Nutrient distribution across the insular shelf of La Parguera Puerto Rico: assessment by algal tissue nitrogen

by

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Abstract

A survey was conducted to investigate possible presence of an inshore to shelf edge gradient in % nitrogen (%N) and stable nitrogen isotope ratio ($\delta^{15}N$; ‰ vs. air) in tissues of the rhodophyte *Acanthophora spicifera* and the heterokonts *Lobophora variegata* and *Dictyota bartayresiana* in coastal waters of La Parguera, southwest Puerto Rico. Average %N (1.26 ± 0.08 SD to 3.25 ± 0.10) and $\delta^{15}N$ (2.06 ± 0.04 to 8.16 ± 0.14‰) in *Acanthophora spicifera* was highly variable along the shoreline. The highest inshore values of $\delta^{15}N$ occurred at two stations influenced by secondary sewage input and a bird rookery and lower values (0.81 ± 0.06‰) were observed at mid-shelf locations. *Dictyota bartayresiana* and *L. variegata* did not display clear trends in %N across the insular shelf, however, $\delta^{15}N$ for *Dictyota bartayresiana* was significantly higher inshore (3.48 ± 0.087‰) versus mid-shelf and shelf edge (0.00 ± 0.08‰) and $\delta^{15}N$ for *L. variegata* was significantly higher at mid-shelf (2.13 ± 0.25‰) versus shelf edge locations (0.34 ± 0.24‰). These results indicate that anthropogenic effects on tissue nutrients are spatially distributed across the shelf, being higher near shore and not evident at the shelf edge.

Resumen

Se llevó a cabo un estudio sobre la presencia de un gradiente desde la costa hasta el veril insular de La Parguera, Puerto Rico, respecto al porciento (%N) y la razón del isótopo estable de nitrógeno ($\delta^{15}N$) en el tejido de la rodofita *Acanthophora spicifera* y los heterocontes *Lobophora variegata* y *Dictyota bartayresiana.* El %N (1.26 ± 0.08 - 3.25 ± 0.10%) y $\delta^{15}N$ (2.06 ± 0.04 to 8.16 ± 0.14‰) en *A. spicifera* fue altamente variable a lo largo de la costa. En el interior de la plataforma, los valores más altos de $\delta^{15}N$ ocurrieron en dos estaciones que son influenciadas por descargas sanitarias secundarias y por una colonia de aves. Los valores más bajos de $\delta^{15}N$ (0.81 ± 0.06‰) fueron observados en las localidades de la parte intermedia de la plataforma insular. *Dictyota bartayresiana* y *L. variegata* no mostraron patrones claros en %N a través de la plataforma insular. Sin embargo, el $\delta^{15}N$ de *D. bartayresiana* fue significativamente mayor en la parte interior de la plataforma (3.48 ± 0.087‰) versus la parte intermedia y la exterior (0.00 ± 0.08‰). El $\delta^{15}N$ para *L. variegata* fue significativamente mayor en las localidades de la parte intermedia (2.13 ± 0.25‰) versus las exteriores (0.34 ± 0.24‰). Estos resultados indican que los impactos antropogénicos en los nutrientes de los tejidos están espacialmente distribuidos a lo largo de la plataforma insular, siendo más alto cerca de la costa pero no tan evidente en el veril.

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Introduction

The quantity of nutrients available to marine organisms varies both temporally and spatially and is dependent on the presence of localized sources (Larned, 1998). Anthropogenic, human derived, sources are often associated with terrestrial development. Sewage, agriculture, and construction are common examples of human contributions leading to increased availability of nutrients in marine ecosystems (Fong *et al*., 2001; Lajtha and Michener, 1994). All of these potentially environment altering activities occur in the town of La Parguera, Puerto Rico. West of La Parguera a sewage treatment plant releases well-nitrified secondary sewage effluents into the adjacent mangrove community (Corredor and Morell, 1994) and during the rainy season, September through October, raw sewage frequently leaks directly into the water. Additionally, large areas of land within the watershed are continually being stripped of vegetation as a result of development activities. Thus, the once oligotrophic coastal waters off La Parguera, with its variable rainfall and anthropogenic inputs, are highly susceptible to nutrient fluctuations.

Opportunistic organisms capable of rapid nutrient uptake may be favored in communities affected by nutrient enrichment (Fong *et al.* 1994). Macroalgae, cyanobacteria, and phytoplankton are primary producers that quickly extract nutrients from the water column. Macroalgae are competitive because they posses abilities to rapidly uptake several forms of nutrients simultaneously across their entire surfaces, contain large internal storage pools, and use currents to replenish nutrients for greater uptake (Carpenter ad Capone, 1983). The proposed role of fleshy algae in the decline of reef building corals within areas of coastal eutrophication has caused considerable

controversy (Fong *et al*., 1998; Larned, 1998; Littler *et al*., 1991; Szmant, 2002; Miller *et al.,* 1999). Kaneohe Bay, Hawaii is the first well documented example of a coral reef shifting to a macroalgal-dominated reef. Nutrient enrichment of the bay, by domestic sewage, resulted in the overgrowth of coral by the chlorophyte *Dictyosphaeria cavernosa* (Forsskål) Børgesen (Banner, 1974). Szmant (2002) agreed that in many areas, anthropogenic impacts have increased the flux of nutrients into coastal waters. However, she cautions that various cases of coral reef degradation may be attributed to factors such as previous coral bleachings, massive herbivore die-offs, and a history of over-fishing because these factors increase the availability of substrata for algal colonization or increase the severity of effects from nutrient enrichment. Done (1992) also suggests that over-fishing of herbivorous fish, hurricanes, and fatal diseases of grazers, all of which have occurred in southwestern Puerto Rico, can lead to a shift from coral to macroalgae. Nevertheless, increasing eutrophication in addition to changing herbivory patterns and coral diseases, contribute to a synergistic effect resulting in coral reef deterioration.

A predictive indicator of bioavailable nutrient enrichment that provides an accurate and early warning to changes in the nutrient conditions has become highly desirable for management of sensitive coral reef ecosystems (Costanzo *et al*., 2000; Fong *et al*., 1998; Larned, 1998). Historically, the principle method for measuring nutrient availability has been via water column concentrations of ambient nitrogen (N) and phosphorous (P). Recent literature suggests that this method alone may not be an accurate indicator of nutrient availability because ambient N and P concentrations in the water column fail to indicate the fraction which is bioavailable (Maher and Norris, 1990) and do not necessarily correlate with production or abundance of primary producers (Fong *et*

al., 1998; Larned, 1998). Not all nutrients reaching the marine environment can be utilized metabolically. Approximately 80% of the total dissolved nitrogen (N) in urban/suburban runoff and 20-60% from forests and pastures is bioavailable (Seitzinger *et al*., 2002). The rapid uptake ability of macroalgae and the close recycling of nutrients within coral reef ecosystems also diminish the effectiveness of water column concentrations as nutrient enrichment indicators (Fong *et al.*, 1998; Fong *et al.*, 2001).

Nutrients from anthropogenic sources are usually supplied in pulses to inshore waters by runoff and periodic sewage leaks. This produces transient peaks of nutrient levels in the water column. Within a community of opportunistic autotrophs which rapidly strip the water of nutrients, nutrients cannot accumulate unless the loading rates are extremely high, thus direct periodic sampling of the water column will often under or overestimate the nutrient conditions, dependent on whether the "peaks" are hit or missed (Costanzo *et al*., 2000; Fong *et al*., 1994). The concentration of tissue nutrients in macroalgae may be a more useful indicator of nutrient availability because particular species respond to nutrient enrichment by taking in nutrients, growing, and storing excess (Fong *et al*., 1998). Costanzo *et al.* (2000) reported that water column concentrations of nutrients returned to ambient conditions rapidly after a pulse, but the effects on the tissue nitrogen content of *Gracilaria sp.* remained for days. This indicates that brief nutrient pulses not detected by traditional sampling methods continue to influence biological activities after the water column nutrient event has passed.

Macroalgal tissue is responsive to changes in the amount of nutrients and integrates the changes over a period of time. Species specific maximum uptake volume, determined by the size of storage vacuoles, and rates of assimilation determine the period

of time over which algae can integrate the response. Using algal tissue nutrients as bioindicators of enrichment allows for less frequent sampling than what would be necessary for the same accuracy in direct water column sampling (Fong *et al*., 1994). It may also be effective in identifying the location of the nutrient source because the percent change in tissue nitrogen concentration measured among several locations allows for the assessment of a net increase or decrease in nutrient enrichment between these areas (Fong *et al*., 1998).

Abundance of stable nitrogen isotope ($\delta^{15}N$) in algal tissue can also be used to determine local sources of enrichment because terrestrial sources of nutrients generally have higher $\delta^{15}N$ values than ambient seawater and rainwater (McClelland and Valiela, 1998; Umezawa *et al.*, 2007). This difference is created during physical and chemical processes where a selection against or for a particular isotope occurs (i.e., ^{15}N versus ^{14}N). This partial separation of isotopes is termed isotopic (or isotope) fractionation. Isotopic fractionation results from discrimination against particular isotopes, such as an inequality of rate constants for a reaction involving two different isotopic species (Schidlowski *et al.*, 1983; Costanzo *et al.*, 2001). Typically, the lighter species reacts more quickly than the heavier species. Biological reactions involving N can discriminate against the heavier isotope by 40‰ (Lajtha and Michener, 1994). Agricultural practices increase $\delta^{15}N$ by increasing oxidation of soil organic N (Kreitler and Browning, 1983). Denitrification performed by many soil inhabiting microbes results in an increase of $\delta^{15}N$ in the soil that is transported to the ocean in runoff (McClelland and Valiela, 1998). In order to use algae to identify sources of nutrient enrichment, a low level of fractionation within the alga is desired to avoid altering the $\delta^{15}N$ signal of the source. The uptake and

assimilation of inorganic N by algae has the potential to fractionate nitrogen isotopes, favoring ^{14}N in a number of processes. Under particular environmental conditions, nutrient uptake can select against slow, heavy isotopes as molecules of inorganic N passively diffuse through the boundary layer and across the cell membrane. The degree of fractionation is a result of the balance between external nutrient concentration and algal uptake mechanics. In phytoplankton, little or no isotopic discrimination is observed at this stage (Carpenter and Capone, 1983; Lajtha and Michener, 1994). Enzymatic reactions observed during photosynthesis and respiration provide strong rates of fractionation in phytoplankton. Despite the potential for fractionation by algae, changes in $\delta^{15}N$ will not be observed if kinetics are unsaturated and adequate agitation of the boundary layer occurs (Lajtha and Michener, 1994). Thus, in a tropical marine environment where N is considered a limiting nutrient and moderate water energy is observed, it is likely that algae do not fractionate N to a large degree and thus can be used as bioindicators of $\delta^{15}N$ in coastal waters (Cohen and Fong, 2005; Lajitha and Michener, 1994).

Measurements of algal tissue nutrients can provide information about nutrient conditions in the marine environment. Tissue total %N indicates the quantity of nutrients available and $\delta^{15}N$ identifies potential sources of the nutrients. Thus, objectives of this study were to:

- Determine algal tissue %N of benthic marine algae in the vicinity of La Parguera.
- Determine algal tissue $\delta^{15}N$ of benthic marine algae in the vicinity of La Parguera.
- Determine possible cross-shelf patterns in distribution of %N in algal tissue.
- Determine possible cross-shelf patterns in distribution of $\delta^{15}N$ in algal tissue.
- Determine candidate species for indicators of nutrient inputs.

