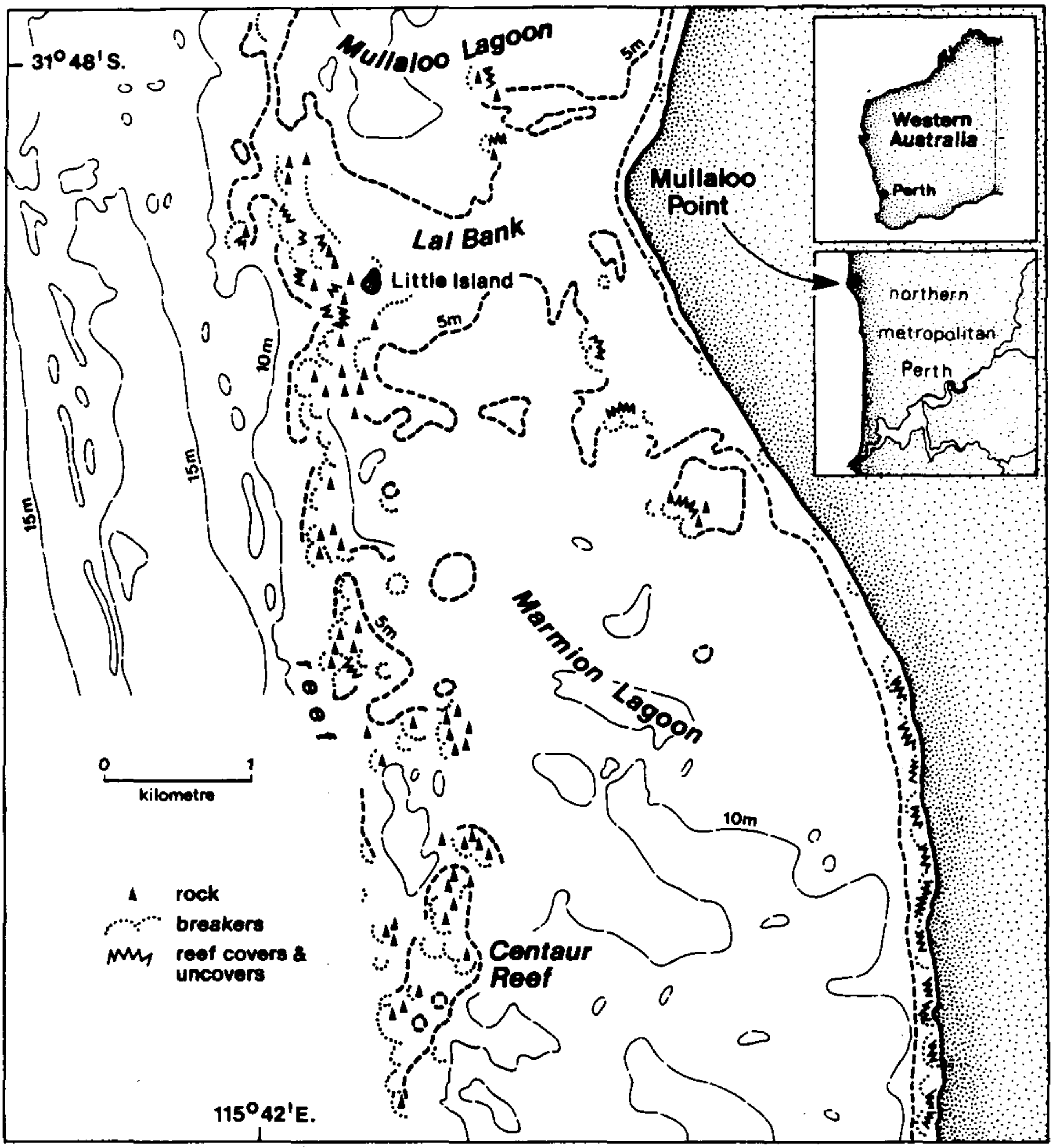

Figure 1. Study site off northern suburbs of Perth, Western Australia.

