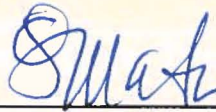


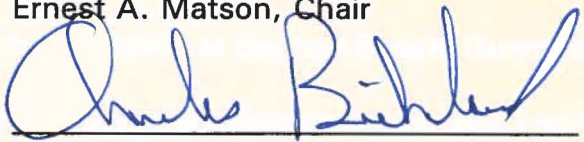
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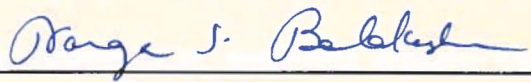
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AN ABSTRACT OF THE THESIS presented by Andrew S. E. Quenga for the Degree of Master of Science in Biology, March 19, 1993.

Title: Nitrogen fixation across a Guam reef flat estuary.

Approved: 
Ernest A. Matson, Chair, Thesis Committee

N_2 fixation rates were measured in sediments at Gognga Beach, Guam, where runoff and upwelling of $NO_3^- + NO_2^-$ -rich groundwater continuously enriches and dilutes intertidal sediments. N_2 fixation rates were compared with porewater salinity, $NO_3^- + NO_2^-$ concentration, sediment organic content, inorganic content, and grain size over distances from the beach and away from the source of ground water. N_2 fixation rates were inversely correlated with porewater $NO_3^- + NO_2^-$ concentration and directly correlated with porewater salinity. Laboratory experiments on the effects of increasing NO_3^- and decreasing salinity (separately) on N_2 fixation rates showed that lowered salinity decreased N_2 fixation but increased $NO_3^- + NO_2^-$ had no effect. N_2 fixation in the sediments at Gognga Beach is lower with proximity to this source of terrestrial N, does not appear to be affected by normally high NO_3^- , and is a minor fraction of the N budget of Tumon Bay, Guam.

UNIVERSITY OF GUAM

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Nitrogen Fixation Across a Guam Reef Flat Estuary

by

Andrew Sergio Esteban Quenga

A thesis submitted in partial fulfillment of the
requirements of the degree of

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TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	iv
LIST OF FIGURES	v
INTRODUCTION	1
MATERIALS AND METHODS	4
Study site	4
Sediment and water sampling	4
Nitrogen fixation	6
Sediment chemistry and grain size	8
Water chemistry	9
Effects of artificial enrichment and dilution	9
Data analyses	10
RESULTS	11
Sedimentary N₂ fixation	11
Sediment chemistry and grain size	11
Porewater chemistry	15
N₂ fixation with NO₃⁻ enrichment and fresh water dilution	15
DISCUSSION	19
LITERATURE CITED	24

LIST OF TABLES

	PAGE
Table 1: Percent organic and ash content by weight (means \pm 1 S.D., n = 9) of Gognga Beach sediments over distance from shore	13
Table 2: Percent of grain size fractions by weight (means \pm 1 S.D., n = 9) of Gognga Beach sediments over distance from shore	14

LIST OF FIGURES

	PAGE
Figure 1: Map of the study site, Gognga Beach and transect T, in Tumon Bay, Guam	5
Figure 2: Sedimentary N_2 fixation rates (means \pm 1 S.D., n = 9) over distance from shore at Gognga Beach	12
Figure 3: Porewater $NO_3^- + NO_2^-$ and salinity (means \pm 1 S.D., n = 3) over distance from shore at Gognga Beach	16
Figure 4: Gognga Beach sedimentary N_2 fixation rates (means \pm 1 S.D., n = 9) vs. porewater $NO_3^- + NO_2^-$ and salinity (means, n = 3)	17
Figure 5: Effects of artificial dilutions of seawater on sedimentary N_2 fixation rates (means \pm 1 S.D., n = 6 or 7)	18

INTRODUCTION

Although coral reef waters typically contain very little combined inorganic nitrogen, productivity is characteristically very high (Odum and Odum, 1955; Johannes *et al.*, 1972). This apparent paradox suggests that (1) efficient recycling of N occurs, (2) present analytical methods are inadequate, (3) the wrong N compounds are being measured, or (4) some combination of these possibilities. Coral reefs generally receive N compounds from upwelling and advection of open ocean water, terrestrial runoff, and *in situ* N₂ fixation (Weibe *et al.*, 1975).

Surface ocean water typically contains only trace amounts of nutrients, however, flow of large quantities of ocean water over reefs may deliver substantial amounts (D'Elia, 1988). Upwelling may deliver large amounts of nutrients, but upwelling is not prevalent at oceanic high islands or atolls (Birkeland, 1984). Terrestrial runoff, however, can supply important quantities of nutrients to coral reefs and other nearshore zones. In addition, reefs in the vicinity of high islands that have porous carbonate substrata are continually supplied with NO₃⁻-rich ground water. This source may represent a significant input of combined N to high island coral reefs (Marsh, 1977; D'Elia *et al.*, 1981).

On reefs in otherwise oligotrophic waters, N₂ fixation may contribute important quantities of combined N. Studies at Enewetak Atoll showed that N₂ fixation in cyanobacterial mats contributed much to coral reef community N requirements (Webb *et al.*, 1975; Weibe *et al.*, 1975; Mague and Holm-

Hansen, 1975; Potts and Whitton, 1977). Studies of reef zones at Shark Bay (Australia), Christmas Island, Canton Atoll (Smith, 1984), and at St. Croix, U.S. Virgin Islands (Adey and Steneck, 1985) concluded that N_2 fixation must contribute the available N because inputs from other sources were so low.