Literature Review

Experimentally tested measurements of nutrient levels using algal tissue include growth, percent total phosphorus (TP), ratio of nitrogen to phosphorous (N:P), ratio of carbon to nitrogen (C:N), and percent total N (%N). Of these approaches: growth, TP, N:P, and C:N were found to be less useful as indicators of nutrification than %N. Duke *et al*. (1989) reported an uncoupling between growth and nutrient uptake in algae, therefore growth is not useful in the identification of an increase in the nutrient status of the water column. Fong *et al*. (1998) also found that growth was not a good indicator of enrichment because changes in wet and dry weight of *Enteromorpha* tissue did not accurately represent changes in enrichment of the adjacent water column. The TP of algal tissue is generally not a good indicator of water nutrient status in tropical regions because phosphorous typically limits primary production. If phosphorous is the limiting nutrient, it will not reflect the concentration in the water column because it is assimilated at the same rate it is acquired (Fong *et al*., 2001). Fong *et al*. (1994) found that a small change in water column N:P results in a much larger change in tissue N:P and Larned (1998) found that tissue N:P of several species of algae were found to be higher than the ratio in the adjacent water column. Fong *et al*. (2001) warn against the use of N:P because *Acanthophora* and *Dictyota* displayed apparent increases in tissue N inshore versus offshore using two different mechanisms. *Acanthophora* had a higher tissue N:P inshore vs. offshore due to a higher concentration of tissue N inshore. *Dictyota* had a higher tissue N and P concentrations offshore vs. inshore but had a higher N:P inshore due to a much greater differential between tissue N and P inshore to offshore. A measure of tissue C:N is also not a good indicator of nutrient fluctuations because this ratio can vary widely

during growth of an alga. High inputs of C from CO_2 (i.e. mixing of the water column with the atmosphere) can greatly increase the rate of growth and assimilation, thereby rapidly decreasing the tissue N and increasing C:N without a decrease in water column nutrients (Lourenco *et al*., 2004; Carpenter and Capone, 1983). Light irradiance levels can also influence C:N on a similar scale to N availability (Carpenter and Capone, 1983).

Percent tissue N in algae is an accurate bioindicator of changes in water column nutrient concentration. Fong *et al*. (1994) found a positive linear relationship between N enrichment rates supplied to a microcosm and the tissue N concentration of *Enteromorpha* within the microcosm. This linear relation only holds true in conditions where N is not the limiting nutrient. When N is limiting, the concentration of tissue N is not a good indicator of the rate of enrichment because N is not allowed to accumulate in the tissue. Fong *et al*. (1998) found that algal tissue N decreased with an increasing distance from sources of enrichment. The change in tissue % N, before vs. after enrichment, was a better indicator than the actual %N per dry weight because it incorporated the initial conditions, allowing for detection of a net increase or decrease.

Acquisition of N generally follows three steps: N first diffuses across the boundary layer, proceeds by uptake across the cell membrane, and finally is assimilated into amino acids directly from NH_4 or indirectly after the reduction of NO_3^- or NO_2^- to NH_4. Three major pools of N occur in macroalgae. The structural pool is the most conservative. It contains molecules such as nucleic acids and structural proteins of membranes that are required for life to exist. The physiological pool stores enzymes, photosynthetic pigments, and other molecules important for physiological and metabolic processes. The size of the organism determines this critical internal N concentration. Any

N taken up in excess of the amount necessary to maintain the growth of the organism enters the storage pool.

All cells in an alga are not metabolically similar. Rates of photosynthesis and nutrient uptake are higher in meristematic regions and outer cellular layers (Carpenter and Capone, 1983). Intracellular nitrogen is found in both inorganic and organic forms within algal tissue. Intracellular inorganic nitrogen (IIN) is found as nitrate, nitrite, and ammonium. The ratio among these three varies with growth, but nitrite concentration is always the lowest (Lourenco et al., 2004). Intracellular inorganic N is important in cells containing large vacuoles. Nitrate containing vacuoles can contribute up to 50% cellular N in nutrient replete conditions (Geider and La Roche, 2002). In *Gracilaria*, these IIN stores are used rapidly, within one week, when low nutrient conditions resume (Jones *et al*., 1996). Organic nitrogen is found in proteins, nucleic acids, and amino acids (Geider and La Roche, 2002). Naldi and Wheeler (1999) found that protein contributed 41-89% of the increase in total N due to enrichment. When *Gracilaria* is enriched with ammonium, the majority of the increase in tissue N is seen in the form of amino acids and protein. When nutrients return to a depleted state, amino acids are the first source of N utilized (Bird *et al*., 1982; Jones *et al*., 1996).

The rates of ammonium (NH_4^+) and nitrate (NO_3^-) uptake by algal tissue vary. When algae are exposed to a sudden pulse in nutrients, NH_4^+ uptake is initially enhanced and NO_3^- uptake is suppressed. This initial surge uptake occurs at a very rapid rate. Surge uptake is probably the absorption of ions onto the surface of the thalli and into the tissue. In a study by Pedersen and Borum (1997) the highest rate of surge uptake was recorded at 271 μmol Ng^{-1} dw h^{-1} for *Ceramium rubrum* C. Agardh. Among all species of

macroalgae in this study, the rate of surge uptake increased and reached saturation with the increase of water nutrient concentration, but there was large species specific variation. The amount of ions absorbed into the tissue and stored in the intracellular pools, is controlled by negative feedback (McGlathery *et al*., 1996; Pedersen and Borum, 1997). More constant rates of uptake resume after algae have utilized the pulse and water column nutrients return to ambient concentrations (Pedersen and Borum, 1997). Algae are better able to accumulate stores of N when growth is limited by temperature or light (Martínez and Rico, 2002; Rosenberg and Ramus, 1982).

Ephemeral, fast-growing, algae with thin, erect branching thalli and simple morphology tend to have faster rates of N uptake and a higher affinity for N at low concentrations than do larger, slow-growing algae (Fong *et al*., 2001; Fujita, 1985; Pedersen and Borum, 1997; Wallentinus, 1984). Ephemeral species take advantage of short term nutrient pulses through the use of surge uptake in order to compensate for periods of low nutrients (Fujita, 1985). The maximum N uptake rate is a direct function of the surface to volume ratio, suggesting ephemeral, thinner forms are optimal (Duke *et al*., 1989; Rosenberg and Ramus, 1982). Some algae with relatively fast rates of uptake are limited in their capacity to store N. The N storage capacity for an alga varies inversely with the surface area to volume ratio, suggesting that larger, thicker algae are more suitable indicators (Duke *et al*., 1989; Larned, 1998; Rosenberg and Ramus, 1982).

Algae can serve as either passive or active indicators of water column nutrient concentrations. Passive indicators are collected from natural populations whereas active indicators are moved from their natural environment and incubated elsewhere before collection for analysis. Perhaps persistent algae with a higher storage capacity for N are

better suited as passive indicators because they can indicate nutrification over a longer time frame. Although, low dose or short lived nutrient pulses may only affect the N content of algae with higher rates of uptake. A high rate of uptake is more important than N storage capacity when considering an active bioindicator because the algae are only deployed for a short period of time.

Certain characteristics of algae should be avoided when selecting a bioindicator of water column nutrient enrichment. Siphonous algae and seagrasses such as *Caulerpa* and *Thalassia* obtain a large portion and possibly all N from the rhizosphere in the sediment leading to an overestimation of the nutrient concentration in the water column (Williams, 1981; Patriquin, 1973). Mat-forming algae strip nutrients from the water within the mat, leaving the upper layers nutrient-depleted and thus are also a poor reflection of the adjacent water column (McGlathery *et al*., 1997; Fong *et al.*, 2001). For example, Fong *et al*. (2001) found that the N concentration of tissue in a mat-forming alga, *Dictyota*, was higher offshore than inshore, which did not reflect the distribution of nutrients in the water column.

Experiments exploring the use of algae as bioindicators have used the green alga *Halimeda* and frequently use *Ulva* and *Enteromorpha*. Despite the small percentage of living tissue within the thalli of *Halimeda incrassata* (J.Ellis) J.V.Lamour, Fong *et al*. (2001) found that % tissue N dry weight (dw) was significantly higher inshore than offshore. Fujita (1985) found that *Ulva lactuca* Linnaeus has a range of ca. 1-5.5% N dw and took up ammonium rapidly at all tested nutrient concentrations immediately after collection from the field. Björnsäter and Wheeler (1990) confirmed a 1-5.5% N dw for *Ulva fenestrata* Postels and Ruprechet and also applied this range to *Enteromorpha*

intestinalis (Linnaeus) Link. Fong *et al*. (1994) found a lower range in values for *Enteromorpha* spp., 0.7-1.35% N dw, and a strong relationship between nutrient supply, water column nutrients, and tissue N content. Fong *et al*. (1998) chose *E. intestinalis* for an *in situ* enrichment experiment because it is eurythermal, euryhaline, has a rapid rate of N uptake, a large storage capacity for N, and is tolerant to desiccation and conditions of low light (Fujita, 1985). They concluded that *E. intestinalis* has potential for being developed into a bioindicator of eutrophication.

Generally, total tissue N of red algae correlates more closely with its concentration in the water column than the tissue of green or brown algae (Bird *et al*., 1982; Björnsäter and Wheeler, 1990; D'Elia and DeBoer, 1978; Jones *et al*., 1996). In *Acanthophora spicifera* (Vahl) Børgesen, % total tissue N is significantly higher in the inshore waters of La Parguera than offshore, reflecting the total known N and nitrate concentrations in the water column. The range in % tissue N is smaller for *A. spicifera*, 1.10-2.67% N dw, than for other red algae (Fong *et al*., 2001). *Gracilaria* displays broad changes in response to water column nutrient concentration flux (Bird *et al*., 1982; Björnsäter and Wheeler, 1990; D'Elia and DeBoer, 1978; Jones *et al*., 1996), with a large range in % tissue N, 0.6-3.5% N dw (Bird *et al*., 1982; Costanzo *et al*., 2000; Fujita, 1985). Pedersen and Borum (1997) add that *Gracilaria* takes up nutrients directly proportionally to the amount added to the water column across a larger nutrification range than slower growing, fleshy forms of algae. *Gracilaria* uptakes N more slowly than *Ulva* but has a larger capacity for N storage (Rosenberg and Ramus, 1982). When compared to the performance of *Enteromorpha* as an active bioindicator, *Gracilaria* maintains more initial mass and is a better detector of N input (Fong *et al*., 1998). Bird *et*

al. (1979) also endorses the use of *Gracilaria* as a bioindicator because it is able to tolerate a wide range of salinity, temperature, and light irradiance.

While studies dealing with the quantity of nutrients introduced into coastal waters have focused on total %N within algal tissue, fewer studies on the source of nutrients have evaluated tissue $\delta^{15}N$. By convention, dinitrogen present in the atmosphere has a $\delta^{15}N$ value of 0‰ and is used as the standard reference for $\delta^{15}N$ comparisons. Values greater than 0‰ indicate enrichment in ^{15}N and values less than 0‰ indicate depletion, with respect to atmospheric N. Nutrient depleted oceanic water, where nitrogen fixation may be a major source of nitrogen, typically displays values very close to or less than 0‰ (Kreitler, 1979; Lajtha and Michener, 1994). Fertilizers manufactured with atmospheric N to produce NH_4 will not produce a $\delta^{15}N$ value different than 0‰ (Lajtha and Michener, 1994). Kreitler (1979) and Kreitler and Browning (1983) report that $\delta^{15}N$ nitrate values from 2-8‰ are indicative of influence from soil cultivation without the use of fertilizer and values from 10-22‰ are influenced by animal waste and septic seeping.

