

**PROCEEDINGS  
OF THE  
NINTH SYMPOSIUM  
ON THE  
NATURAL HISTORY OF THE  
BAHAMAS**

**Edited by:**

David L. Smith  
Sherilyn Smith

**Conference Organizer**

Kenneth C. Buchan

**Production Editors**

David L. Smith  
Sherilyn Smith  
Vincent J. Voegeli

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# USING $\delta^{15}\text{N}$ OF MARINE PLANTS TO UNDERSTAND TERRESTRIAL NITROGEN SOURCES IN AN OLIGOTROPHIC SHALLOW WATER MARINE SYSTEM, SAN SALVADOR, BAHAMAS

Scott D. Wankel

Stanford University, California, USA

## ABSTRACT

Recently there has been increasing concern regarding the role of terrestrial anthropogenic nutrient sources in the ecology of oligotrophic marine systems. San Salvador Island, Bahamas, represents a nutrient-depleted carbonate marine system deriving much of its nitrogen from nitrogen fixing epiphytic and subsurface cyanobacteria. Because San Salvador has no freshwater streams able to transport large amounts of terrestrial nitrogen, riverine inputs do not contribute significantly to marine dissolved inorganic nitrogen (DIN). On the north end of the island, however, a domestic sewage outfall represents a potentially significant anthropogenic source of DIN. The  $\delta^{15}\text{N}$  value of a sewage source is usually higher than organic N or DIN from N fixation. The aim of this pilot study was to assess the usefulness of  $\delta^{15}\text{N}$  analysis of various marine plant species to identify and characterize the distribution of terrestrial and anthropogenic nutrient sources.

Since macroalgae and seagrass are sessile, and therefore represent relatively long-term indicators of nutrient sources in the water column, it was hypothesized that the  $\delta^{15}\text{N}$  of the marine plants growing in areas receiving relatively  $^{15}\text{N}$ -enriched terrestrial nutrients (i.e. sewage inputs, etc.) would reflect this source of DIN. This pilot study was designed as a quick and inexpensive assessment of the usefulness of  $\delta^{15}\text{N}$  for differentiating among deep marine, coastal marine, terrestrial and anthropogenic nitrogen sources. Samples were collected from 17 sites around the island in an effort to characterize the spatial patterns of  $\delta^{15}\text{N}$  in these shallow, nutrient poor waters surrounding San Salvador.

## INTRODUCTION

Worldwide, researchers have documented dramatic declines in the health of coral reef ecosystems. Investigations into the exact causes for declines in reef health have revealed various interacting elements, including disease, changes in sea surface temperatures and eutrophication. Oligotrophic coastal environments are especially sensitive ecosystems, which are easily perturbed by increases in nutrient loading. Although San Salvador is a small and relatively isolated island, terrestrial sources of nutrients from the island, perhaps both natural and anthropogenic, may represent relatively localized significant nutrient sources to its coastal system. Recent declines in coral reef species diversity and abundance on San Salvador seem to be correlated with increases in macroalgae growth around and on reef surfaces suggestive of increased nutrient concentrations.

Stable nitrogen isotopes are a useful tool for tracing nutrient sources and cycling in aquatic systems (Caraco *et al.* 1998, Cifuentes *et al.* 1988, Hall *et al.* 1999, Mariotti *et al.* 1984, McClelland and Valiela 1998, Peterson and Howarth 1987, Rivera-Monroy *et al.* 1995, Rogers 1999, Spies *et al.* 1989, Van Dover *et al.* 1992, Wainright *et al.* 1996). They have been used to help distinguish between marine and terrestrial nutrient inputs as well as to elucidate foodweb structure. Although the biogeochemical processes (e.g. denitrification, nitrification and ammonia volatilization) that alter dissolved inorganic nitrogen in groundwater, surface water and ocean water can be complex, many researchers have been successful with the use of  $\delta^{15}\text{N}$  as a tracer of terrestrially derived DIN. The development of continuous flow isotope ratio mass spectrometry

has allowed quick, low cost analyses of nitrogen isotopes for use in ecological studies.

Tropical waters generally represent very nutrient-depleted environments (Berner 1990, D'Elia and Wiebe 1990), yet both coral reef and seagrass communities have been shown to exhibit high primary productivity (Capone *et al.* 1979) and even net export of nitrogen (Johannes *et al.* 1972). This situation implies that these communities have very efficient means for absorbing (and recycling) nitrogen from nutrient-poor water and/or that there is a significant input of nitrogen from N-fixing organisms (Capone and Taylor 1977, Capone *et al.* 1977, Mague and Holm-Hansen 1975). Indeed, both the limestone substrate of coral reefs and the leaf surfaces of seagrass meadows support a wide variety of N-fixing cyanobacteria (Goldner 1980, Mague and Holm-Hansen 1975, McRoy *et al.* 1973, Wiebe *et al.* 1975).

Very little research has been done using stable nitrogen isotopes to trace nutrient sources in the oligotrophic waters of the Bahamas. The aim of this study was to assess the usefulness of  $\delta^{15}\text{N}$  analyses in determining nitrogen sources and cycling mechanisms in relatively isolated tropical marine systems – as represented by San Salvador. It was hypothesized that sewage inputs supply a disproportionately large amount of inorganic nutrients to the surrounding coral reef systems of San Salvador. Nitrogen isotope analyses of various marine plant species were assessed to determine both source- and species-specific differences in  $\delta^{15}\text{N}$ . In particular, sewage treatment effluent at the northern end of the island was expected to contrast well with plant  $\delta^{15}\text{N}$  values regulated largely by nitrogen fixation. Species-specific differences in  $\delta^{15}\text{N}$  values are presumably related to preferential source utilization and/or species-specific fractionation factors. This study provides a baseline of information about San Salvador for future studies involving stable nitrogen isotopes and nutrient cycling in other tropical carbonate marine systems.