Factors that affect rates of N_2 fixation may therefore be of particular importance to reef productivity. Previous work has examined effects on N_2 fixation of the availability of molybdenum, iron, phosphorus, sulfate, organic matter, carbon and salinity, and O_2 -low "microzones" (Jones, 1974; Howarth and Cole, 1985; Paerl, 1985; Marino *et al.*, 1990; Paulson *et al.*, 1991). Traditionally, however, attention has been more focused on the effects of the availability of other types of N on rates of N_2 fixation. For example, a steady supply of reduced N compounds may inhibit N_2 fixation perhaps by providing a less energetically costly form of N to use (Fogg, 1949; Van Raalte *et al.*, 1974; Stewart *et al.*, 1975; Bothe, 1982; Falkowski, 1983; Howarth *et al.*, 1988). Inputs of sewage sludge and urea to estuarine salt marshes lowered N_2 fixation within the sediments (Van Raalte *et al.*, 1974). Stewart *et al.* (1975) found that NH_4^+ decreased N_2 fixation by some cyanobacteria by inhibiting the formation of heterocysts which are the site of N_2 fixation. Conversely, in the absence of combined N some species of cyanobacteria form heterocysts (Fogg, 1949).

Since high island coastal waters generally receive combined N from runoff, N_2 fixation rates in near shore reefs and sediments may be affected. Also, rain water that percolates through porous carbonate leaches nitrate from topsoil, percolates through the substrata and rests upon denser, underlying seawater. Ground water leaks out of these Ghyben-Herzberg aquifers onto beaches and upwells through intertidal sediments and enriches the interstitial waters with $NO_3^- + NO_2^-$ and dilutes the salinity of the interstitial water (Johannes, 1980; D'Elia, 1981; Lewis, 1987).

In northern Guam, the delivery of NO_3^- -rich aquifer water and accompanying enrichment of sediments and porewaters is quite extensive. Guam aquifer water near Tumon Bay contains *ca.* $114\text{-}\mu\text{M}$ $NO_3^- + NO_2^-$ and is a major source of fixed N for nearshore areas (Matson, 1991). No known studies to date have examined the potential effects of groundwater intrusion on N_2 fixation rates in coastal sediments. I hypothesized that N_2 fixation rates in nearshore sediments affected by groundwater intrusion are lowered by the occurrence of this groundwater NO_3^- , the dilution of seawater, or both; and that therefore terrestrial NO_3^- might replace *in situ* N_2 fixation as a major N source. To test this, I examined (1) N_2 fixation rates in reef sediments naturally subjected to continuous high nitrate delivery *via* groundwater intrusion, and (2) potential changes in N_2 fixation rates that may be additionally attributed to freshwater intrusion, nutrient content, or sediment grain sizes.

MATERIALS AND METHODS

Study site

The northern half of Guam is composed nearly entirely of upraised limestone. This porous substratum provides for an extensive aquifer system, a Ghyben-Herzberg lens, from which NO_3^- -rich water leaks to the beaches and sediments of the moats and reefs of northern Guam. Appreciable amounts of these aquifer waters drain and seep into northeastern Tumon Bay at Gognga Beach (Fig. 1) and are transported through the estuarine moat and to the reef flat largely in tidal and wind driven circulation.

Sediment and water sampling

To determine if sedimentary N_2 fixation was affected by aquifer water intrusion, sediment organic or ash (inorganic) content, or grain size, sediments and pore water were collected on a 300 m transect from the low water mark to the inner reef flat at Gognga Beach (Fig. 1). Three sediment cores and one porewater sample were collected at 5 sites (0, 25, 50, 200, and 300 m) along this transect. This sampling regime was repeated three times between May and June, 1992.

Sediments were collected with core samplers made from 50 ml PlastiPak[®] syringes with the closed end cut off. Samples were collected by pushing the samplers about 5 cm into the sediment and then capping the ends with rubber stoppers. The sediment cores were kept submerged in a cooler with ambient sea water and immediately (*ca.* 1 h) returned to the