Unfortunately, the majority of research conducted on $\delta^{15}N$ in marine plants has focused on phytoplankton rather than macroalgae. Lajitha and Michener (1994) reported that phytoplankton studies provide a wide range of observations for the magnitude of isotopic fractionation in N uptake. Some of the reported discrepancies may result from employment of different culture conditions. Movement of N across the plasmalemma causes little or no fractionation, whereas the subsequent assimilation of N fractionates strongly. Additionally, the fractionation of N in diatoms is higher in unstirred versus well-mixed cultures (Lajitha and Michener, 1994). In summary, Lajitha and Michener (1994) concluded that data indicate fractionation for inorganic nitrogen uptake may vary

significantly as a consequence of growth conditions and species. Cohen and Fong (2005) published one of the few investigations describing fractionation within macroalgae. Short-term experiments suggest that under a variety of ratios and concentrations of N addition, *Enteromorpha intestinalis* takes up ^{14}N and ^{15}N in equal proportion. These results oppose fractionation reports of phytoplankton by Lajitha and Michener (1994). Cohen and Fong (2005) also found that in the presence of NH_4^+ and NO_3^-, algae utilize NH_4^+ first. This may confound $\delta^{15}N$ results if two sources distinct in $\delta^{15}N$ also present different ratios of NH_4^+ and NO_3^-. Schmidt *et al.* (2004) used $\delta^{15}N$ in macroalgae to study the effect of seabirds on the nutrient regime of cays along the Australian Great Barrier Reef. Cays with high densities of seabirds are thought to show extreme N enrichment due to the uric acid in seabird guano which is elevated in $\delta^{15}N$ to an average of 9.9‰. In the marine environment, uric acid is hydrolyzed to NH_4^+ and gaseous NH_3. This reaction induces an increase in pH and thus a greater loss of NH_3 to the atmosphere, causing accumulation of ^{15}N in the water column (Costanzo *et al.,* 2001). Schmidt *et al.* observed that macroalgae from cays with and without seabirds had similar $\delta^{15}N$ values (2.0-3.9‰) suggesting similar sources of nutrients or a failure of macroalgae to use the isotopically enriched N. However, an elevated value of 5.2‰ was found at a significant distance from the reef. The authors suggested that the increase in $\delta^{15}N$ may indicate that there are locations where macroalgae can utilize isotopically enriched N. In this case, they offer the explanation that aquifers may carry nutrients away from the cay and release them into the water column at some distance. This investigation failed to point out at which locations the 6 different macroalgal species were tested to determine $\delta^{15}N$ levels at the cay and away from the cay. It is possible that the locations resulting in elevated values

were a result of a different species being sampled. Costanzo *et al.* (2001) performed two experiments using levels of $\delta^{15}N$ in marine plants to map the effects of multiple sewage outfalls on an Australian bay. Their first experiment entailed sampling natural populations of seagrass, mangrove and macroalgae, while their second called for incubation of the red algae *Catenella nipae* Zanardini at sites arranged radially around the river mouths delivering sewage to the bay. In both methods, high values of $\delta^{15}N$ (ca. 10‰) were found around river mouths and decreased with distance from the source. Measurements greater than 3‰ were described as having been influenced by sewage. Results of this study support the use of macroalgae as an indicator of nutrient source, however similar to the previously mentioned study performed by Schmidt *et al.* (2004) the composition of species sampled at each location was not discussed in the report. This is an important point to consider when differences in N uptake and fractionation between species sampled may contribute to the results. There have been no studies which have dealt with the validity of lumping different species or genera together for $\delta^{15}N$ analysis.

Room for improvement of the use of algae as bioindicators lies in the accuracy of data collected at long distances from the source of eutrophication. In areas with low nutrient conditions, flux from a nutrient source may be hidden by input from sediments. At present, relative measures of % tissue N among sites more closely reflect the water column conditions than quantitative measurements of water nutrient analysis (Fong *et al*., 1994; Fong *et al*., 1998). The development of greater accuracy in quantitative measurements would benefit programs utilizing algae as a means for monitoring eutrophication.

Methods

La Parguera is located on the southwest coast of Puerto Rico (17°57'N, 67°02'W) (Fig. 1). A range of hills within 2 km of the coast isolates the local water shed from further inland sources. Mangrove forests occupy much of the coastline as well as colonize small cays leeward of patch reefs in the inner and mid-shelf. The insular shelf extends approximately 10 km from shore, reaches an average depth of 20 m and supports spur and groove reefs along the perimeter. The prevailing currents flow east to west and the sewage treatment plant is located less than 1 km downstream from the town center of La Parguera.

A total of 21 sites across the La Parguera region were included in this study. Collection sites were: Sewage Plant (17° 58.491N, 67° 03.843W), Boat Ramp (17° 58.268N, 67° 02.732W), Guayacan (17° 56.963N, 67° 05.809W), Phosphorescent Bay (17° 58.018N, 67° 00.881W), Magueyes (17° 58.207N, 67° 02.774W), Collado (17° 57.228N, 67° 04.733W), Pelotas (17° 57.542N, 67° 04.416W), Conserva (17° 57.769N, 67° 03.639W), El Corral (17° 56.866N, 67° 00.175W), Enrique (17° 57.309N, 67° 03.191W), Media Luna (17° 56.096N, 67° 02.911W), Turrumote (17° 56.191N, 67° 01.293W), Margarita (17° 55.405N, 67° 05.801W), Algal Plain (17° 54.771N, 67° 03.638W), El Hoyo (17° 52.559N, 67° 02.619W), Weinberg (17° 53.429N, 66° 59.320W), Buoy (17° 53.300N, 66° 59.879), Romero (17° 56.825N, 66° 58.527W), Bird Island (17° 57.992N, 67° 02.251W), Margarita Basin (17° 90012, 67° 05610), and El Palo (17° 56.638N, 67° 05.209W) (Fig. 1). Sampling was conducted within three consecutive days starting on July 11th 2007. No rainfall occurred in La Parguera for 21

days prior to sampling (Caricomp data provided by Ernesto Weil, personal communications).

La Parguera, Puerto Rico

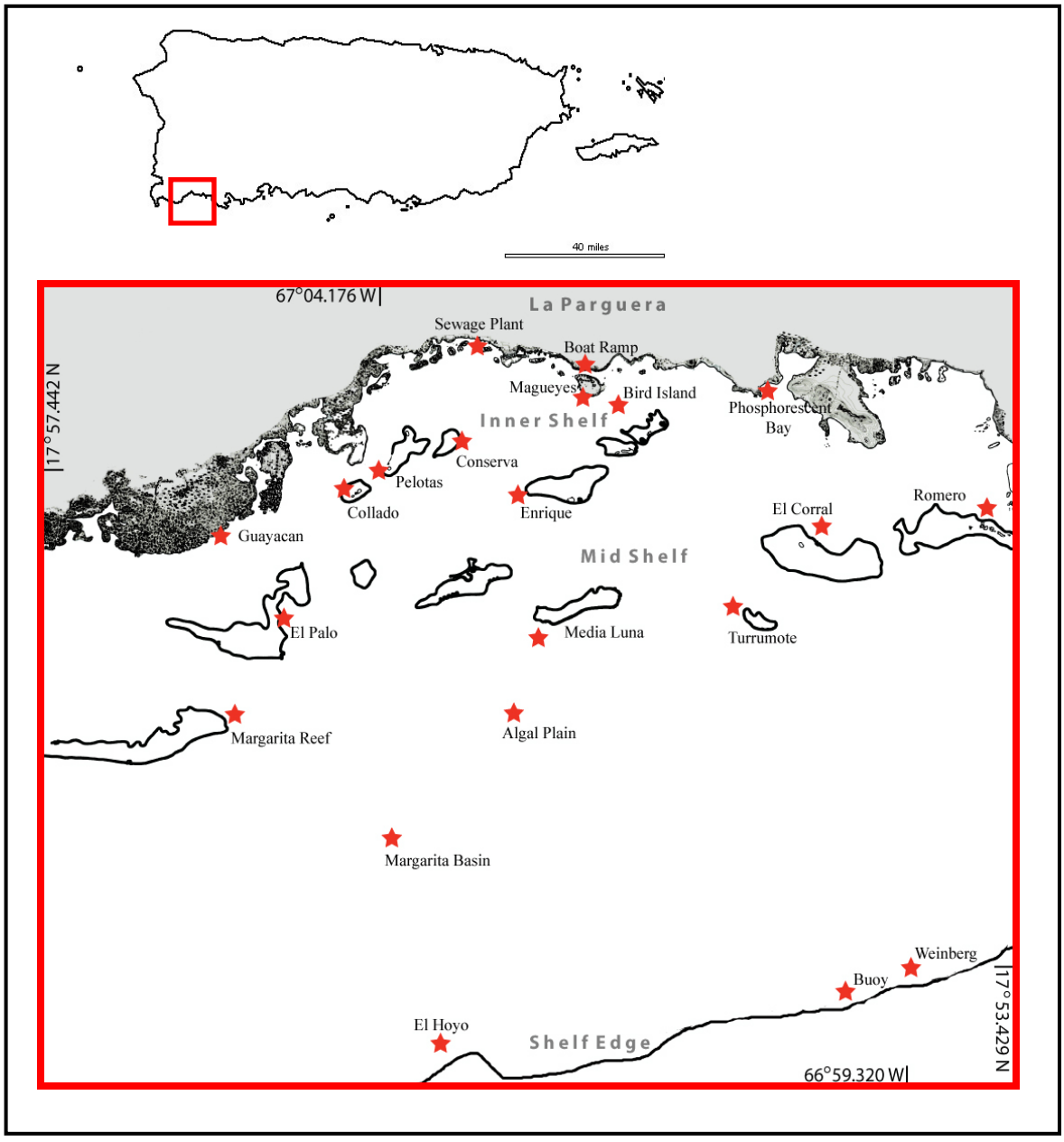

Fig. 1. Map of Puerto Rico and La Parguera study sites.

A total of 34 candidate algal species were collected in over 50 coral reef dives in a pilot study for this investigation. Three of the species collected were selected for nutrient analysis. The process of elimination began by selecting for algal species that had high potential for success as bioindicators of nutrient enrichment. Thus, preference was shown towards species that possessed a relatively high capacity for N storage within their tissue (Larned 1998). The N storage capacity for an alga varies inversely with the surface area to volume ratio, suggesting that larger, thicker algae are most suitable. However, ephemeral, fast-growing, algae with thin, erect branching thalli and simple morphology tend to have faster rates of N uptake and a higher affinity for N at low concentrations than do larger, slow-growing algae (Fong *et al*., 2001; Fujita, 1985; Pedersen and Borum, 1997; Wallentinus, 1984). Algae with a variety of morphologies were therefore examined in this study. Year-round abundance and broad distributions across the insular shelf were also desirable traits. This resulted in the following species selected for analysis: *Dictyota bartayresiana* Lamouroux (Heterokontophyta) as it has the broadest cross-shelf distribution, being common from adjacent to shore to the edge of the insular shelf; *Lobophora variegata* (Lamouroux) Womersley ex Oliveira (Heterokontophyta) which is common in habitats of various light and depth conditions at mid-shelf and outer-shelf locations; *Acanthophora spicifera* (Vahl) Børgesen) (Rhodophyta) due to its rapid growth rate and prevalence from near shore to mid-shelf reefs. Additionally, *A. spicifera* was selected because total tissue N of red algae has been demonstrated to have closer correlation to water column concentration than the tissue of green or brown algae (Bird *et al*., 1982; Björnsäter and Wheeler, 1990; D'Elia and DeBoer, 1978; Jones *et al*., 1996). Due to the fact that *D. bartayresiana* frequently grows in mats, less dense tufts were

collected. For all species, caution was taken to select algae that were not growing in shaded habitats because organisms that are not continuously undergoing a high rate of photosynthesis have an increased opportunity for storage of nutrients (Fong *et al*., 2001). This was difficult to achieve with *A. spicifera* because this alga typically grows on mangrove prop roots where shading is almost certain for part of each day. At the majority of these sites, collections were made from the outermost roots subject to minimal shading.