#### Nitrogen Biogeochemistry

Biosynthetic processes in plants generally result in decreased  $\delta^{15}\text{N}$  values as photosynthesis fractionates (through various

enzymatic pathways) the available nutrients and preferentially uses  $^{14}\text{N}$ . However, photosynthetic fractionation tends to be low during periods of high growth rates or under nutrient limited conditions (Fogel and Cifuentes 1993). Therefore, in a nutrient poor system, photosynthetic fractionation should be near a minimum. Thus, under the oligotrophic conditions of San Salvador, the isotopic composition of the marine algae should be close to the  $\delta^{15}\text{N}$  values of the DIN sources being utilized.

Nitrogen isotopic composition of N-fixing cyanobacteria generally mimics atmospheric  $\text{N}_2$   $\sim 0.0\text{‰}$  (France *et al.* 1998, Wada and Hattori 1976). In contrast, pelagic phytoplankton, which generally utilize nitrogen that is upwelled from deepwater sources in the form of nitrate tend to be relatively  $^{15}\text{N}$ -enriched (as high as 18‰). The isotopic composition of oceanic biogenic nitrogen in tropical oligotrophic systems average around 5 – 10‰ (France 1995, Van Dover *et al.* 1992, Wada 1980). Therefore such a source should be isotopically distinct from nitrogen originating from bacterial fixation. In the Gulf of Mexico, for example,  $\delta^{15}\text{N}$  values of 0.4 to 2.3‰ were found in *Halodule wrightii* (seagrass) communities at Laguna Madre, whereas phytoplankton from a coastal area just outside the bay showed high  $\delta^{15}\text{N}$  values of 8 to 9.3‰ showing the strong distinction between oceanic nitrate and coastal N-fixing isotopic signatures in tropical systems (Wada 1980, Yamamuro *et al.* 1995).

Seagrass and macroalgae communities comprise many plant species that are capable of utilizing bacterially fixed sediment nitrogen. The algae and seagrass sampled in this study included both epilithic and benthic macroalgae species. Although epilithic algae (such as *Padina sanctae-crucis*) are restricted to extracting nutrients exclusively from the water column, benthic primary producers exhibiting rhizoidal growth forms (such as *Halimeda incrassata*, *Udotea flabellum* and *Thalassia testudinum*) can potentially obtain nutrients from the pore waters by use of a system of root-like rhizoid structures (Littler and Littler 1990). Capone *et al.* (1979) estimated that 25 - 50% of the nitrogen demand for seagrass meadow leaf

production was supplied by microbial anaerobic sediment N-fixation in the rhizosphere. Thus comparison of the epilithic species *P. sanctae-crucis* with the other rhizoidal species also offers a perspective on the utilization of pore water nutrients by these macroalgae.

## METHODS

### Study Area

The Commonwealth of the Bahamas is situated in the subtropical southeast Atlantic Ocean and consists of approximately 700 islands and 2,400 cays. This carbonate archipelago is located approximately 130km southeast of Florida, USA. San Salvador is a small island (population approx. 800) located in the southeastern portion of the Bahamas (24°3'N; 74°30'W) isolated from the larger shallower carbonate platforms to the northeast and surrounded by 4000m-deep water.

The island is approximately 11km (E-W) and 19km (N-S) and is surrounded by a series of fringing reefs that form several protected embayments. These bays include Graham's Harbor and Rice Bay in the north, Long Bay in the east, Snow Bay and French Bay in the south and Fernandez Bay in the west (Figure 1). Each of these prominent bays were sampled in addition to a number of other smaller patch reefs offshore at East Beach in the northeast, Grotto Beach in the southwest, Sue Point and Barker's Point in the northwest and Dump Reef in the north (in Graham's Harbor). Additional sites were located at mouth of the Pigeon Creek tidal estuary in the southeast and in the north part of Graham's Harbor (Gaulin Reef).

In 1951, the United States government established a down-range missile-tracking base and a submarine tracking facility on the island. Today the original structures of this naval base serve as the Bahamian Field Station (Gerace *et al.* 1999). Sewage runs through a primary treatment settling tank before it is released offshore through an outfall pipe into Graham's harbor. Additional sampling sites were located in the vicinity of this outfall in an effort to characterize this point source of nutrients to the coastal system (Figure 1). Furthermore, increases in tourism and the general population

of the island are leading to increased resource use and concern for the local environment.

### Sample collection

Samples of marine algae and seagrass were collected in June 2000. The most abundant species found at each site (*Halimeda incrassata*, *Udotea flabellum*, *Padina sanctae-crucis* and *Thalassia testudinum*; Figure 1) were collected by scuba divers who handpicked fresh, healthy looking, representative samples in water depths ranging from 3 - 6 meters. Divers attempted to collect at least three specimens of each species from each site. The four most ubiquitous species were processed and analyzed for stable isotope composition. Samples were chosen from open, well-circulated areas within each site. Bottom sediments of all sites consisted primarily of coarse grained, white, sand-sized carbonate fragments. Sites were located within 100 - 400m of the shore (with the exception of Gaulin Reef in Graham's Harbor). Samples were kept on ice during collection and kept frozen until processing in the laboratory (US Geological Survey, Menlo Park, CA).

### Sample processing

All samples were gently rinsed and hand-cleaned in de-ionized water to remove inorganic and organic contaminants. Samples were then freeze-dried, ground into a homogenous powder and thoroughly mixed before analysis. *U. flabellum* and *H. incrassata* were vapor acidified (HCl) for 18 hours to remove carbonate prior to analysis of organic N (modified from (Yamamuro *et al.* 1995)).  $\delta^{15}\text{N}$  values of the samples were determined using continuous flow isotope ratio mass spectrometry (CF-IRMS). Weighed samples of ground material (1 - 3 mg) were combusted in a Carlo Erba NA1500 elemental analyzer and analyzed as  $\text{N}_2$  by a Micromass Optima mass spectrometer.