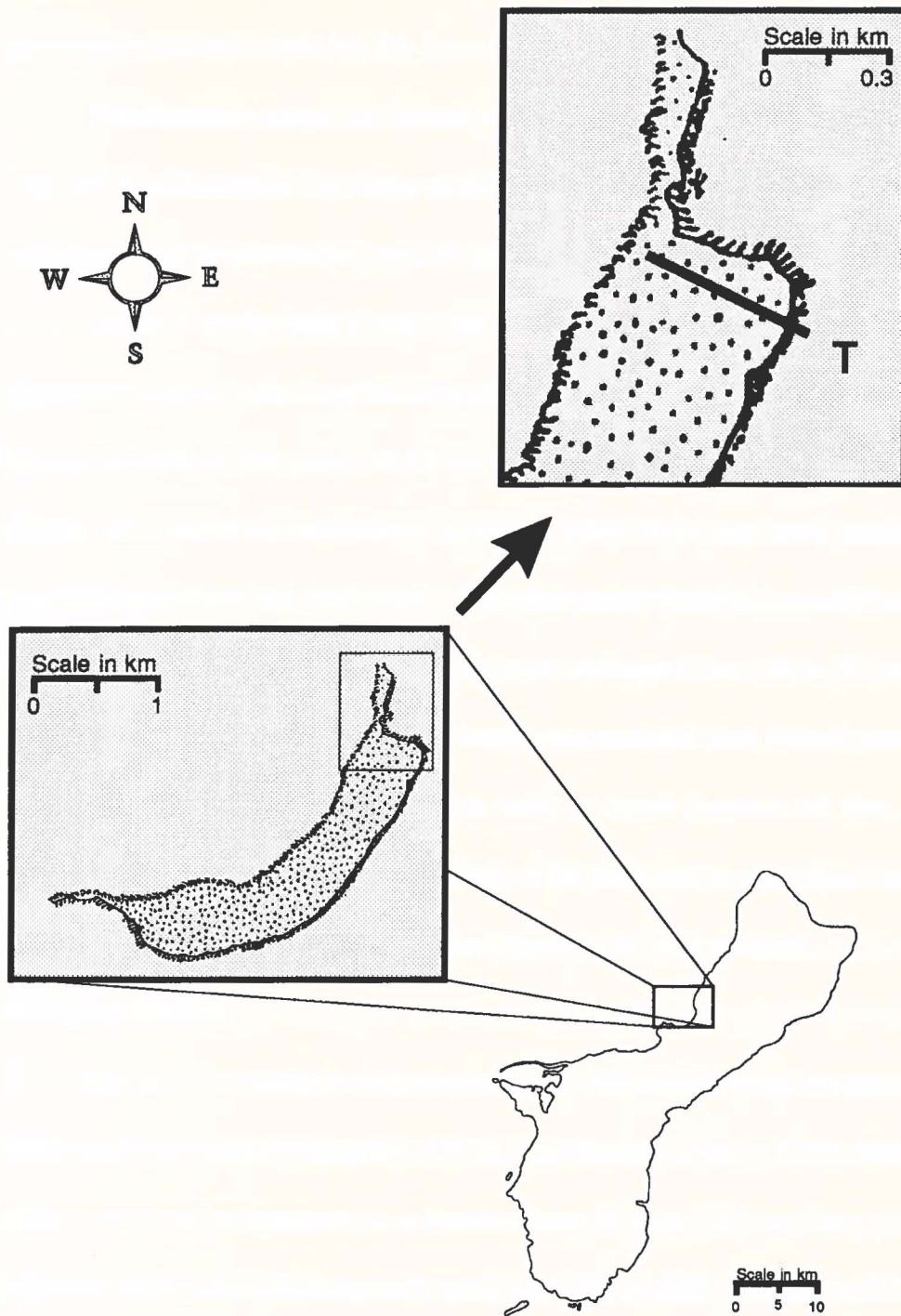


Figure 1. Map of the study site, Gognga Beach and transect T, in Tumon Bay, Guam.

University of Guam Marine Laboratory and equilibrated (uncapped) in a flow-through seawater tank for 24 h.

Porewaters were collected with a sampler similar in design to the "spear" described by Corredor and Morell (1985). It consisted of an 80 cm length of polyvinylchloride pipe (3 cm in diameter) capped shut at the top and covered with perforated plexiglass at the obliquely-cut bottom end. Plastic aquarium tubing was inserted into the side of the top of the pipe and pushed to the bottom of the pipe. A 50 ml syringe attached to the distal end of the tubing was used to withdraw samples after the pipe was pushed to the desired depth into the sediment. Samples were filtered immediately through rinsed, sterile Millex 0.22 μm pore size syringe filters into 30 ml Nalgene polyethylene sample bottles that had been soaked and rinsed several times with ambient seawater then once with a small portion of the porewater sample that had just been collected. The porewater samples were sealed, submerged, transported to the laboratory, and refrigerated.

Nitrogen fixation

N_2 fixation rates in sediments were calculated from measurements of acetylene reduction (Postgate, 1972; Van Raalte et al., 1974) detected with a flame ionization detector on a Perkin-Elmer Sigma 300 capillary gas chromatograph with a 2 m Spherocarb column (100 to 120 mesh), at 175 °C. The theoretical ratio of 3 mol C_2H_2 reduced to 1 mol N_2 fixed (Howarth et al.,

1988) was used to convert to N_2 fixed which was expressed as $\eta\text{mol } N_2 \text{ fixed g dry sediment}^{-1} \text{ h}^{-1}$ based on the 48 h incubation period.

Sedimentary N_2 fixation was measured immediately following the 24 h equilibration period. The sediment samplers were made into air-tight systems that allowed injection and withdrawal of gases *via* syringe by inserting a 4 cm length of tubing that had been fitted with a serum stopper through a hole in the top stopper. Sediment tubes were capped and 10 to 15 ml of sea water and 10 to 15 ml headspace air were allowed to remain. Three ml of headspace air was withdrawn and replaced with 3 ml of acetylene generated from commercial calcium carbide in deionized water through the serum stopper. The tubes were shaken for 30 s, and incubated for 48 h in an outdoor flow-through seawater tank to maintain natural temperature and light conditions.

After the incubation period, an aliquot of gas was withdrawn from each sediment tube. To collect and store gas samples, 2 ml glass scintillation vials were filled with distilled water and capped with rubber serum stoppers. Gas samples were injected into the vials and the water was displaced through a syringe needle stuck through the stopper. These were stored under refrigeration for less than one week until ethylene concentration was measured. Immediately following collection of gas samples, sediments were dried at 50 °C, and weighed.