Acanthophora spicifera occurred at 12 sites across the inshore and mid shelf reefs. *Dictyota bartayresiana* occurred at 16 sites reaching across the inshore, mid-shelf and edge of the insular shelf and *Lobophora variegata* occurred at 8 sites from the mid-shelf to the edge of the insular shelf. Where available, at least six replicates of each alga were collected. Distance between replicates varied among sites due to frequency of species occurrence. In most cases, replicates were at least 5m apart.

Algal samples were collected by SCUBA and snorkeling, transported back to lab in seawater-filled plastic bags kept on ice, briefly rinsed in fresh water to remove salts and nutrients from the surface (Fong *et al*., 1994; Fong *et al*., 1998; Fong *et al*., 2001; Jones *et al.,* 1996), blotted with paper towels to remove excess water, placed in glass scintillation vials, and stored at -20°C until freeze-dried (Virtis Model 25SL Freeze Drier). Following freeze drying, algae were ground with a mortar and pestle (Cohen and Fong, 2005). Samples were analyzed for total %N and $\delta^{15}N$ by a CHN analyzer coupled to an isotope-ratio mass spectrometer at the Cornell University Isotope Laboratory and the University of California, Davis Stable Isotope Facility. Delta ^{15}N is defined as:

$$\delta^{15}N\ (‰) = [(R_{sample}/R_{air}) - 1] \times 10^3$$

where *R* is equal to the atomic $^{15}N/^{14}N$ ratio. Non parametric multiple comparison analysis with t-distribution was performed using the Unistat 5.5 statistical package for Excel to distinguish significantly different values among sites. Unless otherwise stated, significance was at the 5% level.

Over 10cm of rainfall in the days following the sampling period (Caricomp data provided by Ernesto Weil, personal communications) allowed for a unique opportunity to test for change in algal tissue nutrient concentration resulting from runoff. *Acanthophora spicifera* was sampled on July 20th, 2007 at both Magueyes and Boat Ramp to compare nutrient concentration pre- and post-rain. Single-factor ANOVA was used to detect significant differences pre- and post-rain at both sites.

Results

Inshore measurements of %N in *Acanthophora spicifera* varied greatly (1.27% ± .17 to 3.25% ± .24) (Fig. 2). Higher values were associated with well developed mangrove stands and lower values corresponded with lesser developed mangrove areas. Percent N means at mid-shelf locations appear to be lower than most means along the shoreline, however multiple comparisons analysis formed 10 subsets of nonsignificantly different sites (Fig. 3) and three of these subsets contain locations from both the shoreline and mid-shelf.

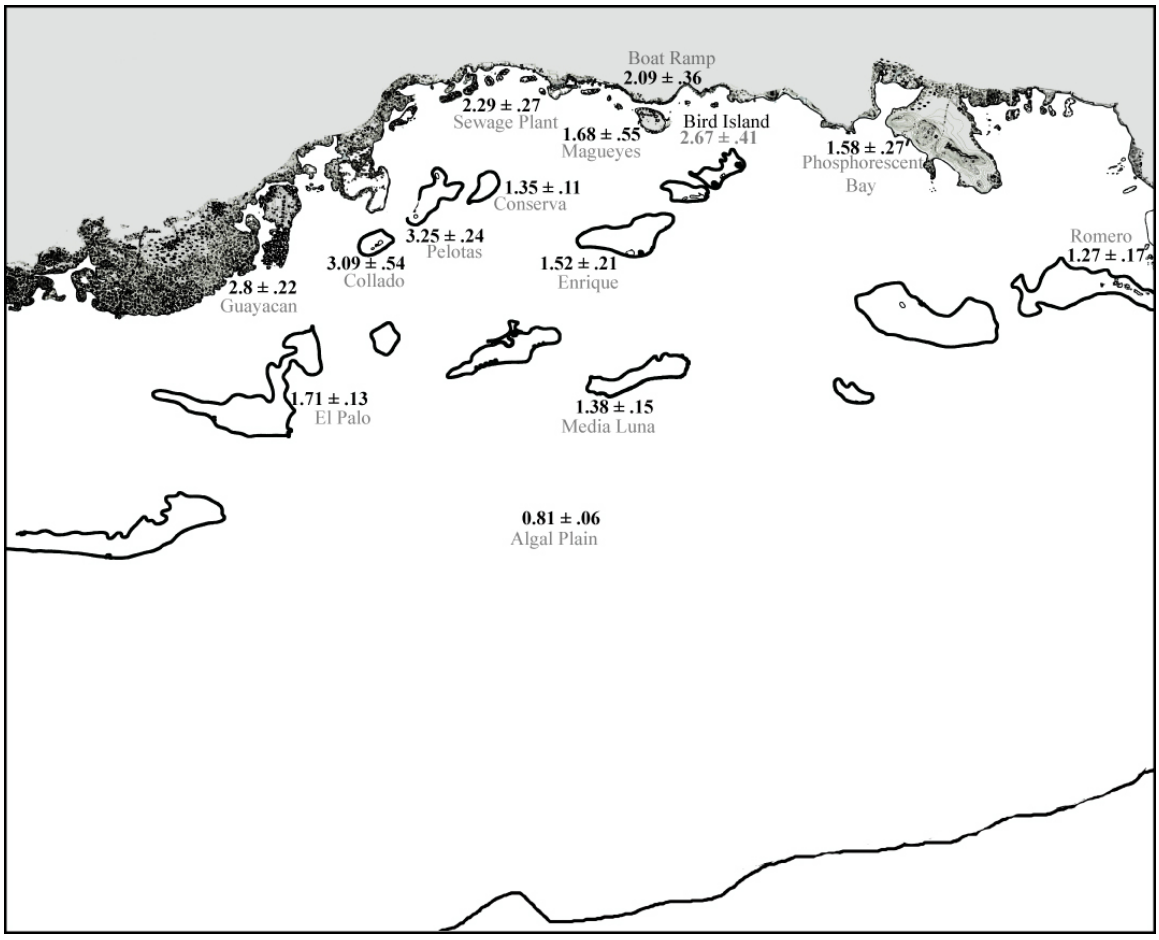

Fig. 2. Means of %N in tissue of *Acanthophora spicifera* across the insular shelf.

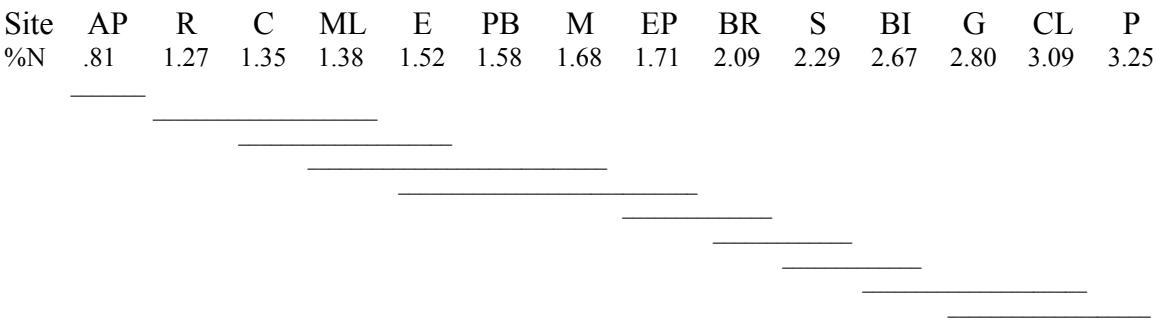

Fig. 3. Nonsignificantly different subsets produced by multiple comparisons analysis of %N in tissue of *Acanthophora spicifera* across the insular shelf. (See Fig. 2 for location of sites)
AP=Algal Plain; R=Romero; C=Conserva; ML=Media Luna; E=Enrique; PB=Phosphorescent Bay; M=Magueyes; EP=El Palo; BR=Boat Ramp; S=Sewage Plant; BI=Bird Island; G=Guayacan; CL=Collado; P=Pelotas

Percent N of *Dictyota bartayresiana* and *Lobophora variegata* did not display clear trends across the insular shelf (Fig. 4 and 6). Four of the 5 nonsignificantly different subsets for *D. bartayresiana* contain locations near the shoreline and at the shelf edge (Fig. 5). *Lobophora variegata* produces values with similar lack of a gradient. Two of the 4 nonsignificantly different subsets contain locations at the mid-shelf and shelf edge (Fig. 7). The lowest value of *L. variegata* (0.64% ± .07) was found at a mid-shelf location.

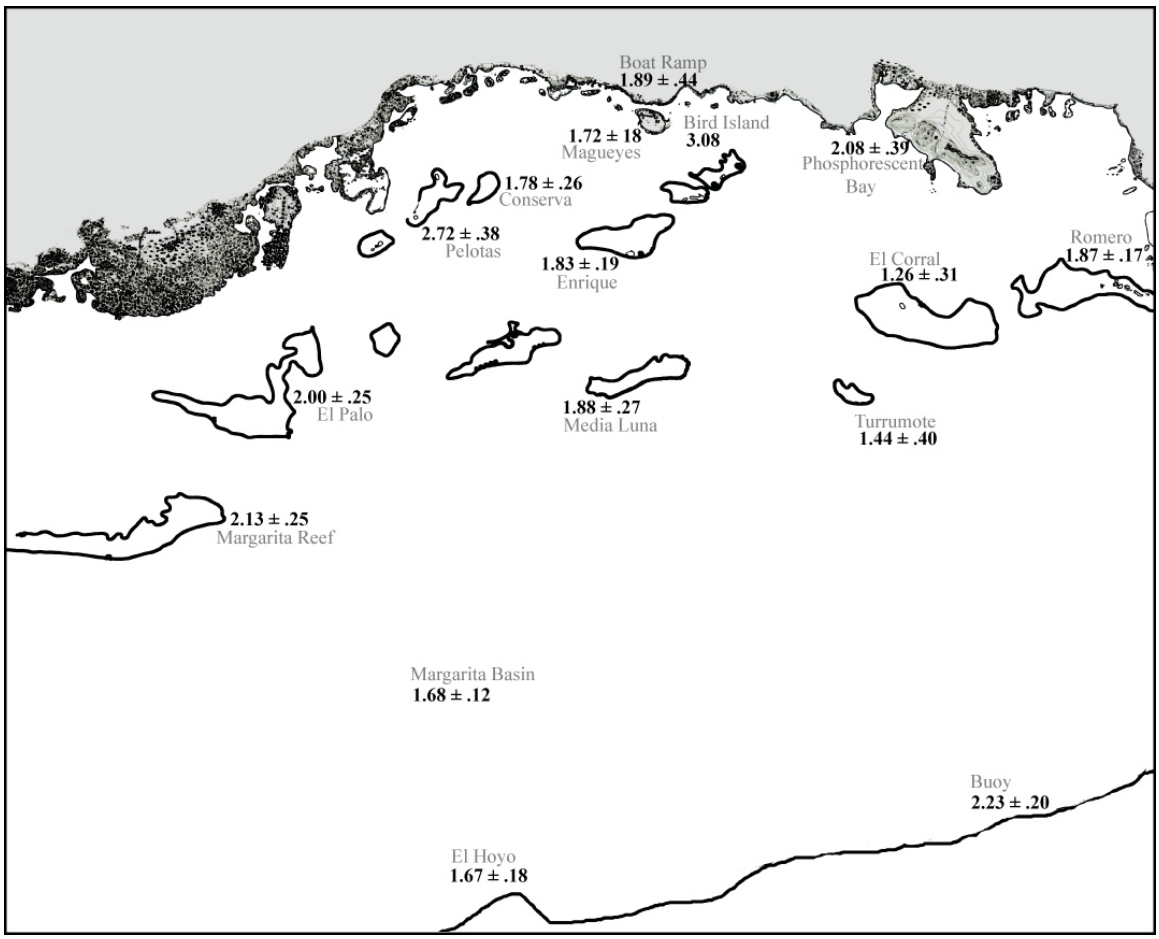

Fig. 4. Means of %N in tissue of *Dictyota bartayresiana* across the insular shelf.