METHODS AND MATERIALS

Study Area

The study area was the Marmion Lagoon, which is about 20 km north of the Swan River close to the northern metropolitan area of Perth (Fig. 1). The area has been the site of a number of associated studies on the biota and the physico-chemical features of the coastal waters, including nutrients associated with submarine groundwater discharge (Johannes and Hearn, 1985), nutrient remineralisation processes (McLachlan *et al.*, 1985) and nutrient recycling from decomposing accumulations of marine macrophyte (Hansen, 1984). Those authors have provided detailed descriptions of the lagoon, its plant communities and the offshore limestone reefs (Table 1). The hard reef substrates support algal communities, dominated by littoral algal turfs and stands of kelp (*Ecklonia radiata*) in the sublittoral zone. The soft carbonaceous sediments of the lagoon support beds of seagrass dominated by *Posidonia sinuosa* and *Amphibolis antarctica*, which are heavily epiphytised. The sand beaches of the shoreline are broken by limestone cliffs and platforms; the latter support cyanobacterial mats in the supralittoral spray zone, turfing algae and cyanobacterial mats in the littoral and sublittoral zones, and *E. radiata* with seasonally abundant *Sargassum* spp. in the sublittoral zone.

Samples

Representative samples of sediments, algae, seagrasses, epiphytes and decomposing macrophyte aggregations were collected between May and December 1985, and assayed for acetylene reduction capabilities.

Surface sediment cores (6 cm diameter, 2 cm depth; about 25 g dry weight) were collected from each soft substrate area, viz., bare sand and sediments from seagrass beds of the lagoon, and beach sediments from the upper and lower littoral. The latter were enriched with organic materials from decomposing swash and beach wrack accumulations, mixed into the sediments by wave action. Anoxic sediments were not included in the seagrass-bed samples.

Turfing algae and cyanobacterial mats attached to parent rock (approximately 45 cm^2 per incubation sample) were collected from coastal rock platforms.

Detached plant materials were sieved to obtain samples (10-12 g fresh weight) representative of the swash zone (>25 mm mesh) and the beach wrack accumulations (<25 mm mesh). Lamina from *in situ* kelp (*E. radiata*) were cut to similar sample weight for comparison with swash and wrack materials.

Leaves of *P. sinuosa* and leaves and stems of *A. antarctica* (about 8 g fresh weight) were also assayed. One group of leaves was chosen that was apparently free of epiphytes but presumably included a surface film of microorganisms.

Seagrass epiphytes were represented by each of three sample types: leaves of *P. sinuosa* and *A. antarctica* with about 75% surface cover of predominantly encrusting coralline algal epiphytes; plastic artificial seagrass "leaf" with epiphyte communities similar to those of the *P. sinuosa* leaves (Rosser, in press); and stems of *A. antarctica* carrying a high biomass of articulated coralline and fleshy algal epiphytes.

Table 1.

Estimated area and annual biomass of dominant plant communities and sediments in the Marmion Lagoon. (Calculated from Kirkman, 1981; Hansen, 1984; Johannes & Hearn, 1985. a - Includes turf communities on the surface of lagoonal reefs. b - Annual turnover rate of 1.6 x standing biomass; Kirkman, 1981, 1984).

Habitat	Area		Average standing biomass (kg dry weight)
	(m^2)	(%)	
<u>Supralittoral</u>			
Cyanobacterial mat	1.3×10^3		
<u>Littoral</u>			
Cyanobacterial mat	5.9×10^3		
Algal turf[a]	89.2×10^3		
Sand sediment			
- upper littoral	14.3×10^3		
- lower littoral	14.3×10^3		
Beach wrack accumulation and swash material			177.2×10^3
<u>Sublittoral</u>			
Algal communities	4.45×10^6	17.1	
- *Ecklonia radiata*[b]			7.12×10^6
Seagrass communities	4.95×10^6	19.0	
- *Posidonia* (30% epiphytised)	3.96×10^6		1.39×10^6
- *Amphibolis*	0.99×10^6		0.40×10^6
stem epiphytes			0.25×10^6
Bare sand	16.50×10^6	63.4	