Controls for N₂ fixation measurements included sediment cores wrapped in black electrical tape (to estimate non-photosynthetic N₂ fixation), and sediment cores without acetylene (to measure for natural production of ethylene). Additionally, reef water without sediments was tested to estimate possible N₂ fixation in the water column.

Sediment chemistry and grain size

Subsamples of sediment from above were analyzed for percent organic content, and percent ash (inorganic) content. Total organic and ash content were determined by first weighing 4 to 6 g of each sample into a beaker and then adding sufficient 20% HCl over a 24 to 48 h period to dissolve the carbonate fraction. The residual material was collected on precombusted (500 °C for *ca.* 2-h) and preweighed Gelman type A/E glass fiber filters and dried at 50 °C. The residue was then combusted at 500 °C for 2 h and reweighed. This remaining material was considered to be ash and the material lost after combustion was considered to be organic matter (Matson, 1989). Sediment grain size was measured by shaking the remaining core sample through sediment sieves (1.905, 0.447, 0.116, and 0.038 mm). Each fraction was weighed and relative percentage was determined.

Water chemistry

To determine the extent of aquifer water intrusion at Gognga Beach, porewater was analyzed for $\text{NO}_3^- + \text{NO}_2^-$ by shaking with cadmium (Jones, 1984), and salinity of interstitial water was determined with a refractometer.

Effects of artificial enrichment and dilution

Because porewaters at the study site were diluted with brackish, NO_3^- -rich aquifer water, laboratory experiments were performed to test N_2 fixation in sediments exposed to artificially raised NO_3^- concentrations and exposed to artificially lowered salinity. Sediment cores were collected 100 m from shore at Gognga Beach, and transported immediately to the laboratory. Cores were then placed (uncapped) in triplicate or quadruplicate in each of 4 enrichment chambers (5 gallon buckets) that contained 0, 25, 50, and 100 μM NaNO_3 in 12 l of full-strength seawater. To ensure uniform distribution of enriched seawater through the sediment, the tubes were initially removed, shaken, and returned to their respective chambers. Air was bubbled into the chambers for circulation. After a 3 d equilibration period, N_2 fixed was measured as described previously. Enrichment experiments were done twice, each time with new sediments.

To determine the affects of reduced salinity on sedimentary N_2 fixation, new sediments were collected and the same procedures were followed. Salinities of 0, 10, 20 and 35 ‰ sea water were prepared with ambient

seawater and deionized water. Dilution experiments were performed twice, each time with new sediments.

Data analyses

Sedimentary N_2 fixation rates, organic content, ash content, and porewater $NO_3^- + NO_2^-$ and salinity were examined for changes over distances from shore. Means of N_2 fixation rates were examined for changes associated with changes in organic content, ash content, concentration of pore water $NO_3^- + NO_2^-$, and salinity. Means of N_2 fixation rates in artificial enrichment and dilution treatments of seawater were examined for changes associated with varying concentrations. The Welch equality of means test (BMDP statistical analysis software) was used to determine if differences were significant ($p < 0.05$)

RESULTS

Sedimentary N₂ fixation

Sedimentary N₂ fixation rates increased significantly ($F_{4,16} = 9.69$, $p = 0.000$) with distance from shore and the highest rates were observed between 50 m ($0.0095 \text{ } \eta\text{mol g}^{-1}\text{h}^{-1}$) and 300 m ($0.016 \text{ } \eta\text{mol g}^{-1}\text{h}^{-1}$, Fig. 2). No significant differences were observed between N₂ fixation rates of sediments collected at high and low tides. No detectable N₂ fixation occurred in the dark tubes or in tubes with sea water alone.

Sediment chemistry and grain size

Data from analyses of organic and ash contents are presented in Table 1. Organic and inorganic contents did not change significantly with distance from shore ($F_{4,19} = 0.11$, $p = 0.978$, and $F_{4,19} = 1.17$, $p = 0.354$ respectively), and did not correlate with N₂ fixation rates. Examination of sediment grain sizes showed that although percentages of size fractions changed significantly between distances (Table 2), sizes were not correlated with rates of N₂ fixation.