Site	EC	T	EH	MB	M	C	BR	E	ML	R	EP	PB	MR	B	P	BI
%N	1.26	1.44	1.67	1.68	1.72	1.78	1.89	1.83	1.88	1.87	2.00	2.08	2.13	2.23	2.72	3.08

Fig. 5. Nonsignificantly different subsets produced by multiple comparisons analysis of %N in tissue of *Dictyota bartayresiana* across the insular shelf. (See Fig. 4 for location of sites) EC=El Corral; T=Turrumote; EH=El Hoyo; MB=Margarita Basin; M=Magueyes; C=Conserva; BR=Boat Ramp; E=Enrique; ML=Media Luna; R=Romero; EP=El Palo; PB=Phosphorescent Bay; MR=Margarita Reef; B=Buoy; P=Pelotas; BI=Bird Island

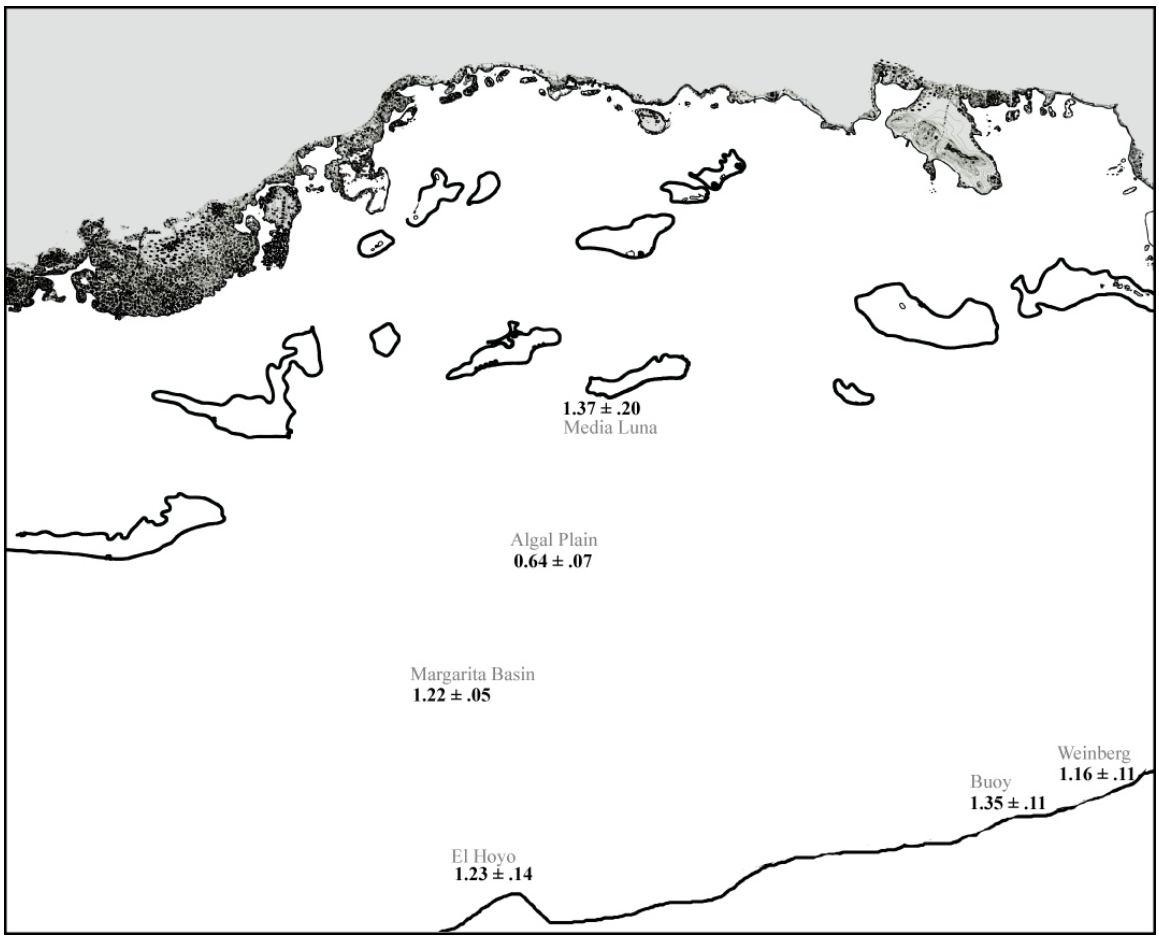

Fig. 6. Means of %N in tissue of *Lobophora variegata* across the insular shelf.

Site	AP	W	MB	EH	ML	B
%N	0.64	1.16	1.22	1.23	1.37	1.35

Fig. 7. Nonsignificantly different subsets produced by multiple comparisons analysis of %N in tissue of *Lobophora variegata* across the insular shelf. (See Fig. 6 for location of sites) AP=Algal Plain; W=Weinberg; MB=Margarita Basin; EH=El Hoyo; ML=Media Luna; B=Buoy

Similar to %N, $\delta^{15}N$, inshore measurements of *Acanthophora spicifera* varied greatly (1.77‰ ± 0.22 to 8.16‰ ± .34) (Fig. 8). However, a reverse trend was observed with higher values associated with lesser developed mangrove areas (including the town

of La Parguera) and lower values corresponding with well developed mangrove stands. The highest inshore values of $\delta^{15}N$ occurred at two stations influenced by secondary sewage input (Sewage Plant: 8.16‰ ± 0.34) and a bird rookery (Bird Island: 5.91‰ ± 0.48). The seven nonsignificantly different subsets created by multiple comparison analysis show a clear progression from higher $\delta^{15}N$ values along the eastern portionof the shoreline to lower values at mid-shelf and the western portion of the shoreline (Fig. 9). A single anomaly to this trend occurred at the inshore location Conserva where the lowest $\delta^{15}N$ value (1.77‰ ± 0.22) was found.

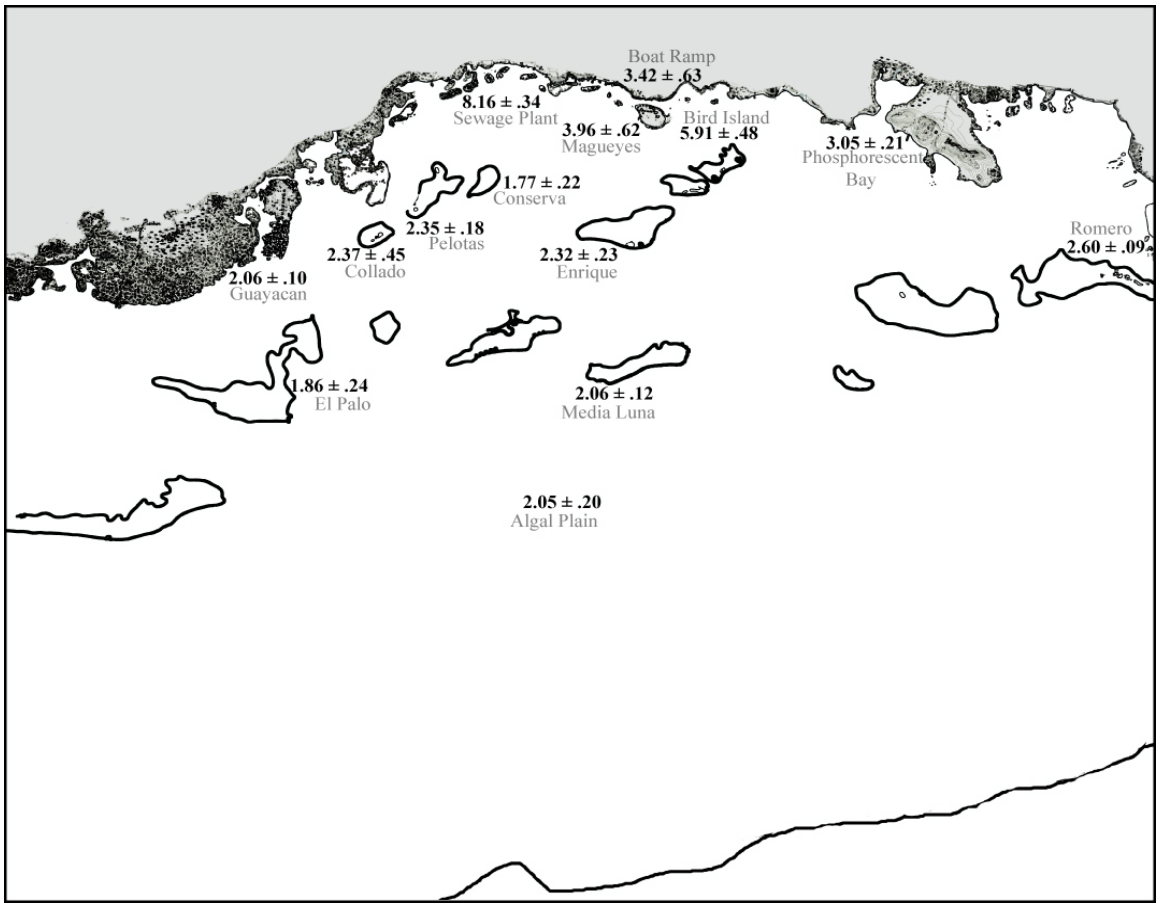

Fig. 8. Means of $\delta^{15}N$ in tissue of *Acanthophora spicifera* across the insular shelf.

Site	C	EP	AP	G	ML	E	P	CL	R	PB	BR	M	BI	S
$\delta^{15}N$	1.77	1.86	2.05	2.06	2.06	2.32	2.35	2.37	2.60	3.05	3.42	3.96	5.91	8.16

Fig. 9 Nonsignificantly different subsets produced by multiple comparisons analysis of $\delta^{15}N$ in tissue of *Acanthophora spicifera* across the insular shelf. (See Fig. 8 for location of sites) C=Conserva; EP=El Palo; G=Guayacan; AP=Algal Plain; ML=Media Luna; CL=Collado; E=Enrique; P=Pelotas; R=Romero; PB=Phosphorescent Bay; BR=Boat Ramp; M=Magueyes; BI=Bird Island; S=Sewage Plant

Delta ^{15}N ratios in *Dictyota bartayresiana* and *Lobophora variegata* also display a clear trend across the insular shelf (Fig. 10 and 12). Nine subsets of *D. bartayresiana* indicate a decrease in $\delta^{15}N$ from shoreline to shelf edge (Fig. 11). Two anomalies to the pattern exist at near shore locations, Conserva and Phosphorescent Bay, where values are similar to those at mid-shelf locations. The four subsets of *L. variegata* follow a similar decreasing trend from mid-shelf to shelf edge (Fig. 13).