Ratios of  $^{15}\text{N}:^{14}\text{N}$  are expressed relative to  $\text{N}_2$  in air for nitrogen. Isotopic ratios were calculated as:

$$\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

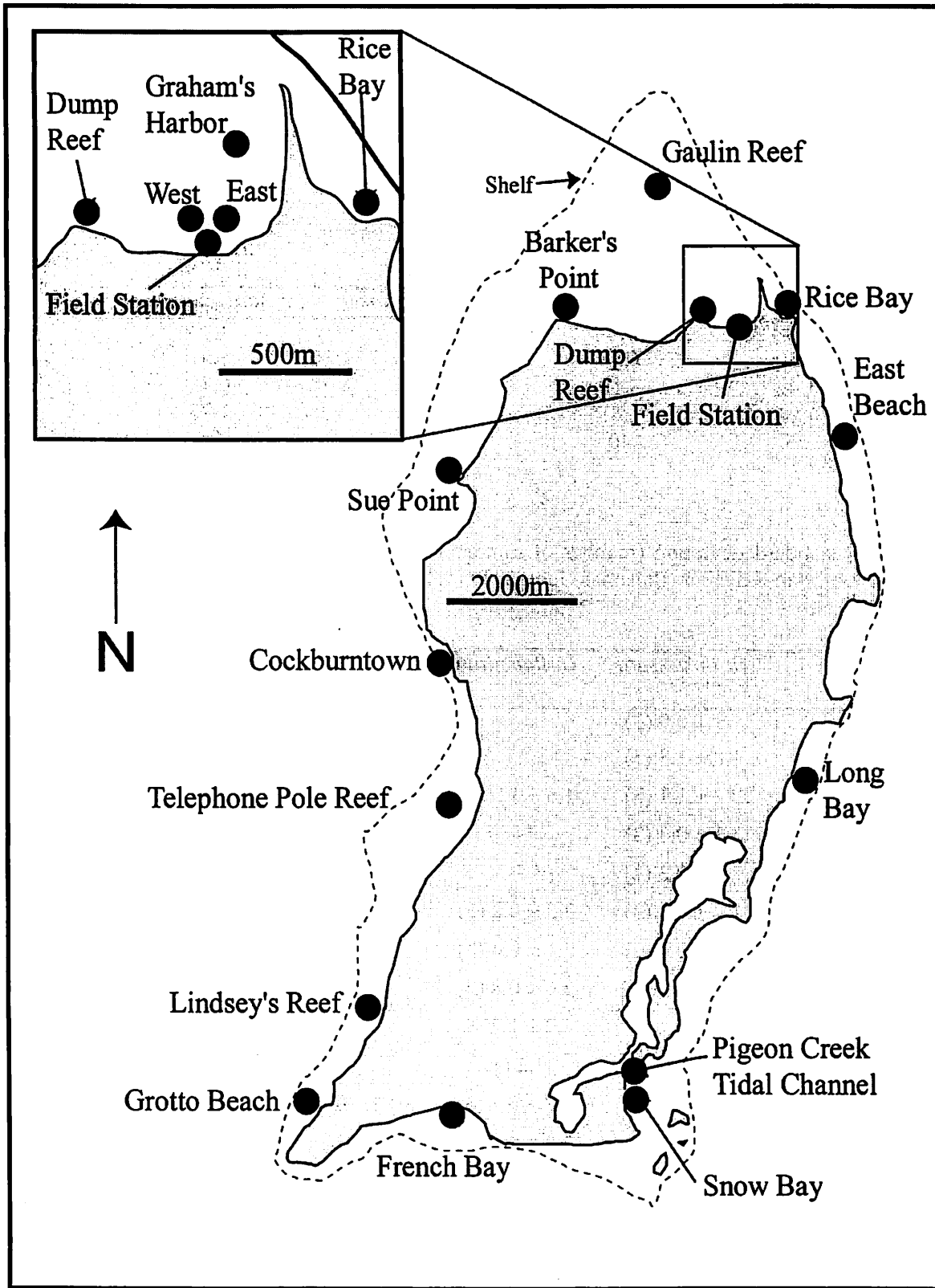


Figure 1. Map of San Salvador, Bahamas showing study sites.

Where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the  $^{15}\text{N}/^{14}\text{N}$  ratio in the sample and standard, respectively. Delta values are expressed in parts per thousand or permil (‰).

## RESULTS AND DISCUSSION

The low mean  $\delta^{15}\text{N}$  values (Table 1) of macroalgae and seagrass on San Salvador from

**Table 1.** Summary statistics for  $\delta^{15}\text{N}$  (values in ‰).

	<i>H. incrassata</i>	<i>U. flabellum</i>	<i>T. testudinum</i>	<i>P. sanctae-crucis</i>
Min	-2.93	-0.84	-1.00	-0.54
Max	5.30	6.58	4.43	5.31
Mean	1.42	3.10	1.38	0.75
Std. Dev.	1.94	1.61	1.26	1.77
n	55	40	38	26

**Table 2.** Mean  $\pm$  standard deviation (number of samples) of  $\delta^{15}\text{N}$  of macroalgae and seagrass from sampling sites around San Salvador, Bahamas, June 2000 (values in ‰). Note some species not present at all sites.

