



Macroalgae blooms and $\delta^{15}\text{N}$ in subtropical coastal lagoons from the Southeastern Gulf of California: Discrimination among agricultural, shrimp farm and sewage effluents

Alejandra Piñón-Gimate^a, Martín F. Soto-Jiménez^{b,*}, María Julia Ochoa-Izaguirre^c, Eynar García-Pagés^a, Federico Páez-Osuna^b

^a Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Unidad Académica Mazatlán, Apdo. Postal 811, Mazatlán, Sinaloa 82040, Mexico

^b Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Unidad Académica Mazatlán, Apdo. Postal 811, Mazatlán, Sinaloa 82040, Mexico

^c Facultad de Ciencias del Mar, Universidad Nacional Autónoma de Sinaloa, Paseo Claussen s/n, Mazatlán, Sinaloa 82000, Mexico

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ABSTRACT

Macroalgae blooms of *Gracilaria vermiculophylla*, *Hypnea spinella* and *Spyridia filamentosa* have been found in coastal lagoons in the SE Gulf of California. Agriculture, livestock, shrimp and poultry farms and sewage contribute anthropogenic nitrogen to the systems. The $\delta^{15}\text{N}$ of these sources, water column and macroalgae were studied in order to identify the N supply for macroalgae blooms. $\delta^{15}\text{N}$ of three species of macroalgae (4.3–13.6‰) were enriched compared to the water column ($\delta^{15}\text{N}-\text{NO}_3^-$ 3.7–6.8‰), probably because of fractionation from the macroalgae. $\delta^{15}\text{N}$ of POM (1.4–10.3‰) was similar to the water column but the relationship was unclear. Depending on the site, macroalgae showed different $\delta^{15}\text{N}$ values since some sites receive more or less influence from one given source of the associated watershed, which is reflected in the different $\delta^{15}\text{N}$ values of the macroalgae of the same system and in the relative contributions of the sources.

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1. Introduction

Sources of dissolved inorganic nitrogen (DIN, as ammonia, nitrate, or nitrite) typically limit the distribution, productivity, and abundance of primary producers, including single celled phytoplankton and larger macroalgae (Ryther and Dunstan, 1971; Nelson et al., 2003; Thornber et al., 2008). Inputs of N through sewage and fertilizer runoff frequently increase the rate of primary production in coastal systems (Doering et al., 1995; Taylor et al., 1999; Thornber et al., 2008) which can lead to large blooms of phytoplankton and/or macroalgae (Harlin, 1995; Fletcher, 1996; Nixon and Buckley, 2002; Berman et al., 2005). As a result of anthropogenic nutrient enrichment, dense mats of macroalgae are increasing in abundance and frequency in coastal waters around the world (Valiela et al., 1997; Bricker et al., 2003; Lapointe et al., 2005). A patchy distribution of macroalgae can be associated with localized pulses of nutrients, like the mats of *Caulerpa*, *Gracilaria*, *Hypnea* and *Spyridia* (biomass $>40\text{ g m}^{-2}$) found in the coastal lagoons of Sinaloa, Mexico (Piñón-Gimate et al., 2008). Understanding how nitrogen enters a water body, and the way it is subsequently used by primary producers, is of great importance

in assessing the impacts of anthropogenic vs. “natural” sources of nutrient to marine systems (Rogers, 2003; Kamer et al., 2004; Savage and Elmgren, 2004). The measurement of stable nitrogen isotope ratios ($\delta^{15}\text{N}$) in macroalgae can be used to assess the spatial extent and degree of land-based nutrient enrichment in coastal waters by “fingerprinting” the source of N when the various $\delta^{15}\text{N}$ signatures are known (Heaton, 1986; Lapointe and Bedford, 2007).

Globally, many studies have used $\delta^{15}\text{N}$ as a tool to discriminate between natural and anthropogenic N sources that could in turn support excessive macroalgal growth (e.g., Lapointe, 1997; McClelland and Valiela, 1998; Costanzo et al., 2001; Umezawa et al., 2002; Gartner et al., 2002; Savage and Elmgren, 2004). However, in subtropical ecosystems these types of studies are still scarce (e.g. Barile, 2004; Lapointe et al., 2004; Lapointe et al., 2005).

In this study, we investigated the nitrogen contents and the $\delta^{15}\text{N}$ signatures of three important species of macroalgae that frequently grow excessively and form blooms in three coastal lagoons along the southeast Gulf of California coastline. The region supports fisheries, tourism, mining, intensive agriculture, and shrimp aquaculture. These activities and the presence of around 5 million inhabitants constitute a serious threat to the rich and complex biodiversity of the Gulf ecoregion (Páez-Osuna et al., 2003). In order to determine the primary source of DIN for bloom forming macroalgae we compared the $\delta^{15}\text{N}$ signatures of three macroalgae species

* Corresponding author. Tel.: +52 669 985 2845; fax: +52 669 982 6133.
E-mail address: martin@ola.icmyl.unam.mx (M.F. Soto-Jiménez).

with the DIN and $\delta^{15}\text{N}$ values of the water column directly influenced by land-based anthropogenic N derived from sewage, shrimp farming and agriculture as the primary nitrogen source supporting macroalgal growth.