Total area of lagoon and intertidal areas = $26.03 \times 10^6 m^2$

Total volume of the lagoon waters = $223 \times 10^6 m^3$

Assays

Nitrogen fixation (acetylene reduction) was determined by standard methods (see Wiebe *et al.*, 1975: Flett *et al.*, 1976) in light and dark conditions. Each sample was incubated with 130 ml 0.2 μm filtered seawater in a 260 ml gas-tight glass jar fitted with a rubber seal in the glass base. The jar was inverted to stand on its bakelite lid and incubated in a thermostatted water shaker bath either in photosynthetically saturating light (325 μein, m^{-2}, sec^{-1}) or in the dark. At time zero, 13 ml of air was replaced with acetylene gas. Ethylene concentration in the gas phase of time course samples (40 min intervals for 5 h) were determined with a Pye Unicam 204 gas chromatograph equipped with a Poropak 'R' column. Each plant or sediment sample type was assayed in a single experiment, using ten replicates (5 light, 5 dark) at ambient seawater temperatures (17.0 - 18.0°C, winter; 20.5 - 21.5°C, summer).

Following incubation, the surface areas of sediment cores, algal turf and cyanobacterial mat samples were measured. The dry weights of seagrasses, epiphytes, algae, swash and wrack materials were determined after drying to constant weight at 100°C.

Data Analyses

In most experiments, ethylene production was calculated with reference to an internal methane standard and corrected for gas solubilities (Flett *et al.*, 1976). However, preliminary experiments showed methane was produced by aged beach wrack and some sediments from seagrass beds. Here, ethylene production was calculated with reference to each of the introduced acetylene and methane gas concentrations; these values were not significantly different (Student t-test for paired comparisons, $p < 0.05$; Steel & Torrie, 1960), as the magnitude of acetylene consumption and methane production during incubation was small compared to the concentration of the gas additions. Control assays without acetylene atmosphere were run with each sample type to assess any non-nitrogenase production of ethylene (see Taylor, 1983); ethylene was not observed in any sample within 5 h of incubation.

Rates of ethylene production were determined from the linear data of time course graphs (between 40 and 300 min incubation) and are expressed as a function of either area (cm^2) or biomass (g dry weight) of the incubated sample.

Significance of difference between light- and dark-treated samples was determined by the Student t-test with the significance level held at $p < 0.05$. The t'-test (Steel & Torrie, 1960) was applied where non-equality of variance was demonstrated (littoral cyanobacterial mats, 5-day-old beach wrack, *A. antarctica* leaf plus epiphytes, and stem plus epiphytes).

Table 2. Light and dark rates of acetylene reduction in winter samples from plant communities and sediments of the Marmion Lagoon. (Mean value ± standard deviation, n=5. Significance between light and dark treatments by Student t-test shown at 5% level, *; 1% level, **; 0.1% level, ***; ns, not significant).

Sample type	Acetylene reduction activity (nmol C_2H_4 produced, h^{-1})					
	per unit area (cm^2)			per unit dry weight (g)		
	Light	Dark		Light	Dark	
<u>Supralittoral</u>						
Cyanobacterial mat	1.032±0.210	0.798±0.168	**			
<u>Littoral</u>						
Cyanobacterial mat	21.048±3.240	0.594±0.150	***			
Algal turf	0.528±0.138	0.324±0.114	***			
Beach Wrack						
- 5 days old				0.774±0.336	0.252±0.084	**
- 20 days old				0.378±0.162	0.360±0.222	ns
<u>Sublittoral</u>						
Swash material				0.348±0.114	0.330±0.126	ns
Ecklonia radiata	0.114±0.054	0.144±0.072	ns			
Artificial seagrass						
+ epiphytes	4.512±0.78	1.506±0.732	***			
Posidonia sinuosa						
- leaf only	0.186±0.030	0.156±0.018	**			
- young, + ephipytes	0.558±0.258	0.372±0.018	*			
- old, + epiphytes	4.104±0.948	1.872±0.756	***			
Amphibolis antarctica						
- leaf only	0.054±0.024	0.012±0.006	**			
- leaf + epiphytes	1.008±0.270	0.744±0.180	**			
- stem + epiphytes	42.600±33.600	4.722±1.542	**			
<u>Sediments</u>						
Beach sand						
- upper littoral	0.009±0.002	0.009±0.002	ns			
- lower littoral	0.010±0.003	0.008±0.002	ns			
Lagoon sand, bare	0.042±0.010	0.043±0.013	ns			
P. sinuosa sediment	0.050±0.010	0.057±0.019	ns			
A. antarctica sediment	0.057±0.007	0.061±0.010	ns			