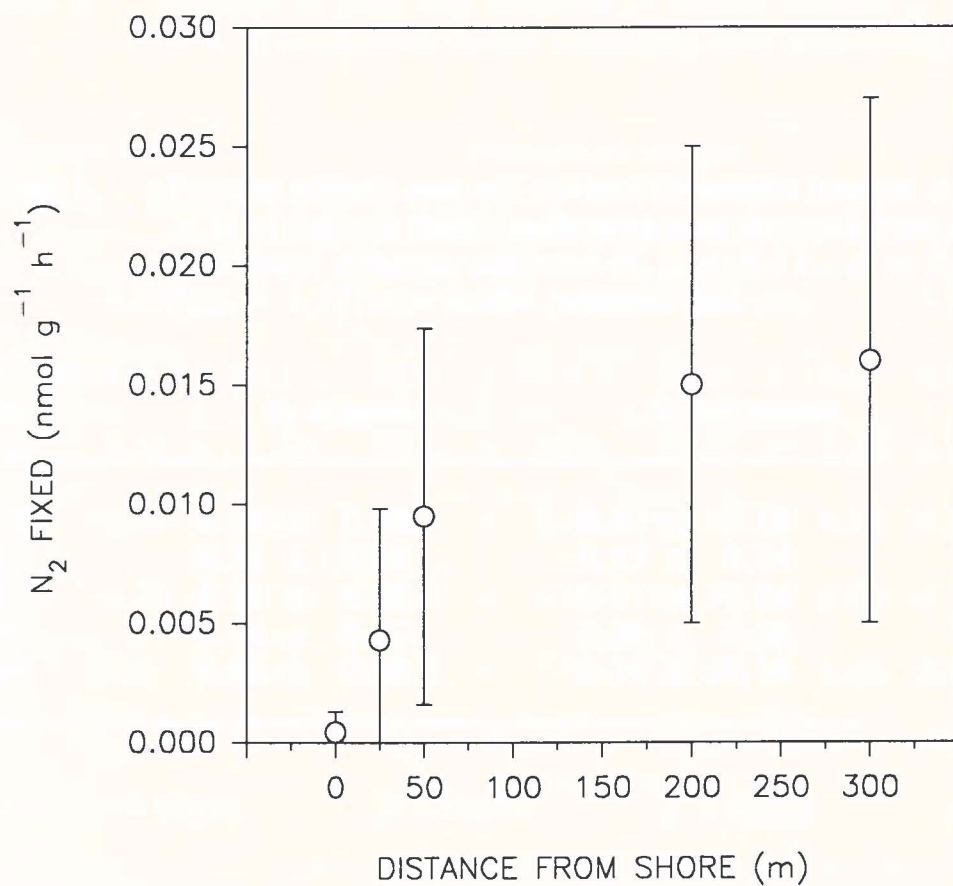


Figure 2. Sedimentary N₂ fixation rates (means \pm 1 S.D., n = 9) over distance from shore at Gognga Beach. Rates increased significantly over distance ($F_{4,16} = 9.69$, $p = 0.000$, Welch equality of means test).

Table 1. Percent organic and ash content by weight (means \pm 1 S.D., n = 9) of Gognga Beach sediments over distance from shore. No significant changes occurred ($F_{4,19} = 0.11$, $p = 0.978$, and $F_{4,19} = 1.17$, $p = 0.354$ respectively).

m from shore	% organics	% inorganics
0	0.59 \pm 0.38	0.33 \pm 0.19
25	0.71 \pm 0.85	0.27 \pm 0.34
50	0.71 \pm 0.66	0.31 \pm 0.26
150	0.74 \pm 0.57	0.38 \pm 0.38
300	0.65 \pm 0.26	0.16 \pm 0.16

Table 2. Percent of grain size fractions by weight (means \pm 1 S.D., n = 9) of Gognga Beach sediments over distance from shore.

Distance (m)	Percent by weight			
	0.04 mm	0.12 mm	0.44 mm	1.90 mm
0	24.4 \pm 13.1	33.2 \pm 10.6	38.0 \pm 13.6	4.42 \pm 2.10
25	4.11 \pm 1.16	39.4 \pm 6.02	52.0 \pm 5.36	4.41 \pm 1.22
50	2.41 \pm 2.02	27.2 \pm 5.60	63.7 \pm 6.12	6.67 \pm 1.78
200	4.70 \pm 3.75	25.8 \pm 6.71	64.7 \pm 8.02	4.79 \pm 1.08
300	15.0 \pm 11.4	59.8 \pm 7.51	22.2 \pm 5.90	2.92 \pm 1.16
	$F_{4,18} = 8.17$	$F_{4,20} = 31.25$	$F_{4,20} = 63.72$	$F_{4,20} = 6.85$
	$p = 0.000$	$p = 0.000$	$p = 0.000$	$p = 0.001$

Porewater chemistry

Porewater $\text{NO}_3^- + \text{NO}_2^-$ concentration and salinity changed significantly over distance from shore (Fig. 3, $F_{4,4} = 32.2$, $p = 0.002$, and $F_{4,7} = 272$, $p = 0.000$ respectively). Highest concentrations of $\text{NO}_3^- + \text{NO}_2^-$ were at 0 m ($120 \pm 20 \mu\text{M}$) and decreased by half at 25 m then dropped to low levels after 50 m ($5.6 \pm 5.2 \mu\text{M}$). Salinity of porewaters was lowest at 0 m (10 ‰), increased at 25 and 50 m and was equal to reef water at 200 and 300 m (35 ‰). Figure 4 shows significant changes in N_2 fixation rates with means of porewater $\text{NO}_3^- + \text{NO}_2^-$ concentrations ($F_{4,16} = 9.69$, $p = 0.000$) and salinities ($F_{4,16} = 9.69$, $p = 0.000$).

N_2 fixation with NO_3^- enrichment and fresh water dilution

NaNO_3 concentrations up to $100 \mu\text{M}$ had no effect on N_2 fixation ($F_{3,12} = 0.36$, $p = 0.784$). Seawater dilutions significantly affected N_2 fixation rates (Fig. 5, $F_{3,12} = 5.39$, $p = 0.014$). Highest rates were observed in full strength seawater ($6.0 \times 10^{-3} \pm 3.5 \times 10^{-3} \eta\text{mol g}^{-1} \text{h}^{-1}$). Lowest rates were in 0 ‰ ($0.95 \times 10^{-3} \pm 1.6 \times 10^{-3} \eta\text{mol g}^{-1} \text{h}^{-1}$).