Site	<i>H. incrassata</i>	<i>U. flabellum</i>	<i>T. testudinum</i>	<i>P. sanctae-crucis</i>
Rice Bay	1.1 $\pm$ 0.9 (3)	--	1.0 $\pm$ 0.4 (3)	0.6 $\pm$ 0.0 (3)
East Beach	1.8 $\pm$ 1.3 (3)	3.5 (1)	1.8 $\pm$ 0.23(4)	0.1 $\pm$ 0.6 (3)
Long Bay	0.7 $\pm$ 1.1 (3)	1.6 $\pm$ 1.3 (3)	0.0 $\pm$ 0.2 (3)	0.8 $\pm$ 0.2 (3)
Pigeon Creek Channel	3.1 $\pm$ 0.3 (3)	--	1.8 $\pm$ 0.9 (3)	--
Snow Bay	2.1 $\pm$ 0.3 (2)	--	0.3 $\pm$ 0.2 (2)	--
French Bay	1.4 $\pm$ 0.1 (3)	2.5 $\pm$ 0.2 (3)	1.4 $\pm$ 0.5 (2)	--
Grotto Beach	0.5 $\pm$ 0.4 (3)	3.1 $\pm$ 0.4 (3)	--	-0.5 $\pm$ 0.4 (4)
Lindsey's Reef	-2.3 $\pm$ 0.6 (3)	2.6 $\pm$ 0.8 (3)	--	-0.2 $\pm$ 0.6 (3)
Telephone Pole Reef	1.2 $\pm$ 0.4 (3)	3.2 $\pm$ 1.2 (3)	--	--
Sue Point	1.2 $\pm$ 1.0 (2)	3.2 (1)	--	0.1 $\pm$ 0.5 (2)
Barker's Point	2.4 $\pm$ 1.9 (6)	3.8 $\pm$ 0.5 (3)	-0.3 $\pm$ 0.8 (3)	--
Dump Reef	4.2 $\pm$ 1.7 (3)	2.3 $\pm$ 2.6 (3)	1.6 $\pm$ 0.2 (4)	0.8 $\pm$ 0.6 (4)
BFS West Transect	4.5 $\pm$ 0.7 (5)	2.9 $\pm$ 0.9 (3)	2.1 $\pm$ 0.4 (4)	--
BFS Outfall	4.6 $\pm$ 0.5 (3)	5.3 $\pm$ 2.4 (4)	4.3 $\pm$ 0.2 (3)	5.3 $\pm$ 0.1 (2)
BFS East Transect	2.8 $\pm$ 0.5 (5)	4.8 $\pm$ 0.7 (4)	1.5 $\pm$ 0.4 (3)	--
Graham's Harbor	3.8 (1)	3.5 $\pm$ 1.0 (4)	1.5 $\pm$ 0.3 (2)	--
Gaulin Reef	1.6 $\pm$ 1.0 (4)	2.9 $\pm$ 0.1 (2)	1.3 $\pm$ 0.6 (2)	-0.2 $\pm$ 0.2 (2)

this study strongly imply this coastal marine system's overall dependence on N-fixation. It is generally accepted that none of these species is capable of fixing nitrogen independently, but rather that the source of inorganic nitrogen used in the plant biomass derives from bacterially fixed atmospheric N<sub>2</sub>. Minagawa and Wada (1984) found the  $\delta^{15}\text{N}$  of water fern (*Azolla pinnata*) associated with N-fixing cyanobacteria, *Anabaena azollae*, to be as low as -1.9‰, highlighting the relationship of the cyanobacteria with the associated primary producer. Similar patterns recorded in tropical coastal systems have been interpreted as evidence for the importance of N-fixation in tropical marine systems (Gu *et al.* 1996, Yamamuro 1995, Minagawa and Wada 1984, Martinez *et al.* 1983, Wada 1980).

In this study, there was a remarkable range of  $\delta^{15}\text{N}$  within each species. *H. incrassata* shows a range of 8‰ (Figures 2 and 3). Comparisons among species at sites with the same species reveal that both *P. sanctae-crucis* and *T. testudinum* are significantly lower in  $\delta^{15}\text{N}$  than *U. flabellum* ( $p < 0.001$ ) and *H. incrassata* ( $p < 0.05$  and  $p < 0.001$ , respectively). As an epilithic species, *P. sanctae-crucis* should assimilate inorganic nitrogen directly from the water column. The low  $\delta^{15}\text{N}$  values for this species indicate that this water column nitrogen is derived dominantly from N-fixation (France *et al.* 1998, Yamamuro *et al.* 1995). Regarding the low values for the seagrass *T. testudinum*, blades have been shown to serve as significant substrates for N-fixing cyanobacteria – with corresponding N<sub>2</sub> fixation rates exceeding those of *Halimeda* as a substrate by a factor of 10 (Goldner 1980). This localized source of N-fixation on seagrass blades probably contributes significantly to the biomass of the seagrass itself through localized water column contributions and through recycling of bacterial biomass within the sediments.

#### Source-specific Patterns in $\delta^{15}\text{N}$

Fractionation by autotrophic organisms tends to be low during times of either high growth rates or nutrient limited conditions (Fogel and Cifuentes 1993). Therefore, in the nutrient poor waters around San Salvador, the

primary producers should have higher  $\delta^{15}\text{N}$  values than in a region where nutrients are not limiting. Consequently under oligotrophic conditions the  $\delta^{15}\text{N}$  of aquatic plants should approach that of the inorganic nitrogen sources being utilized.

Mean values of each species from non-field station sites (considered pristine) were 1.5‰ for *Halimeda incrassata*, 3.0‰ for *Udotea flabellum*, 1.1‰ for *Thalassia testudinum* and 0.2‰ for *Padina sanctae-crucis*. For all species the highest  $\delta^{15}\text{N}$  values were found immediately adjacent to the field station. At the sewage outfall pipe, increases in  $\delta^{15}\text{N}$  above the unimpacted site means were 2.31‰, 1.11‰, 1.49‰ and 5.10‰ for *H. incrassata*, *U. flabellum*, *T. testudinum*, and *P. sanctae-crucis*, respectively.