RESULTS

Acetylene reduction activity occurred in all samples from plant communities and sediments of the Marmion Lagoon (Table 2). Activity was not detected in samples of particulate organic matter filtered from the water column (5 L). Generally, the activity in plant community samples was greater than in that of sediments by several orders of magnitude. No seasonal differences in acetylene reduction activity were detected in samples from lagoon sediments, littoral cyanophyte mats, turfing algae and seagrass epiphytes assayed in winter (June/July) and summer (December).

The highest activities were found in submerged cyanobacterial mats from the littoral zone and epiphytes associated with seagrasses. *Posidonia sinuosa* and artificial seagrass, which developed similar epiphyte communities, showed similar acetylene reduction activity. The encrusting coralline algae and surface film that develop on young seagrass leaves of each species showed relatively low rates of activity compared with the older epiphyte communities, which are characterised by fleshy and articulated coralline algae.

The decrease in acetylene activity with age of beach wrack material probably reflected changes in the microbial associations that occur in the decomposition process in the stranded plant materials (Hansen, 1984).

The difference between light- and dark-treated samples was used as an indicator of the relative significance of autotrophic cyanobacteria and heterotrophic bacterial populations as the dominant biological group effecting acetylene reduction (see Thomson & Webb, 1984)(Table 2). Plant material of the older beach wrack, the turbulent swash zone, *Ecklonia radiata* lamina, and all sediment samples showed no significant differences in acetylene reduction activity between light and dark treatments. This suggests that heterotrophic bacteria were the primary nitrogen fixers. All other samples from plant associations showed that the acetylene reduction rates were light-stimulated, indicating that photosynthetic cyanobacteria have the primary role in nitrogenase activity.

DISCUSSION

Nitrogen fixation (acetylene reduction) associated with the plant communities and sediments of the Marmion Lagoon is ubiquitous, but is generally low. These nitrogenase rates fit the lower range of published values for sediments and similar types of seagrass associations in temperate regions (Capone, 1983).

Heterotrophic bacteria appear to be the dominant nitrogenase agents in lagoonal and littoral sediments, older beach wrack, swash zone materials and on *Ecklonia radiata*. Physical disturbance resulting from varying degrees of turbulence in these habitats may preclude the establishment of microzones conducive to the development of cyanobacteria (Carpenter & Price, 1976; Paerl, 1985). The high concentrations of particulate organic matter in each of these habitats resulting from transport, accumulation and decomposition of algae, epiphytes and seagrass materials (Robertson & Hansen, 1982; Hansen, 1984) could provide a niche more suited to heterotrophic bacteria than cyanobacteria. That heterotrophic bacteria are the site of nitrogenase activity (albeit low) associated with *E. radiata*, is consistent with observations of slow but continuous release of mucoidal materials from the laminar surface. This would provide a dynamic environment enriched with organic carbon.

Diazotrophic cyanobacteria are commonly associated with marine plants (see Capone, 1983). The nitrogenase activity in plant communities in the Marmion Lagoon is due primarily to cyanobacterial associations, except in established and high-activity niches (e.g., older seagrass epiphytes) where heterotrophic bacteria make a significant contribution. This may be a response not only to a more stable physical regime but also to the increased availability of dissolved organic carbon released by the plants (Horner & Smith, 1984).