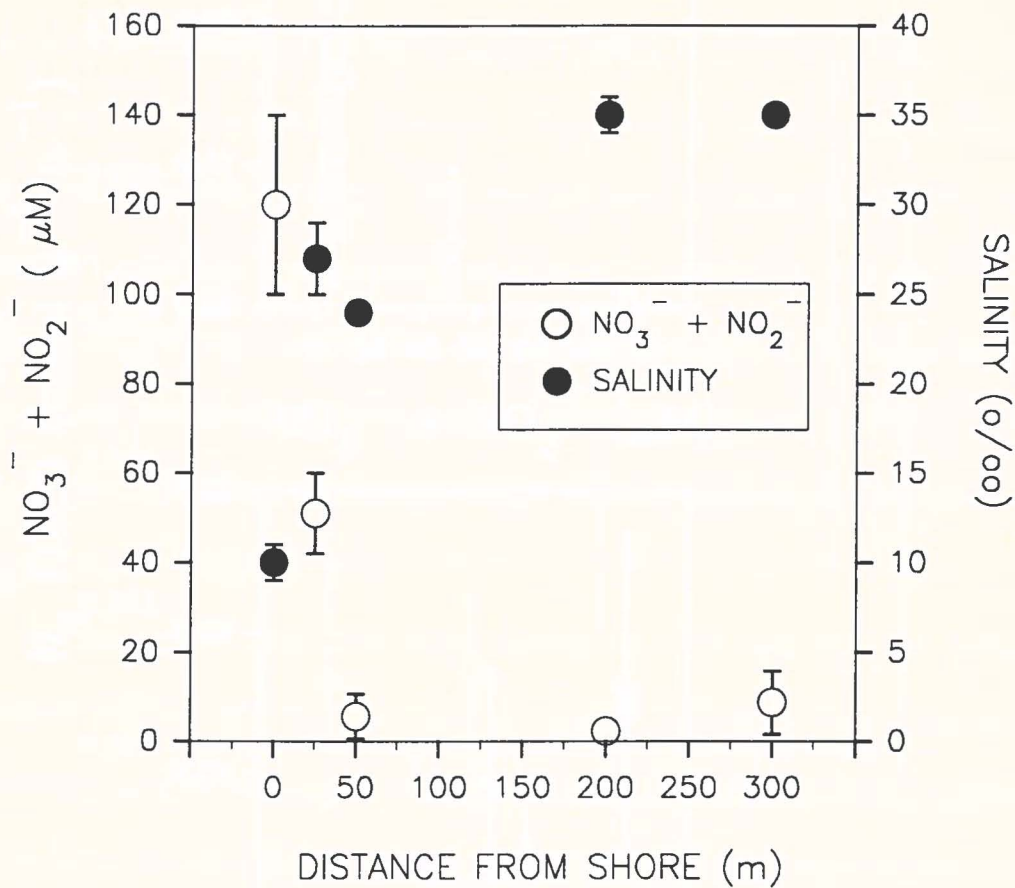


Figure 3. Pore water $\text{NO}_3^- + \text{NO}_2^-$ and salinity (means \pm 1 S.D., $n = 3$) over distance from shore at Gognga Beach. Concentrations of each changed significantly over distance ($F_{4,4} = 32.2$, $p = 0.002$, and $F_{4,4} = 272.2$, $p = 0.000$, Welch equality of means test).