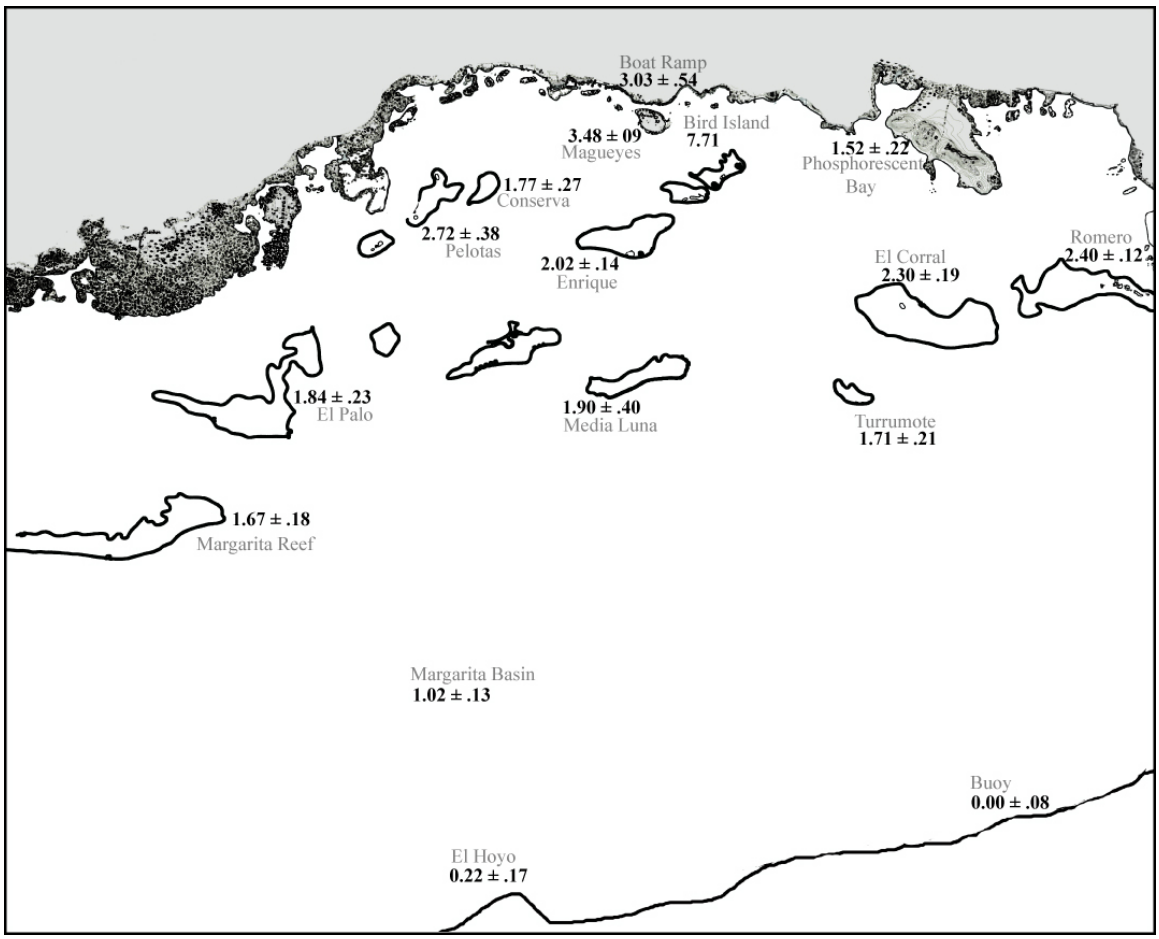

Fig. 10. Means of $\delta^{15}N$ in tissue of *Dictyota bartayresiana* across the insular shelf.

Site	B	EH	MB	PB	MR	T	C	EP	ML	E	EC	R	P	BR	M	BI
$\delta^{15}N$	0.00	0.22	1.02	1.52	1.67	1.71	1.77	1.84	1.90	2.02	2.30	2.40	2.72	3.03	3.48	7.71

Fig. 11. Nonsignificantly different subsets produced by multiple comparisons analysis of $\delta^{15}N$ in tissue of *Dictyota bartayresiana* across the insular shelf. (See Fig. 10 for location of sites) B=Buoy; EH=El Hoyo; MB=Margarita Basin; PB=Phosphorescent Bay; MR=Margarita Reef; T=Turrumote; C=Conserva; EP=EL Palo; ML=Media Luna; E=Enrique; EC=El Corral; R=Romero; P=Pelotas; BR=Boat Ramp; M=Magueyes; BI=Bird Island

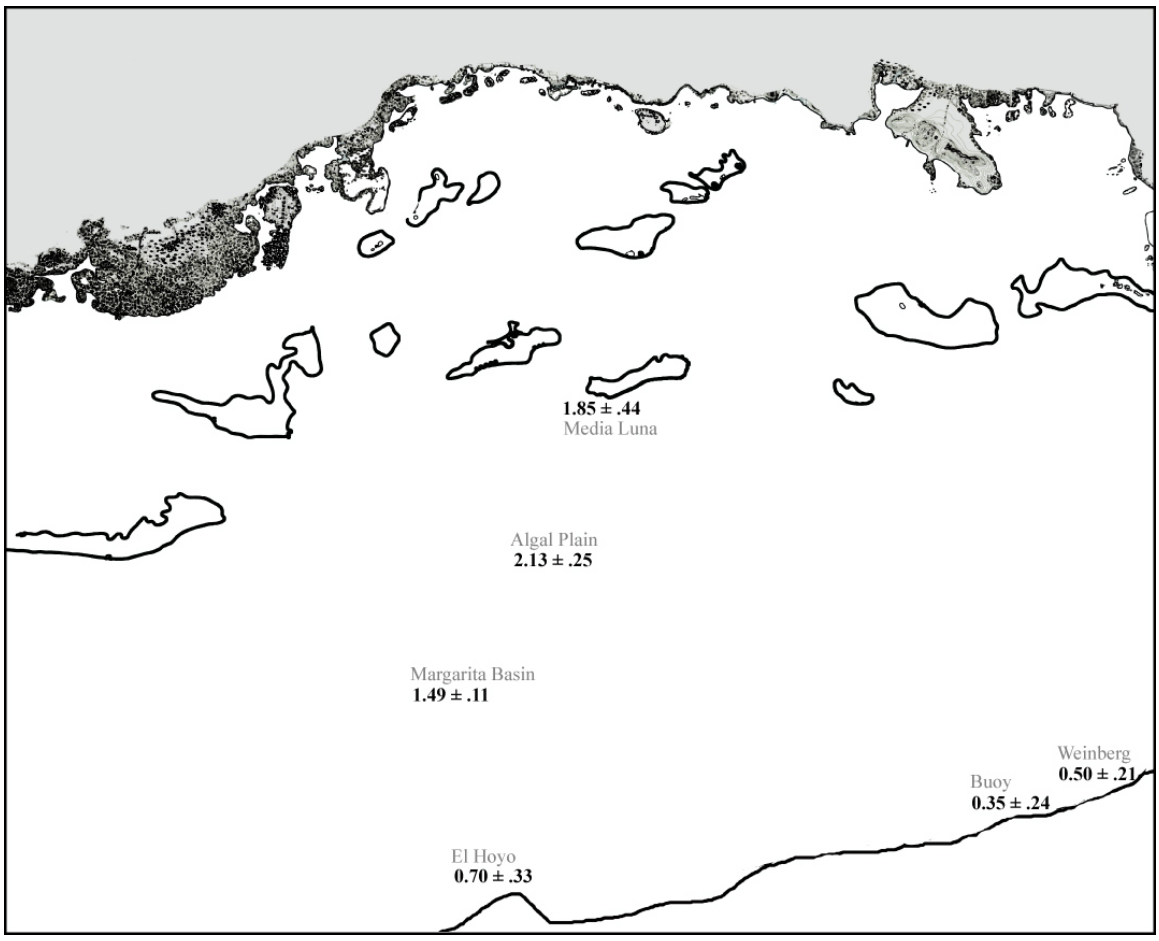

Fig. 12. Means of $\delta^{15}N$ in tissue of *L. variegata* across the insular shelf.

Site	B	W	EH	MB	ML	AP
$\delta^{15}N$	0.35	0.54	0.70	1.49	1.85	2.13

Fig. 13. Nonsignificantly different subsets produced by multiple comparisons analysis of $\delta^{15}N$ in tissue of *Lobophora variegata* across the insular shelf. (See Fig. 12 for location of sites) B=Buoy; W=Weinberg; EH=El Hoyo; MB=Margarita Basin; ML=Media Luna; AP=Algal Plain

Mean post-rain %N increased non significantly (p=.53) at Magueyes and decreased non significantly (p=.81) at Boat Ramp (Table 1). Mean post-rain $\delta^{15}N$

increased at both sites but only resulted in a significant difference at Boat Ramp (p=.022) (Table 2).

Table 1. Percent N in *Acanthophora spicifera* at Magueyes and Boat Ramp pre- and post-rain event. Single-factor ANOVA.

	Boat Ramp		Magueyes	
	Post-Rain n=6	Pre-Rain n=5	Post-Rain n=6	Pre-Rain n=6
Mean %N	2.05	2.09	1.87	1.68
SD	0.26	0.36	0.44	0.55
	p=0.81		p=0.53	

Table 2. Delta ^{15}N in *Acanthophora spicifera* at Magueyes and Boat Ramp pre- and post-rain event. Single-factor ANOVA.

	Boat Ramp		Magueyes	
	Post-Rain n=6	Pre-Rain n=5	Post-Rain n=6	Pre-Rain n=6
Mean $\delta^{15}N$	4.31	3.42	4.55	3.96
SD	0.44	0.63	0.67	0.62
	p=0.02		p=0.14	

Discussion

Despite high variability, %N in *Acanthophora spicifera* tissue indicates a weak nutrification pattern across the inner and mid shelf, however, variability for *Dictyota bartayresiana* and *Lobophora variegata* do not clearly cross shelf patterns of nutrification. High values of %N for *A. spicifera* in developed mangrove stands may be due to higher rates of productivity in the immediate area, including a large invertebrate community that is capable of supplying large amounts of nutrients in the form of excretory wastes (Bracken *et al.,* 2007). Nitrogen recycling within the mangrove community may also be responsible for the elevated observed values (Carpenter and Capone, 1983). The lower values found in some mid-shelf locations may indicate lower nutrient concentrations. The range of %N in *A. spicifera* reported in this study (0.81-3.25%) is larger than that reported by Fong *et al*., (2001) for approximately the same study area (1.10-2.67%).

Marked variability of %N in *Dictyota bartayresiana* and *Lobophora variegata* across the insular shelf may be the result of a number of possibilities. The first of which, could be representative of truly variable nutrient conditions across the insular shelf. This lack of a trend in %N can also occur if algae are N limited and thus can not accumulate N in storage vacuoles, preventing a reflection of different nutrient regimes. Otero (personal communication) indicated that previous experimentation on nutrient limitation within the sampling area indicated a co-limitation of nitrogen, phosphorus, and iron and these limitations may be responsible for the variations of algal tissue %N. A third possibility is saturation of N in algal tissue across the insular shelf. Within a system experiencing low levels of N, sufficient water energy may provide access to more N than can be taken up

or stored in internal storage vacuoles (Larned and Atkinson, 1997). Nitrate releasing cyanobacterial symbionts living within Caribbean reef sponges can also provide a significant amount of nitrate to algae (Corredor *et al.,* 1988). Percent N means reported here for *D. bartayresiana* are similar to means for *D. cervicornis* and *D. dichotoma* presented by Fong *et al*. (2001) for the coastal waters of La Parguera. Mean %N was about 1.5% and 1.6% for *D. cervicornis* and *D. dichotoma*, respectively. The samples, taken in January of 1998, depict higher %N offshore compared to inshore for both *D. cervicornis* and *D. dichotoma*, a trend opposite to that of all other species examined. Results of the present study and of Fong *et al*. (2001) suggest that algal tissue %N in *Dictyota spp.* is not a clear indicator of nutrient loading in La Parguera. However, Fong *et al.* (2001) did find a decrease in %N from the shoreline to mid-shelf in the tissue of other algal species, including *A. spicifera.* Their sampling took place in January, a month typically characterized by calm seas and low wind speeds. July, the month sampled in the current study was very windy, with large swells and currents. Increased water energy provided in July could elevate nutrient flux from sediments or sufficiently increase the supply of N to algal cells, effectively saturating intercellular stores, and thus disrupting any spatial detectable nutrient trends within the water column.