While seasonal variation in nitrogenase activity has been demonstrated in a number of marine communities (see Capone, 1983; Thomson & Webb, 1984), there was no evidence of this in the samples from the Marmion Lagoon. The narrow annual temperature range of seawater (17 - 22°C; Pearce *et al.*, 1985) may modify seasonal variability at nitrogenase sites. Any seasonal changes in the contribution of nitrogen to the Marmion Lagoon by biological nitrogen fixation will be a function of changes in the biomass of the dominant plant communities, which are sites of high nitrogen fixation rates, rather than changes in the population density or nitrogenase activity of diazotrophic microorganisms.

Calculation of the contribution of nitrogen fixation to nitrogen in large areas on the basis of small samples and the acetylene reduction method alone is fraught with difficulty (see Taylor, 1983; Hatcher, 1985). However, such preliminary estimates allow comparison of the relative magnitude of this contribution between habitats, and with other nitrogen-contributing processes. The annual nitrogen contribution to the Marmion Lagoon by biological nitrogen fixation (Table 3) was calculated from the area/volume estimates of plant communities and sediments (Table 1) and their acetylene reduction rates (Table 2), assuming a 12 h day (light)/12 h night and the general C_2H_4:N molar ratio of 3.0 (see Capone, 1983 for a discussion of molar conversion ratios). The principle sources of fixed nitrogen are the lagoonal habitats: sand and *P. sinuosa* sediments, and *A. antarctica* epiphytes.

Table 3. Estimated annual nitrogen fixation for the Marmion Lagoon.

Habitat	Nitrogen fixation (kg N, yr^{-1})
Supralittoral	
Cyanobacterial mat	0.5
Littoral	
Cyanobacterial mat	15.5
Algal turf	25.9
Sand sediment	< 0.1
Beach wrack and swash material	25.6
Sublittoral	
Algal communities	
Ecklonia radiata	58.2
Seagrass communities	
Posidonia sinuosa	57.6
Amphibolis antarctica	243.0
Sediments	
Bare sand	283.2
Posidonia sinuosa	85.8
Amphibolis antarctica	23.9
Total:	819.2

The nitrogen required annually to maintain the observed productivity of macrophyte communities in the Marmion Lagoon has been estimated to be 208 x 10^3 kg N, yr^{-1} (Johannes & Hearn, 1985). Biological nitrogen fixation can make an annual contribution of about 0.4% of the total nitrogen required and is thus a barely significant component of *de novo* nitrogen supply. However, the rates and distribution of denitrification and nitrification processes (Nixon & Pilson, 1983; Smith, 1984) have not been determined in the Marmion Lagoon. The loss of gaseous nitrogen through these processes will reduce the estimates of the combined nitrogen contribution to the benthic communities. They could also effectively negate the small contribution made by diazotrophic cyanophytes and bacteria to the annual nitrogen budget.

Submarine groundwater discharge can deliver about 48% of the "new" nitrogen requirement, as nitrate (Johannes & Hearn, 1985). In addition, the dissolved organic nitrogen (DON, >15 µM nitrate equivalence) associated with the groundwater discharge may contribute to the nitrogen requirements of the plant communities, either directly or indirectly following conversion of refractory nitrogen compounds. Nutrient recycling by intertidal remineralisation processes (McLachlan *et al.*, 1985) and intertidal and nearshore decomposition of marine macrophytes (Hansen, 1984) could account for a further 17 - 19% of the annual nitrogen requirement. Recent studies have shown that winter storm events cause nitrogen enrichment of the water column near the reefs (Kirkman, pers. comm.). However, rates of nutrient regeneration in the water column or associated with the benthos have not been evaluated.

It is apparent that, before a full nitrogen budget can be described for this coastal lagoon system, the additional processes and sources of combined nitrogen must be more fully understood. The source of a net input of at least 30% more nitrogen (the amount required to sustain the annual macrophyte productivity) must be determined. It presumably comes from such sources as DON, regeneration and recycling processes in the water column and on the lagoon floor, and advection and mixing processes in the shelf and coastal waters.

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