The increase (above the typical N<sub>2</sub> fixing values ~0.0‰) in  $\delta^{15}\text{N}$  for *P. sanctae-crucis* at the outfall pipe suggests localized changes in the isotopic composition of the inorganic nitrogen in the water column. No direct measurement of the  $\delta^{15}\text{N}$  of the DIN (or particulate organic nitrogen) in the sewage source was made and thus the exact isotopic composition of the source material is unknown. However, highly decomposed organic matter usually contains very low amounts of nitrogen, with the remaining nitrogen being considerably enriched in <sup>15</sup>N (Heaton 1986, Thornton and McManus 1994). Because *P. sanctae-crucis* should only be representing the DIN fraction in the water column, the heavier  $\delta^{15}\text{N}$  values are consistent with a high  $\delta^{15}\text{N}$  DIN from the sewage source. Also, both the epilithic and rhizoidal species show the same pattern (increases in  $\delta^{15}\text{N}$ ) near the sewage outfall, indicating that direct uptake of heavier DIN from the water column is responsible for the higher  $\delta^{15}\text{N}$  values seen there.

Sewage inputs to the coastal ecosystem on San Salvador seem to be generally evidenced by increases in macroalgae  $\delta^{15}\text{N}$  values above more "pristine" macroalgae  $\delta^{15}\text{N}$  values. The spatial distribution of this source, based on  $\delta^{15}\text{N}$  of macroalgae from around Graham's Harbor, suggests that there is probably a general westward current aiding in transport of nitrogen from the outfall pipe. Enrichments on the north



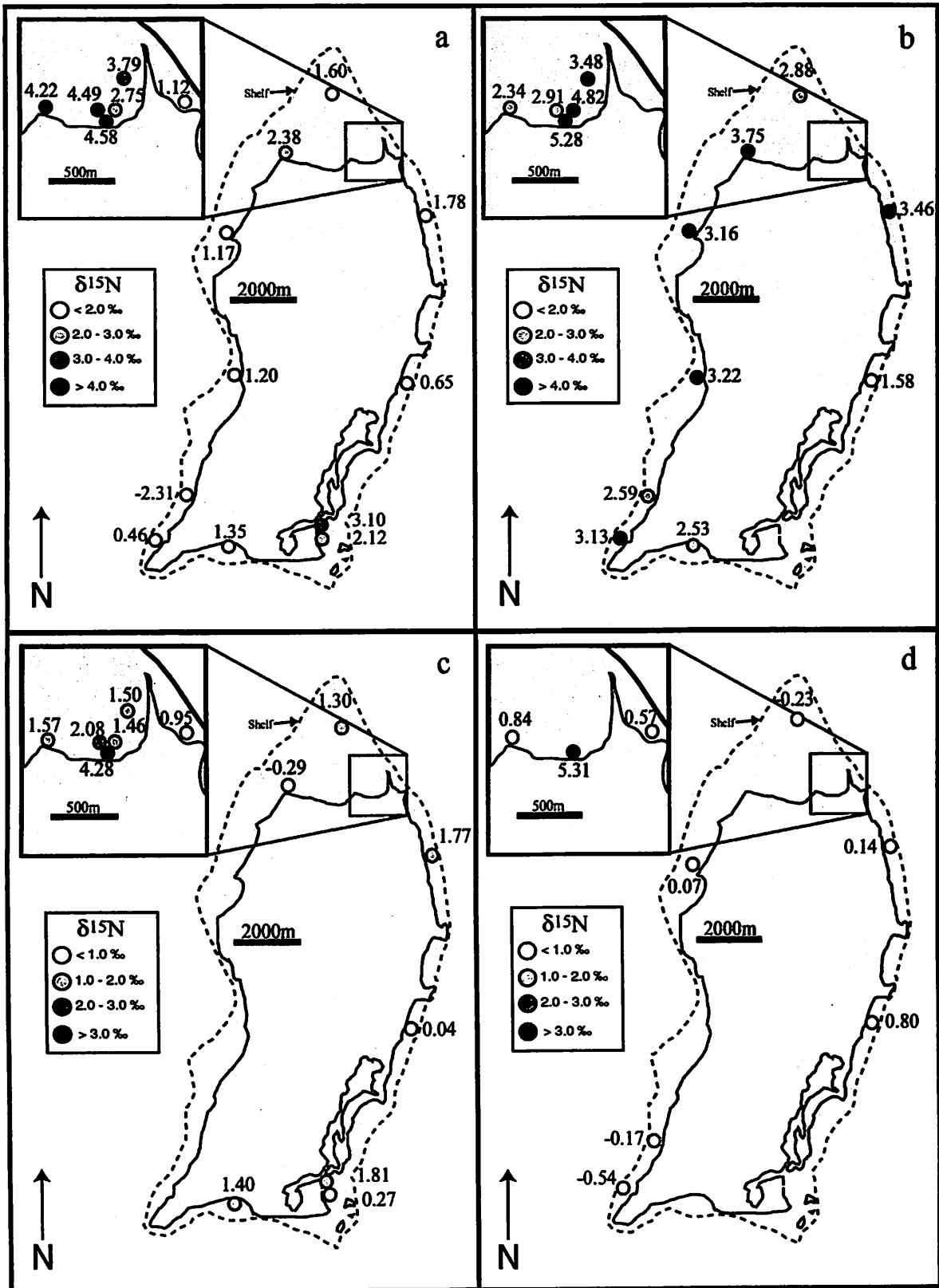


Figure 2. Spatial distribution of mean  $\delta^{15}\text{N}$  values for each site. a) *Halimeda incrassata* b) *Udotea flabellum* c) *Thalassia testudinum* d) *Padina sanctae-crucis*.