Gradients in $\delta^{15}N$ and %N do not necessarily co-fluctuate in the marine environment. This is to be expected because high values of %N can occur with low values of $\delta^{15}N$ (-2 to 2‰) when ammonium based fertilizers are used for farming. Low values of %N can occur with high values of $\delta^{15}N$ (10-20‰) when sewage effluents become diluted in aquifers or other large masses of water (McClelland and Valiela,

1998). The latter scenario may explain the presence of a $\delta^{15}N$ gradient occurring with a lack of a %N gradient in the La Parguera region.

Algal tissue $\delta^{15}N$ values presented in this study range from -0.39 to 8.05‰. This represents the combined $\delta^{15}N$ signature of all algal intracellular nitrogen. Trends of $\delta^{15}N$ for *Acanthophora spicifera, Dictyota bartayresiana,* and *Lobophora variegata* indicate significant anthropogenic impacts in geographically limited areas along the developed shoreline (up to 8.05‰), moderate to low effects at mid-shelf (2-3‰), and no detectable effect at the shelf edge (< 2‰). The highest $\delta^{15}N$ values for *A. spicifera* identify two potential point sources of allochthonous nutrients; a sewage treatment plant and a bird rookery inhabited primarily by cattle egrets, land feeding birds.

Kreitler (1979) and Kreitler and Browning (1983) report that $\delta^{15}N$ nitrate values from 2-8‰ are indicative of influence from soil cultivation without the use of fertilizer and values from 10-22‰ are influenced by animal waste and septic seeping.
Septic seeping occurs in La Parguera but areas of soil cultivation are not observed in the local watershed. Cultivation increases $\delta^{15}N$ by increasing oxidation of soil organic N (Kreitler and Browning, 1983). Digging and overturning of soil as would occur during land development activities in La Parguera, may offer an analogous situation creating similar effects in $\delta^{15}N$. This disturbance may increase aeration of the soil which increases the activity of microbes within the soil. Denitrification performed by many of these microbes results in an increase of $\delta^{15}N$ in the soil which is transported to the ocean in runoff (McClelland and Valiela, 1998). The amount of runoff also increases when compaction of soil is reduced during land development and vegetation removal.

In the present study, $\delta^{15}N$ provides a more defined nutrient trend than %N across the insular shelf of La Parguera. The trend defined by $\delta^{15}N$ does not indicate the quantity of nutrients in the water, as will %N, but implies that anthropogenic impacts are strongest at locations along the shoreline and may not reach the local shelf edge. In areas where algae are not N limited, perhaps $\delta^{15}N$ would provide better evidence for allochthonous nutrient inputs than %N.

Discussion pertaining to pre- and post-rain change in tissue nutrient content of macroalgae is brief due to limited observations. During and after the rain event, sediment plumes in the area of Boat Ramp were evident for more than 24 hours, however, measures of %N did not indicate an increase in nutrient input. It is possible that N stores within the algae were already saturated from previously high nutrient concentrations at both Boat Ramp and Magueyes. Measures of $\delta^{15}N$ indicate a significant increase in allochthonous N at Boat Ramp but not at Magueyes. Algae sampled from Magueyes are located in an area shielded from the visible sediment plume by a land mass and currents that direct the plume away from the site. In this particular sampling event, $\delta^{15}N$ was also a better indicator than %N of new nutrient influx. Delta ^{15}N may perform better as an indicator of nutrient pulses when algae are sampled from locations often saturated with N.

Conclusion

High levels of algal tissue $\delta^{15}N$ (ca. 8‰) in the inshore waters of La Parguera are indications of moderate anthropogenic influence and may be originating from sewage input and/or land development activities. Low levels of $\delta^{15}N$ (less than 0‰) in algal tissues observed at the edge of the insular shelf are typical of nutrient depleted oceanic water (Kreitler, 1979), demonstrating that the influence of anthropogenic effects visible in inshore waters did not have an effect on offshore waters. Results from this study strongly support the use of algal tissue $\delta^{15}N$ as an indicator of anthropogenic influence in coastal waters. More work is needed to fully encourage the use of algal tissue total %N as an indicator of nutrification. Conclusions presented here are based on a limited sample period. Future sampling should be designed to take into account seasonal differences in environmental conditions.

Appendix. 1. Raw values of algal samples collected for this study.

Date	Time	Location	*Genus sp.*	%N	$\delta^{15}N$
7/11/2007	8:00 AM	Turrumote	*Dictyota bartayresiana*	1.77	1.59
7/11/2007		Turrumote	*Dictyota bartayresiana*	1.54	1.61
7/11/2007		Turrumote	*Dictyota bartayresiana*	2.00	1.44
7/11/2007	8:30 AM	Turrumote	*Dictyota bartayresiana*	1.09	1.95
7/11/2007		Turrumote	*Dictyota bartayresiana*	1.01	1.74
7/11/2007		Turrumote	*Dictyota bartayresiana*	1.20	1.96
7/11/2007		El Corral	*Dictyota bartayresiana*	1.23	2.35
7/11/2007		El Corral	*Dictyota bartayresiana*	1.10	2.19
7/11/2007		El Corral	*Dictyota bartayresiana*	1.03	2.52
7/11/2007		El Corral	*Dictyota bartayresiana*	0.92	2.46
7/11/2007		El Corral	*Dictyota bartayresiana*	1.58	2.32
7/11/2007		El Corral	*Dictyota bartayresiana*	1.71	1.99
7/11/2007	10:00 AM	Romero	*Dictyota bartayresiana*	1.94	2.55
7/11/2007		Romero	*Dictyota bartayresiana*	2.03	2.44
7/11/2007		Romero	*Dictyota bartayresiana*	2.04	2.43
7/11/2007		Romero	*Dictyota bartayresiana*	1.78	2.37
7/11/2007		Romero	*Dictyota bartayresiana*	1.59	2.19
7/11/2007		Romero	*Dictyota bartayresiana*	1.83	2.42
7/11/2007		Romero	*Acanthophora spicifera*	1.58	2.50
7/11/2007		Romero	*Acanthophora spicifera*	1.21	2.57
7/11/2007		Romero	*Acanthophora spicifera*	1.07	2.64
7/11/2007		Romero	*Acanthophora spicifera*	1.23	2.53
7/11/2007		Romero	*Acanthophora spicifera*	1.22	2.75
7/11/2007		Romero	*Acanthophora spicifera*	1.30	2.62
7/11/2007		Phosphorescent Bay	*Dictyota bartayresiana*	2.10	1.58
7/11/2007		Phosphorescent Bay	*Dictyota bartayresiana*	1.67	1.70
7/11/2007		Phosphorescent Bay	*Dictyota bartayresiana*	2.45	1.27
7/11/2007	10:50 AM	Phosphorescent Bay	*Acanthophora spicifera*	1.37	3.10
7/11/2007		Phosphorescent Bay	*Acanthophora spicifera*	1.46	3.37
7/11/2007		Phosphorescent Bay	*Acanthophora spicifera*	2.10	2.86
7/11/2007		Phosphorescent Bay	*Acanthophora spicifera*	1.44	3.07
7/11/2007		Phosphorescent Bay	*Acanthophora spicifera*	1.48	3.10
7/11/2007		Phosphorescent Bay	*Acanthophora spicifera*	1.66	2.78
7/11/2007		Bird Island	*Acanthophora spicifera*	2.40	5.77
7/11/2007		Bird Island	*Acanthophora spicifera*	3.30	5.32
7/11/2007		Bird Island	*Acanthophora spicifera*	2.88	5.97
7/11/2007		Bird Island	*Acanthophora spicifera*	2.45	5.82
7/11/2007		Bird Island	*Dictyota bartayresiana*	2.96	5.37
7/11/2007		Bird Island	*Acanthophora spicifera*	2.34	6.66
7/11/2007		Bird Island	*Dictyota bartayresiana*	3.08	7.71
7/11/2007		Enrique	*Acanthophora spicifera*	1.24	2.10
7/11/2007		Enrique	*Acanthophora spicifera*	1.74	2.47
7/11/2007		Enrique	*Acanthophora spicifera*	1.38	2.70
7/11/2007		Enrique	*Acanthophora spicifera*	1.73	2.14
7/11/2007		Enrique	*Acanthophora spicifera*	1.64	2.21
7/11/2007		Enrique	*Acanthophora spicifera*	1.39	2.32
7/11/2007		Enrique	*Dictyota bartayresiana*	1.67	2.18

7/11/2007		Enrique	*Dictyota bartayresiana*	1.85	1.82
7/11/2007		Enrique	*Dictyota bartayresiana*	2.15	2.02
7/11/2007		Enrique	*Dictyota bartayresiana*	1.63	2.16
7/11/2007		Enrique	*Dictyota bartayresiana*	1.76	2.04
7/11/2007		Enrique	*Dictyota bartayresiana*	1.88	1.90
7/11/2007	12:52 PM	El Palo	*Acanthophora spicifera*	1.83	1.63
7/11/2007		El Palo	*Dictyota bartayresiana*	2.18	1.75
7/11/2007		El Palo	*Dictyota bartayresiana*	2.10	2.12
7/11/2007		El Palo	*Dictyota bartayresiana*	2.24	2.13
7/11/2007		El Palo	*Acanthophora spicifera*	1.56	2.15
7/11/2007		El Palo	*Acanthophora spicifera*	1.80	1.94
7/11/2007		El Palo	*Acanthophora spicifera*	1.63	1.72
7/11/2007		El Palo	*Dictyota bartayresiana*	1.56	1.78
7/11/2007		El Palo	*Dictyota bartayresiana*	2.01	1.63
7/11/2007		El Palo	*Dictyota bartayresiana*	1.92	1.60
7/11/2007	1:13 PM	Guayacan	*Acanthophora spicifera*	2.55	2.15
7/11/2007		Guayacan	*Acanthophora spicifera*	2.52	1.97
7/11/2007		Guayacan	*Acanthophora spicifera*	3.04	1.99
7/11/2007		Guayacan	*Acanthophora spicifera*	2.83	2.16
7/11/2007		Guayacan	*Acanthophora spicifera*	2.91	2.13
7/11/2007		Guayacan	*Acanthophora spicifera*	2.96	1.95
7/11/2007		Collado	*Acanthophora spicifera*	3.45	2.23
7/11/2007		Collado	*Acanthophora spicifera*	3.93	1.89
7/11/2007		Collado	*Acanthophora spicifera*	3.14	3.18
7/11/2007		Collado	*Acanthophora spicifera*	2.57	2.36
7/11/2007		Collado	*Acanthophora spicifera*	2.94	2.09
7/11/2007		Collado	*Acanthophora spicifera*	2.53	2.50
7/11/2007		Pelotas	*Acanthophora spicifera*	3.59	2.31
7/11/2007		Pelotas	*Acanthophora spicifera*	3.13	2.05
7/11/2007		Pelotas	*Acanthophora spicifera*	3.43	2.50
7/11/2007		Pelotas	*Acanthophora spicifera*	2.90	2.57
7/11/2007		Pelotas	*Acanthophora spicifera*	3.24	2.40
7/11/2007		Pelotas	*Acanthophora spicifera*	3.19	2.29
7/11/2007	2:22 PM	Pelotas	*Dictyota bartayresiana*	2.43	2.74
7/11/2007		Pelotas	*Dictyota bartayresiana*	2.58	2.61
7/11/2007		Pelotas	*Dictyota bartayresiana*	3.14	3.06
7/11/2007		Conserva	*Dictyota bartayresiana*	1.39	2.04
7/11/2007		Conserva	*Dictyota bartayresiana*	1.83	1.90
7/11/2007		Conserva	*Dictyota bartayresiana*	2.06	1.75
7/11/2007		Conserva	*Dictyota bartayresiana*	2.05	1.25
7/11/2007		Conserva	*Dictyota bartayresiana*	1.62	1.82
7/11/2007		Conserva	*Dictyota bartayresiana*	1.72	1.85
7/11/2007		Conserva	*Acanthophora spicifera*	1.23	1.56
7/11/2007		Conserva	*Acanthophora spicifera*	1.30	1.58
7/11/2007		Conserva	*Acanthophora spicifera*	1.31	2.10
7/11/2007		Conserva	*Acanthophora spicifera*	1.52	1.93
7/11/2007		Conserva	*Acanthophora spicifera*	1.45	1.83
7/11/2007		Conserva	*Acanthophora spicifera*	1.31	1.62
7/11/2007		Sewage Outfall	*Acanthophora spicifera*	2.20	8.45
7/11/2007		Sewage Outfall	*Acanthophora spicifera*	2.74	7.91