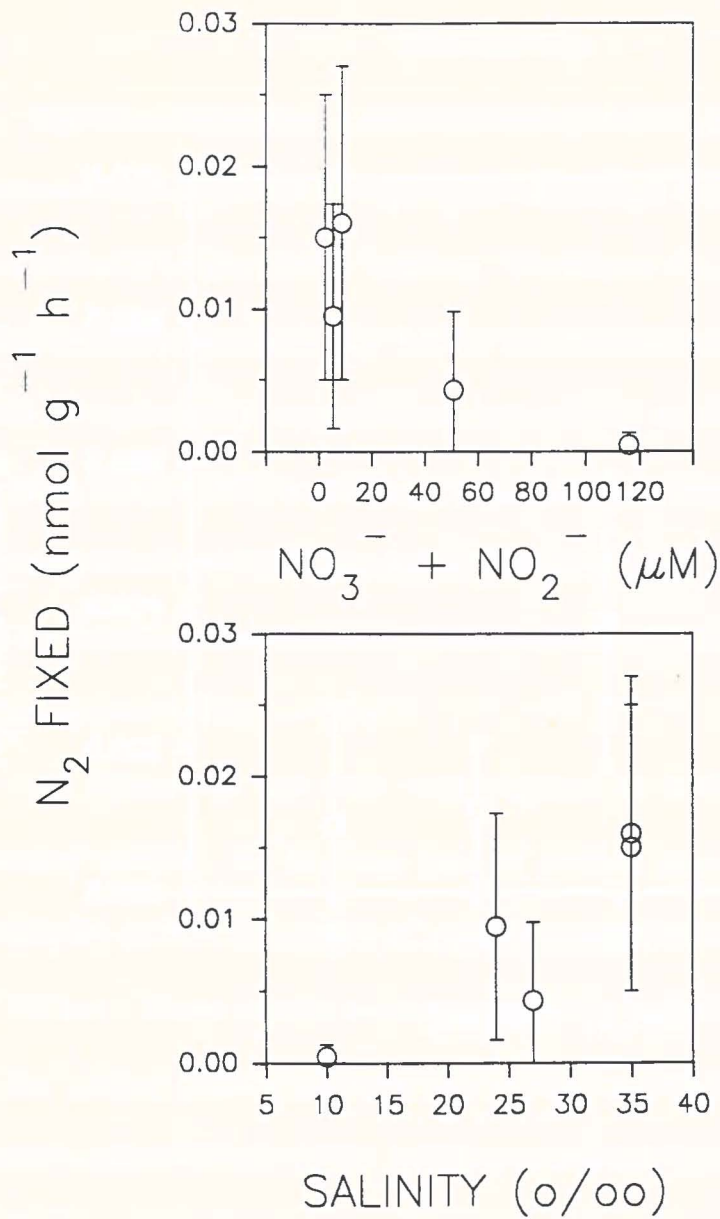


Figure 4. Gognga Beach sedimentary N₂ fixation rates (means \pm 1 S.D., n = 9) vs. porewater NO₃⁻ + NO₂⁻ and salinity (means, n = 3). Rates changed significantly between concentrations ($F_{4,16} = 9.69$, $p = 0.000$, Welch equality of means test).

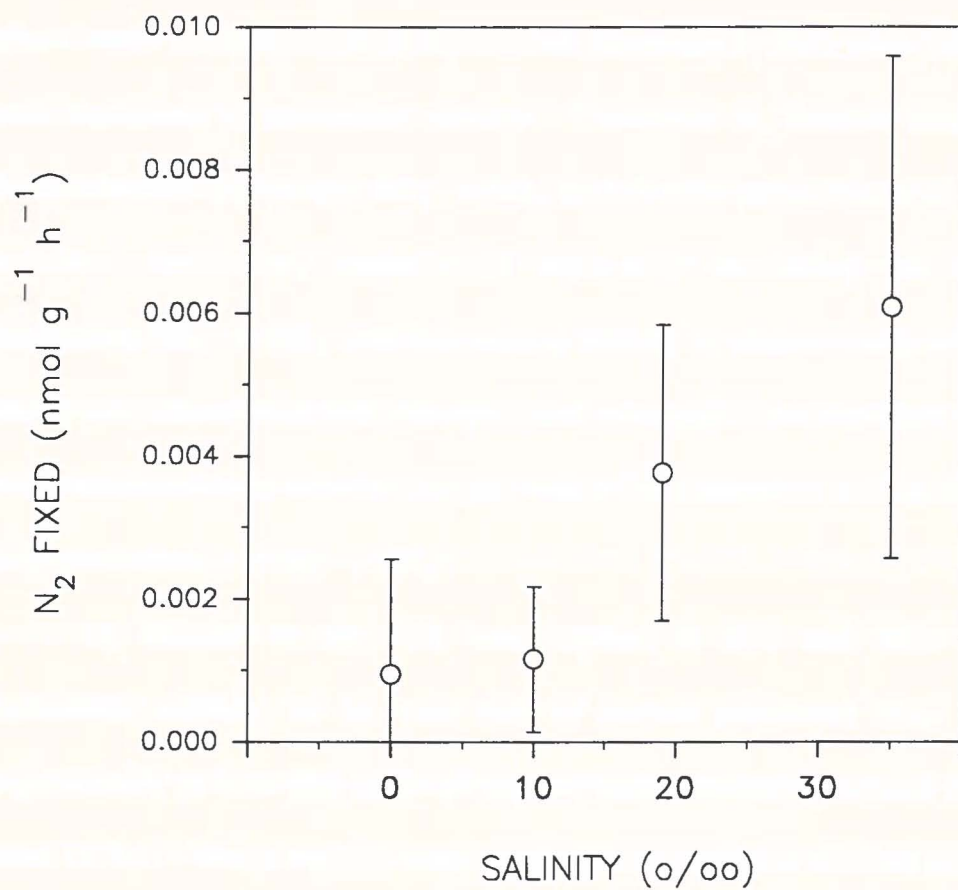


Figure 5. Effects of artificial dilutions of seawater on sedimentary N₂ fixation rates (means \pm 1 S.D., $n = 6$ or 7). Rates changed significantly between salinities ($F_{3,12} = 5.39$, $p = 0.014$, Welch equality of means test).

DISCUSSION

Significant increases in sedimentary N_2 fixation between distances from shore were accompanied by significant increases in porewater $NO_3^- + NO_2^-$ and decreases of porewater salinity. Therefore, N_2 fixation was inversely proportional to porewater $NO_3^- + NO_2^-$ and directly proportional to porewater salinity. Laboratory experiments separately examining the effects of increasing $NO_3^- + NO_2^-$ and decreasing salinity to match levels found at the study site determined that the significant changes in sedimentary N_2 fixation were attributable to salinity changes within the porewater and not to elevated concentrations of $NO_3^- + NO_2^-$.

N_2 fixation in the field was highest at 200 and 300 m (where porewater salinity, 35 ‰, was that of normal seawater) then decreased significantly at 0 m (the point of aquifer water discharge where the salinity was 10 ‰). These results were duplicated in artificial dilution experiments (Fig. 5). Many marine bacteria are unable to tolerate lower than normal seawater salinity (Pratt, 1974) which may explain the low rates of N_2 fixation in sediments at 0 m.