2. Study area

This study included three coastal lagoons of the Sinaloa littoral on the southeastern Gulf of California (Fig. 1). The area covered by the main activities and the population of the associated watershed of each lagoon is shown in the same figure. Sample locations were selected according to accessibility of macroalgal blooms (Piñón-Gimate et al., 2008). No macroalgal blooms were found far from shore or in deep water. Macroalgal blooms were defined as an area longer than 50 m, parallel to the shore, conspicuously covered with beds of benthic macroalgae with a biomass higher than 1 g m^{-2} . All stations were located downstream of major anthropogenic influences (Fig. 1).

Additionally, water samples of known effluents from the Culiacán River watershed (CRW) were collected. The CRW has mixed land uses and its major activities include intensive agriculture, livestock, poultry, and urbanism. The Altata-Ensenada El Pabellón (AE) system is associated with the CRW and receives the discharge from the Culiacán River and numerous streams in the watershed.

3. Material and methods

During the dry and rainy seasons of 2004 and 2005, approximately 4 thalli of 3 important species: *Gracilaria vermiculophylla*, *Hypnea spinella* and *Spyridia filamentosa* (red algae specimens) were collected at each of the 7 selected stations when they were present. The nominal dry season was considered from May to June 2004 and the nominal rainy season from August to October 2004, which are defined by monthly average air temperatures between 27 and 30 °C (CNA, 2005) and rainy season is based on well-defined periods of rainfall (Flores-Verdugo et al., 1993). A total of 33