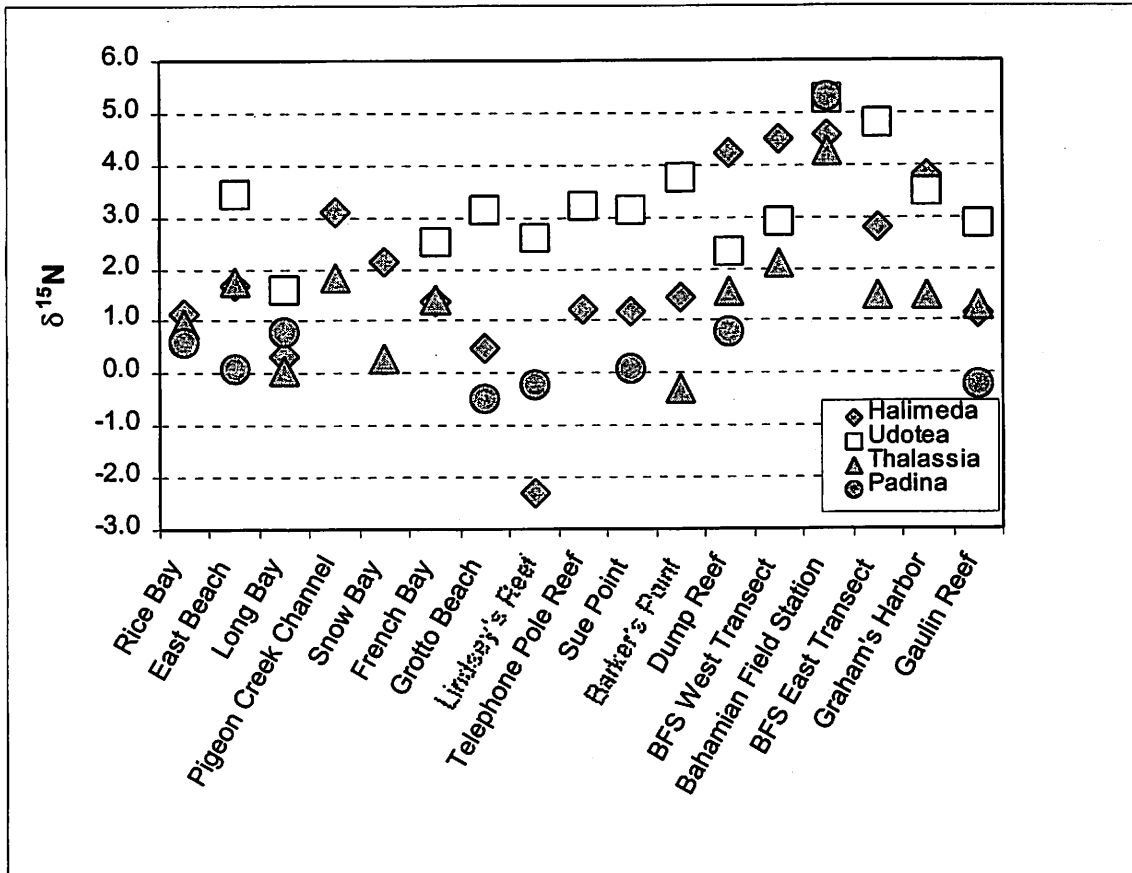


Figure 3. Mean  $\delta^{15}\text{N}$  values of plant species sampled at each site.

side of the island do not seem to extend beyond of Graham's Harbor suggesting that this source is relatively restricted to the north side of the island.

Relatively high  $\delta^{15}\text{N}$  values in *T. testudinum* and *H. incrassata* also occur near the mouth of the Pigeon Creek tidal estuary. One possibility for the high  $\delta^{15}\text{N}$  values seen here is denitrification occurring within the organic rich sediments of Pigeon Creek. This mangrove and seagrass dominated tidal estuary represents a relatively large source of organic matter to the nearby coastal system in the form of leaf detritus. During the decomposition of this organic matter within the anoxic sediments (through anaerobic bacterial respiration), organic nitrogen is incorporated into bacterial biomass and then remineralized to inorganic nitrogen. Through denitrification within the anoxic layer

of the sediments, the inorganic nitrogen pool becomes enriched as denitrifying bacteria selectively metabolize the lighter fraction of the DIN. This process leaves the remaining inorganic nitrogen relatively enriched in  $^{15}\text{N}$  (Kaplan and Cline 1975). When this material is exported by the flows of tides, the relatively high  $\delta^{15}\text{N}$  DIN is integrated into the biomass of the macroalgae and seagrasses near the mouth of the estuary, as seen in *T. testudinum* and *H. incrassata* (Figures 2 and 3).

#### Species-specific Patterns in $\delta^{15}\text{N}$

The rhizoidal macroalgae species (*U. flabellum* and *H. incrassata*), with the ability to utilize pore water DIN in addition to water column DIN, maintain generally higher  $\delta^{15}\text{N}$  values than *P. sanctae-crucis* at all sites (Table 2). Because of the affinity for phosphate to bind

with carbonate sediments, carbonate porewater N:P ratios are typically high (Berner 1974)). Therefore, it is likely that these species are P-limited rather than N limited. In nutrient limited growth conditions, isotopic fractionation of non-limiting components (nitrogen in this case) can increase. For example, Gervais and Riebesell (2001) have shown a distinct carbon isotopic fractionation in marine diatoms when growth was limited by low phosphorus. If the algae in this study were limited in growth rate by P availability, then the available inorganic N should also be fractionated as it is taken up (Fogel and Cifuentes 1993), resulting in lighter rather than the heavier  $\delta^{15}\text{N}$  values as seen here. This suggests a source of isotopically heavier nitrogen within the sediments, perhaps caused by fractionation of nitrogen within the sediment nitrogen cycle. It is possible that bacterial uptake in the sediments preferentially enriches the remaining available inorganic nitrogen assimilated through the rhizomes of the macroalgae, giving rise to higher  $\delta^{15}\text{N}$  values.