Experiments artificially increasing $NO_3^- + NO_2^-$ did not change N_2 fixation rates. Fixed N compounds (NH_4^+ , NO_3^- , urea, amino acids, etc.) reduce N_2 fixation by inhibiting the synthesis of nitrogenase (Fogg, 1949). However, all N compounds do not elicit the same rates of reduction in N_2

fixation. Generally, in cyanobacteria, inhibition of N_2 fixation by NH_4^+ proceeds more rapidly than inhibition by NO_3^- . N_2 fixing activity in the cyanobacterium, *Anabaena cylindrica*, is abolished within 48 h by NH_4^+ and is reduced by NO_3^- only when exposed to it for weeks (Stewart *et al.*, 1975). This is because *A. cylindrica* lacks an active transport system that can handle greater than $10 \mu M NO_3^-$ (Meeks, 1979). Thus the repression of nitrogenase synthesis by NO_3^- may be limited by the rate of NO_3^- uptake (Bothe, 1982). Further, Wheeler and Kirchman (1986) determined that some marine bacteria preferentially assimilate more reduced form of N (such as NH_4^+ and amino acids) over NO_3^- and that growth yields of phototrophic bacteria in NO_3^- are considerably less than in more reduced forms of N (Drews and Imhoff, 1991).

Sediments for the artificial NO_3^- enrichment experiment were collected at 100 m (where groundwater upwelling is less than nearshore) and were exposed to high NO_3^- for only 72 h. This may explain the lack of inhibition of N_2 fixing activity in the lab. Sediments at 0 m, however, are continuously subjected to aquifer water runoff and thereby exposed to high NO_3^- . If the uptake of NO_3^- is limited in N_2 fixers in 0 m sediments, the continuous exposure to NO_3^- by aquifer water intrusion may affect N_2 fixation rates.

In addition to diluting the salinity of porewaters, aquifer water also supplies O_2 to porewaters (Matson, 1989). This may also inhibit N_2 fixation,

since O_2 strongly inhibits nitrogenase synthesis (Bergersen, 1962; Fay, 1992). Additionally, upwelling groundwater, primarily in discreet seeps at the shore line, may provide further aeration by the physical disturbance of the sediments thereby reducing N_2 fixation. In oxygenated, aquatic environments, cyanobacteria-bacteria aggregates may form highly reduced (low O_2) microenvironments in which N_2 fixation is stimulated (Paerl, 1985). Formation of these "microzones" is limited by turbulence resulting in the inhibition of N_2 fixation. In this study, long incubation periods (up to 48 h) may have created anoxic conditions in the sediment tubes resulting in overestimations of N_2 fixation rates.

Aquifer water intrusion and resulting delivery of freshwater and nutrients to reef sediments at Gonga Beach is extensive (Table 3). D'Elia *et al.* (1981) found that inputs of NO_3^- from seepage of groundwater may be an important source of fixed N for high islands coral reefs. Groundwater runoff is not uniform throughout Tumon Bay (Marsh, 1977; Matson, 1989). The northeastern end of the bay, Gonga Cove, contains the highest number of aquifer seeps and therefore receives the most $NO_3^- + NO_2^-$. Circulation of water southward driven by rising tides (Marsh *et al.*, 1981) then disperses $NO_3^- + NO_2^-$ throughout the bay. Tumon Bay receives *ca.* 5 m^3 per day per m of shoreline of aquifer water that averages $114\ \mu\text{M } NO_3^- + NO_2^-$ (Matson, 1989). This results in roughly $17,500\text{ Kg N yr}^{-1}$ entering Tumon Bay *via*

groundwater runoff, exclusive of aquiferwater upwelling through subsurface fissures and cracks in the intertidal zone.

By multiplying the average rate of N_2 fixed $m^{-2} day^{-1}$ by the total area of Tumon Bay then by 76% (the percent cover of sediment in Tumon Bay), the total amount of N made available *via* N_2 fixation primarily as peptides and amino acids (Jones, 1974) in the top 5 cm of sediment in Tumon Bay was determined to be *ca.* 164 Kg yr^{-1} . Matson (1991) reported an average of $2.10 \mu M NO_3^- + NO_2^-$ in Tumon Bay and $1.90 \mu M NO_3^- + NO_2^-$ offshore of Tumon Bay. Given a 0.7 m mean tidal amplitude (Matson personal communication) the volume of water brought in by the high tide to replace water drained at low tide can provide substantial amounts of $NO_3^- + NO_2^-$ even with the relative low concentration of $NO_3^- + NO_2^-$ compared to that of groundwater runoff. Estimates of volume displacement determined here result in *ca.* 41.3 Kg N delivered with each high tide. The net delivery of $NO_3^- + NO_2^-$ (amount delivered at high tide - amount lost at low tide) may not, however, be determinable since $NO_3^- + NO_2^-$ from aquifer leakage is exported from northern Guam into coastal ocean waters (Matson, 1991) and has not yet been distinguished from $NO_3^- + NO_2^-$ in upwelling nutrient-rich thermocline waters.

The delivery of fixed N to Tumon Bay *via* runoff of ground water more than compensates for the apparent inhibition of sedimentary N_2 fixation. In

this study, highest rates of N_2 fixation were found where little or no aquifer water was present. These rates, however, make available far less fixed N than ground water seepage and may not contribute significant amounts to the rest of Tumon Bay.

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