7/11/2007		Sewage Outfall	*Acanthophora spicifera*	2.44	7.59
7/11/2007		Sewage Outfall	*Acanthophora spicifera*	2.27	8.41
7/11/2007		Sewage Outfall	*Acanthophora spicifera*	1.96	8.24
7/11/2007	4:00 PM	Sewage Outfall	*Acanthophora spicifera*	2.14	8.38
7/12/2007	7:30 AM	El Hoyo	*Dictyota bartayresiana*	1.95	0.44
7/12/2007		El Hoyo	*Dictyota bartayresiana*	1.75	0.05
7/12/2007		El Hoyo	*Dictyota bartayresiana*	1.69	0.11
7/12/2007		El Hoyo	*Dictyota bartayresiana*	1.65	0.13
7/12/2007		El Hoyo	*Dictyota bartayresiana*	1.49	0.43
7/12/2007		El Hoyo	*Dictyota bartayresiana*	1.48	0.15
7/12/2007		El Hoyo	*Lobophora variegata*	1.24	0.73
7/12/2007		El Hoyo	*Lobophora variegata*	1.34	0.61
7/12/2007		El Hoyo	*Lobophora variegata*	1.35	0.37
7/12/2007		El Hoyo	*Lobophora variegata*	1.29	0.55
7/12/2007		El Hoyo	*Lobophora variegata*	1.19	0.61
7/12/2007		El Hoyo	*Lobophora variegata*	0.98	1.34
7/12/2007		Buoy	*Lobophora variegata*	1.48	0.31
7/12/2007		Buoy	*Lobophora variegata*	1.35	0.42
7/12/2007		Buoy	*Lobophora variegata*	1.17	-0.01
7/12/2007		Buoy	*Lobophora variegata*	1.31	0.54
7/12/2007		Buoy	*Lobophora variegata*	1.46	0.19
7/12/2007		Buoy	*Lobophora variegata*	1.36	0.63
7/12/2007		Buoy	*Dictyota bartayresiana*	2.43	0.08
7/12/2007		Buoy	*Dictyota bartayresiana*	2.21	0.00
7/12/2007		Buoy	*Dictyota bartayresiana*	2.03	-0.08
7/12/2007		Weinberg	*Lobophora variegata*	1.22	0.40
7/12/2007		Weinberg	*Lobophora variegata*	1.16	0.24
7/12/2007		Weinberg	*Lobophora variegata*	0.98	0.51
7/12/2007		Weinberg	*Lobophora variegata*	1.24	0.35
7/12/2007		Weinberg	*Lobophora variegata*	1.09	0.79
7/12/2007		Weinberg	*Lobophora variegata*	1.26	0.70
7/12/2007		Margarita Basin	*Lobophora variegata*	1.26	1.67
7/12/2007		Margarita Basin	*Dictyota bartayresiana*	1.77	0.89
7/12/2007		Margarita Basin	*Dictyota bartayresiana*	1.78	1.07
7/12/2007		Margarita Basin	*Lobophora variegata*	1.17	1.52
7/12/2007		Margarita Basin	*Lobophora variegata*	1.15	1.55
7/12/2007		Margarita Basin	*Dictyota bartayresiana*	1.73	0.97
7/12/2007		Margarita Basin	*Dictyota bartayresiana*	1.72	1.25
7/12/2007		Margarita Basin	*Lobophora variegata*	1.29	1.39
7/12/2007		Margarita Basin	*Dictyota bartayresiana*	1.51	0.99
7/12/2007		Margarita Basin	*Dictyota bartayresiana*	1.55	0.94
7/12/2007		Margarita Basin	*Lobophora variegata*	1.22	1.37
7/12/2007		Margarita Basin	*Lobophora variegata*	1.24	1.46
7/12/2007	1:30 PM	Algal Plain	*Acanthophora spicifera*	0.74	2.25
7/12/2007		Algal Plain	*Lobophora variegata*	0.50	2.48
7/12/2007		Algal Plain	*Lobophora variegata*	0.74	2.18
7/12/2007		Algal Plain	*Lobophora variegata*	0.64	2.05
7/12/2007		Algal Plain	*Lobophora variegata*	0.62	2.38
7/12/2007		Algal Plain	*Lobophora variegata*	0.69	1.72
7/12/2007		Algal Plain	*Lobophora variegata*	0.64	1.97

7/12/2007		Algal Plain	*Lobophora variegata*	0.65	2.12
7/12/2007		Algal Plain	*Acanthophora spicifera*	0.80	2.01
7/12/2007		Algal Plain	*Acanthophora spicifera*	0.90	2.21
7/12/2007		Algal Plain	*Acanthophora spicifera*	0.77	1.76
7/12/2007		Algal Plain	*Acanthophora spicifera*	0.84	2.04
7/13/2007	12:00 PM	Magueyes	*Acanthophora spicifera*	2.37	2.88
7/13/2007		Magueyes	*Acanthophora spicifera*	1.24	3.88
7/13/2007		Magueyes	*Acanthophora spicifera*	1.08	3.69
7/13/2007		Magueyes	*Dictyota bartayresiana*	1.96	3.45
7/13/2007		Magueyes	*Dictyota bartayresiana*	1.73	3.41
7/13/2007		Magueyes	*Dictyota bartayresiana*	1.87	3.48
7/13/2007		Magueyes	*Dictyota bartayresiana*	1.66	3.61
7/13/2007		Magueyes	*Dictyota bartayresiana*	1.67	3.56
7/13/2007		Magueyes	*Acanthophora spicifera*	1.51	4.26
7/13/2007		Magueyes	*Acanthophora spicifera*	1.53	4.47
7/13/2007		Magueyes	*Acanthophora spicifera*	2.33	4.56
7/13/2007		Magueyes	*Dictyota bartayresiana*	1.45	3.39
7/13/2007	2:00 PM	Margarita Reef	*Dictyota bartayresiana*	1.81	1.89
7/13/2007		Margarita Reef	*Dictyota bartayresiana*	2.57	1.64
7/13/2007		Margarita Reef	*Dictyota bartayresiana*	2.11	1.84
7/13/2007		Margarita Reef	*Dictyota bartayresiana*	2.15	1.62
7/13/2007		Margarita Reef	*Dictyota bartayresiana*	2.32	1.89
7/13/2007		Margarita Reef	*Dictyota bartayresiana*	1.86	1.55
7/13/2007		Margarita Reef	*Dictyota bartayresiana*	2.02	1.48
7/13/2007		Margarita Reef	*Dictyota bartayresiana*	2.23	1.47
7/13/2007	3:00 PM	Media Luna	*Lobophora variegata*	1.43	1.39
7/13/2007		Media Luna	*Lobophora variegata*	1.26	1.96
7/13/2007		Media Luna	*Lobophora variegata*	1.15	2.22
7/13/2007		Media Luna	*Lobophora variegata*	1.36	2.46
7/13/2007		Media Luna	*Lobophora variegata*	1.28	1.70
7/13/2007		Media Luna	*Lobophora variegata*	1.74	1.39
7/13/2007		Media Luna	*Dictyota bartayresiana*	2.35	1.09
7/13/2007		Media Luna	*Dictyota bartayresiana*	1.66	2.03
7/13/2007		Media Luna	*Dictyota bartayresiana*	1.59	1.95
7/13/2007		Media Luna	*Dictyota bartayresiana*	1.99	2.19
7/13/2007		Media Luna	*Dictyota bartayresiana*	1.78	2.08
7/13/2007		Media Luna	*Dictyota bartayresiana*	1.90	2.02
7/13/2007		Media Luna	*Acanthophora spicifera*	1.23	2.05
7/13/2007		Media Luna	*Acanthophora spicifera*	1.35	2.17
7/13/2007		Media Luna	*Acanthophora spicifera*	1.48	2.18
7/13/2007		Media Luna	*Acanthophora spicifera*	1.61	1.84
7/13/2007		Media Luna	*Acanthophora spicifera*	1.39	2.07
7/13/2007		Media Luna	*Acanthophora spicifera*	1.23	2.07
7/13/2007	4:00 PM	Boat Ramp	*Acanthophora spicifera*	2.53	2.77
7/13/2007		Boat Ramp	*Acanthophora spicifera*	1.67	3.41
7/13/2007		Boat Ramp	*Acanthophora spicifera*	2.36	2.88
7/13/2007		Boat Ramp	*Dictyota bartayresiana*	1.70	2.52
7/13/2007		Boat Ramp	*Dictyota bartayresiana*	2.38	2.85
7/13/2007		Boat Ramp	*Dictyota bartayresiana*	1.62	2.60
7/13/2007		Boat Ramp	*Dictyota bartayresiana*	2.53	2.79

7/13/2007		Boat Ramp	*Acanthophora spicifera*	No Data	
7/13/2007		Boat Ramp	*Acanthophora spicifera*	1.80	3.77
7/13/2007		Boat Ramp	*Acanthophora spicifera*	2.11	4.27
7/13/2007		Boat Ramp	*Dictyota bartayresiana*	1.46	3.83
7/13/2007		Boat Ramp	*Dictyota bartayresiana*	1.68	3.58
7/20/2007	3:30 PM	Boat Ramp	*Acanthophora spicifera*	2.28	4.93
7/20/2007		Boat Ramp	*Acanthophora spicifera*	2.38	4.76
7/20/2007		Boat Ramp	*Acanthophora spicifera*	2.14	4.21
7/20/2007		Boat Ramp	*Acanthophora spicifera*	1.93	4.01
7/20/2007		Boat Ramp	*Acanthophora spicifera*	1.76	4.08
7/20/2007		Boat Ramp	*Acanthophora spicifera*	1.79	3.84
7/20/2007		Magueyes	*Acanthophora spicifera*	2.10	3.81
7/20/2007		Magueyes	*Acanthophora spicifera*	2.05	3.85
7/20/2007		Magueyes	*Acanthophora spicifera*	1.35	4.46
7/20/2007		Magueyes	*Acanthophora spicifera*	1.31	4.58
7/20/2007		Magueyes	*Acanthophora spicifera*	1.99	5.50
7/20/2007		Magueyes	*Acanthophora spicifera*	2.39	5.12

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