*H. incrassata*  $\delta^{15}\text{N}$  seems most sensitive to changes in DIN (widest range of  $\delta^{15}\text{N}$  values) and shows  $\delta^{15}\text{N}$  values higher than background levels not only at the field station outfall and transects, but also at Dump Reef and Graham's Harbor grassbed. Relatively higher  $\delta^{15}\text{N}$  values at the Pigeon Creek tidal channel were also most prominent in *H. incrassata*. The difference in  $\delta^{15}\text{N}$  between the two rhizoidal macroalgae *U. flabellum* and *H. incrassata* ( $p = 0.006$ ) implies a difference in DIN utilization. Possibly different forms of DIN ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ) are used depending on fluctuations in ambient concentrations, or reflecting differences in the dependence on pore water nitrogen versus water column nitrogen.

#### COMMENTS AND CONCLUSIONS

The nitrogen isotopic compositions of the four species studied on San Salvador indicate that fixation of atmospheric nitrogen plays a primary role in this coastal marine nitrogen budget. It also suggests that upwelled deepwater nitrate and terrestrial inputs do not contribute significantly to the system. Thus, terrestrial sources or sources that have been significantly

modified by denitrification are possible to trace by use of the  $\delta^{15}\text{N}$  of autotrophic organisms. The  $\delta^{15}\text{N}$  patterns suggest also some spatial heterogeneity in the nitrogen cycling around the island. *Halimeda incrassata* seems to be the most sensitive species to changes in DIN source.

This study indicates that increases in anthropogenic nutrient loading to the coastal system on San Salvador may be having some localized influence resulting in increased macroalgae growth on coral reefs. While it seems these localized sources are relatively limited, it is still not clear whether they represent a significant but diffuse nutrient load to the entire coastal system or whether they remain restricted in influence to relatively small areas.

All species analyzed effectively showed strong  $\delta^{15}\text{N}$  signals from the sewage inputs of the field station. Further investigation into the isotopic composition of the sediments, mangroves, and macroalgae within the estuary along with nutrient concentration data will hopefully reveal more about the biogeochemical processes occurring in Pigeon Creek. Stable isotope analysis is promising as an effective method for characterizing nutrient source and cycling mechanisms.

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LITERATURE CITED

- Berner, R. A. 1974. Kinetic models for the early diagenesis of nitrogen, phosphorus, and silicon in anoxic marine sediments, *in* Goldberg, E. D., ed., *The Sea*: New York, Wiley, p. 427-445.
- . 1990. Coral-reef algae, *in* Dubinsky, Z., ed., *Ecosystems of the World*: New York, Elsevier, p. 253-264.
- Capone, D. G., Penhale, P., Oremland, R., and Taylor, B. 1979. Relationship between productivity and N<sub>2</sub> fixation in a *Thalassia testudinum* community: *Limnology and Oceanography*, v. 24, no. 1, p. 117-125.
- Capone, D. G., and Taylor, B. F. 1977. Nitrogen fixation (acetylene reduction) in the phyllosphere of *Thalassia testudinum*: *Marine Biology*, v. 40, p. 19-28.
- Capone, D. G., Taylor, D. L., and Taylor, B. F. 1977. Nitrogen fixation (acetylene reduction) associated with macroalgae in a coral-reef community in the Bahamas: *Marine Biology*, v. 40, p. 29-32.
- Caraco, N. F., Lampman, G., Cole, J. J., Limburg, K. E., Pace, M. L., and Fischer, D. 1998. Microbial assimilation of DIN in a nitrogen rich estuary: Implications for food quality and isotope studies: *Marine Ecology Progress Series*, v. 167, p. 59-71.
- Cifuentes, L. A., Sharp, J. H., and Fogel, M. L. 1988. Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary: *Limnology and Oceanography*, v. 33, no. 5, p. 1102-1115.
- D'Elia, C. F., and Wiebe, W. 1990. Biogeochemical nutrient cycles in coral-reef ecosystems, *in* Dubinsky, Z., ed., *Ecosystems of the World*: Elsevier, New York, p. 49-74.
- Fogel, M. F., and Cifuentes, L. A. 1993. Isotope fractionation during primary production, *in* Engel, M. H., and Macko, S. A., eds., *Organic Geochemistry*: New York, Plenum Press, p. 73-98.
- France, R. L. 1995. Source variability in  $\delta^{15}\text{N}$  of autotrophs as a potential aid in measuring allochthony in freshwaters: *Ecography*, v. 18, p. 318-320.
- France, R. L., Holmquist, J. G., Chandler, M., and Cattaneo, A. 1998.  $\delta^{15}\text{N}$  evidence for nitrogen fixation associated with macroalgae from a seagrass-mangrove-coral reef system: *Marine Ecology Progress Series*, v. 167, p. 297-299.
- Gerace, D. T., Ostrander, G. K., and Smith, G. W. 1999. San Salvador, Bahamas: CSI - UNESCO.
- Gervais, F., and Riebesell, U. 2001. Effect of phosphorus limitation on elemental composition and stable carbon isotope fractionation in a marine diatom growing under different CO<sub>2</sub> concentrations: *Limnology and Oceanography*, v. 46, no. 3, p. 497-504.
- Goldner, L. L. 1980. Nitrogen fixation (acetylene reduction) in shallow water Bahamian environments: *Bulletin of Marine Science*, v. 30, no. 2, p. 444-453.
- Hall, R. I., Leavitt, P. R., Quinlan, R., Dixit, A. S., and Smol, J. P. 1999. Effects of agriculture, urbanization, and climate on water quality in the northern Great Plains: *Limnology and Oceanography*, v. 44, no. 3, part 2, p. 739-756.
- Heaton, T. H. E. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: A review: *Chemical Geology*, v. 59, p. 87-102.

- Johannes, R. E., Alberts, J., D'Elia, C. F., Kinzie, R. A., III, Pomeroy, L. R., Sottile, S., Wiebe, W., Marsh, J. A., Jr., Helfrich, P., Maragos, J., Meyer, J., Smith, S., Crabtree, D., Roth, A., McClosky, L. R., Betzer, S. B., Marshall, N., Pilson, M. E. Q., Telek, G., Clutter, R. I., DuPaul, W. D., Webb, K. L., and Wells, J. M., Jr. 1972. The metabolism of some coral reef communities: A team study of nutrient and energy flux at Eniwetok: *BioScience*, v. 22, p. 541-543.
- Kaplan, I. R., and Cline, R. M. 1975. Isotopic fractionation of dissolved nitrate during denitrification in the eastern tropical North Pacific Ocean: *Marine Chemistry*, v. 3, no. 4, p. 271-299.
- Larkum, A., Kennedy, I., and Muller, W. 1988. Nitrogen fixation on a coral reef: *Marine Biology*, v. 98, p. 143-155.
- Lindau, C., Delaune, R., Patrick, W., Jr., and Lambremont, E. 1989. Assessment of stable nitrogen isotopes in fingerprinting surface water inorganic nitrogen sources: *Water, Air and Soil Pollution*, v. 48, p. 489-496.
- Littler, M., and Littler, D. 1990. Productivity and nutrient relationships in psammophytic versus epilithic forms of Bryopsidales (Chlorophyta): Comparisons based on a short-term physiological assay: *Hydrobiologia*, v. 204/205, p. 49-55.
- Mague, T., and Holm-Hansen, O. 1975. Nitrogen fixation on a coral reef: *Phycologia*, v. 14, no. 2, p. 87-92.
- Mariotti, A., Lancelot, C., and Billen, G. 1984. Natural isotopic composition of nitrogen as a tracer of origin for suspended organic matter in the Scheldt estuary: *Geochimica et Cosmochimica Acta*, v. 48, p. 549-555.
- McClelland, J., and Valiela, I. 1998. Linking nitrogen in estuarine producers to land-derived sources: *Limnology and Oceanography*, v. 43, no. 4, p. 577-585.
- McRoy, C. P., Goering, J. J., and Chaney, B. 1973. Nitrogen fixation associated with seagrasses: *Limnol. Oceanogr.*, v. 18, p. 998-1002.
- Minagawa, M., and Wada, E. 1984. Stepwise enrichment of  $^{15}\text{N}$  along food chains: Further evidence and the relation between  $\delta^{15}\text{N}$  and animal age: *Geochimica et Cosmochimica Acta*, v. 48, p. 1135-1140.
- Peterson, B. J., and Howarth, R. W. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia: *Limnology and Oceanography*, v. 32, no. 6, p. 1195-1213.
- Rivera-Monroy, V., Day, J., Twilley, R., Vera-Herrera, F., and Coronado-Molina, C. 1995. Flux of Nitrogen and Sediment in a Fringe Mangrove Forest in Terminos Lagoon, Mexico: *Estuarine, Coastal and Shelf Science*, v. 40, p. 139-160.
- Rogers, K. M. 1999. Effects of sewage contamination on macro-algae and shellfish at Moa Point, New Zealand using stable carbon and nitrogen isotopes: *New Zealand Journal of Marine and Freshwater Research*, v. 33, p. 181-188.
- Spies, R., Kruger, H., Ireland, R., and Rice, D. W., Jr. 1989. Stable Isotope ratios and contaminant concentrations in a sewage-distorted foodweb: *Marine Ecology Progress Series*, v. 54, p. 157-170.
- Thornton, S. F., and McManus, J. 1994. Application of organic carbon and nitrogen stable isotope and C/N ratio as source indicators of organic matter provenance in estuarine systems: Evidence from the Tay Estuary,

- Scotland.: Estuarine, Coastal and Shelf Science, v. 38, p. 219-233.
- Van Dover, C. L., Grassle, J. F., Fry, B., Garritt, R. H., and Starczak, V. R. 1992. Stable isotope evidence for entry of sewage-derived organic material into a deep-sea food web: *Nature*, v. 360, p. 153-155.
- Wada, E. 1980. Nitrogen Isotope Fractionation and Its Significance in Biogeochemical Processes Occurring in Marine Environments, in Goldberg, E. D., Horibe, Y., and Saruhasi, K., eds., *Isotope Marine Chemistry*: Tokyo, Japan, Uchida Rokakuho Publishing Company, p. 375-398.
- Wada, E., and Hattori, A. 1976. Natural abundance of  $^{15}\text{N}$  in particulate organic matter in the North Pacific Ocean: *Geochimica et Cosmochimica Acta*, v. 40, no. 2, p. 249-251.
- Wainright, S. C., Fuller, C. M., Michener, R. H., and Richards, R. A., 1996, Spatial variation of trophic position and growth rate of juvenile striped bass (*Morone saxatilis*) in the Delaware River: *Canadian Journal of Fish and Aquatic Science*, v. 53, p. 685-692.
- Wiebe, W. J., Johannes, R. E., and Webb, K. L. 1975. Nitrogen Fixation in a Coral Reef Community: *Science*, v. 188, no. 4185, p. 257-259.
- Yamamuro, M., Kayanne, H., and Minagawa, M. 1995. Carbon and nitrogen stable isotopes of primary producers in coral reef ecosystems: *Limnology and Oceanography*, v. 40, p. 617-621.