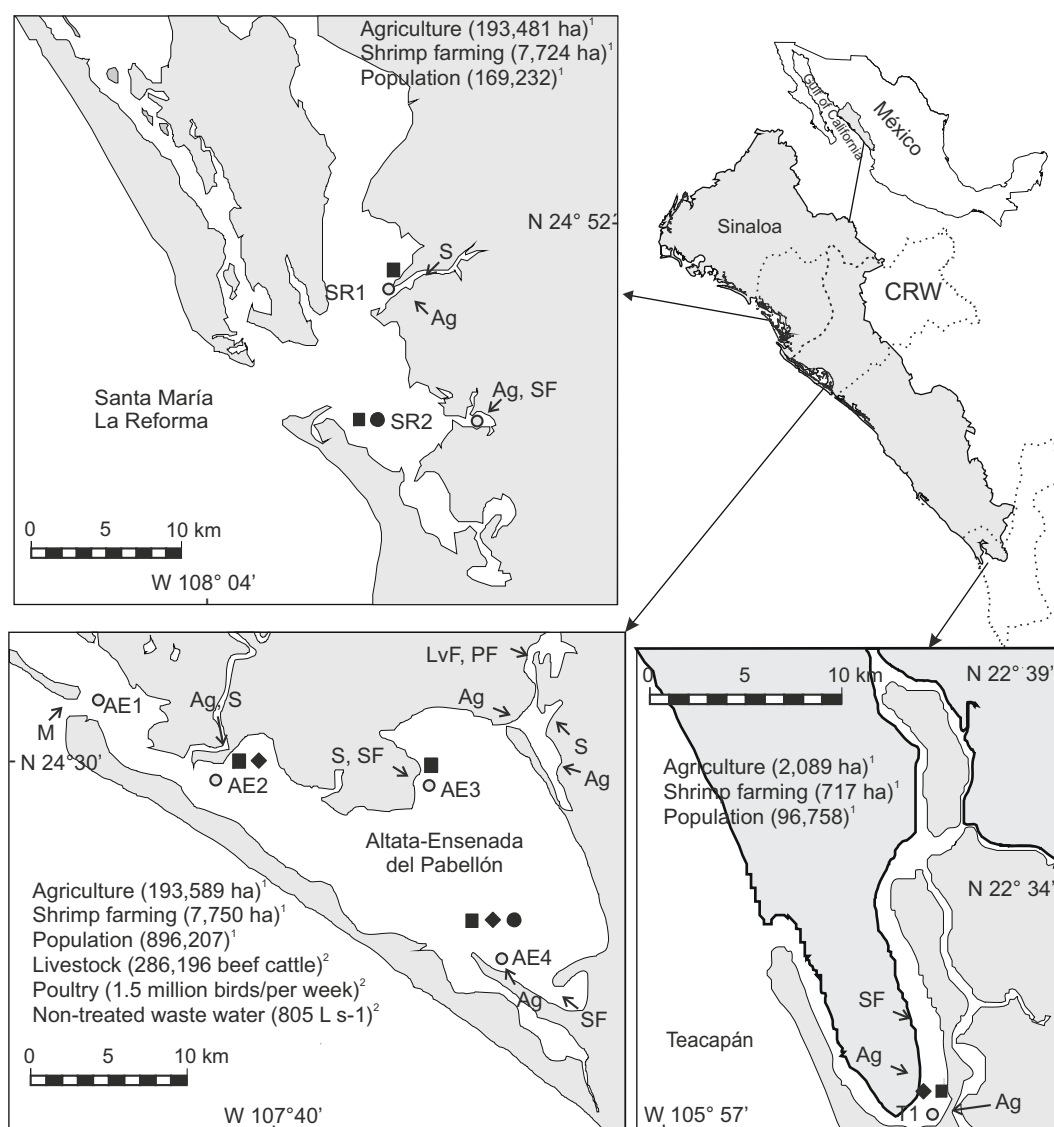


Fig. 1. Sampling stations in three coastal lagoons of Sinaloa where macroalgal blooms were found in 2004 (except for sampling station AE1 which was sampled for marine water). Sampling stations are numbered from north to south and were labeled accordingly: CWR = Culiacán River watershed, SR = Santa María-La Reforma, AE = Altata-Ensenada El Pabellón and T = Teacapán. Ag = Agriculture influence, LvF = Livestock farming loads, PF = Poultry farming loads, S = Sewage influence, SF = Shrimp farm influence, and M = Marine influence (■: *Gracilaria vermiculophylla*, ◆: *Hypnea spinella* and ●: *Spyridia filamentosa*). Activities (number of ha used) and population (number of inhabitants) of the associated watershed are described (¹Páez-Osuna et al., 2007; ²INEGI, 2005a,b).

macroalgae samples were collected belonging to three different species. Macroalgae specimens were attached to the substratum in all cases. Samples were washed in the field with water from the lagoon and separated by hand. In the laboratory, individual thalli samples were dried at 60 °C for 3–5 days and ground into a fine powder with a mortar and pestle.

Water samples were taken in the middle of the bloom and close to the edges of it, during low and high tides. In the field, water samples (2–6 replicates depending on the site location) were properly stored and transported to the laboratory for further determination of nutrients: nitrate, nitrite, ammonia, and dissolved inorganic nitrogen (DIN). For nutrient analysis, techniques used were those described by Strickland and Parsons (1972), and Grasshoff et al. (1983). Water samples were filtered through a pre-combusted (500 °C, 4 h) glass fiber filter (Whatman GF/F of 0.45 µm) with a low strength vacuum pump. Filters were previously weighed to constant weight. Filtration was made until full filter saturation was achieved. Filters were then rinsed with MilliQ water to eliminate salts and then dried at 60 °C for 72 h for constant weight. All of the C and N contents of the filtered matter collected on the filters were considered as particulate organic matter (POM). POM is principally comprised of living or dead phytoplankton, and includes detritus from other organisms. A total of 28 filters were obtained, ground into a fine powder with a mortar and pestle and packed. Filtered water samples were collected in polyethylene bottles and kept frozen for diffusion analysis of NH_4^+ and NO_3^- . Ammonium and nitrate diffusion was carried out to extract and collect inorganic nitrogen from water samples (Holmes et al., 1998; Sigman et al., 1997), respectively. The inorganic N was sequestered into filters GF/D and then packed in tin cups prior to stable-nitrogen-isotope analysis ($\delta^{15}\text{N}$).

Two subsamples of each sample of macroalgae and filters were taken; then the subsamples were exposed to HCl vapor for 4 h at room temperature to remove carbonates (Harris et al., 2001). These samples were weighed, placed into 5 × 9 mm tin cups and kept in closed vials until analysis. For N content, all samples were analyzed at UC Davis Stable Isotope Facility in a Carlo Erba elemental analyzer. For isotope ratios a Europa Scientific ANCA-NT 20-20 Stable Isotope Analyzer with ANCA-NT Solid-Liquid Preparation Module (PDZ Europa, Crews, UK) was used. The analytical precision was 0.2‰ for both N and C, estimated from standards analyzed along with the samples. The $\delta^{15}\text{N}/\delta^{14}\text{N}$ relative ratios to N_2 in air ($\text{At}\%^{15}\text{N} = 0.3663033$) are expressed as $\delta^{15}\text{N}$. Ammonium and nitrates were expressed as ($\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{15}\text{N}-\text{NH}_4^+$, respectively).

A mixing model was used to estimate the proportional contributions of the sources of environmental nitrogen into the macroalgae. The IsoSource software package was used to run mixing equations (Phillips and Gregg, 2003). For this purpose we considered the $\delta^{15}\text{N}$ values of the macroalgae at each sampling station and the ($\delta^{15}\text{N}-\text{NO}_3^-$) values of the water sources in the region based on the influences at each sampling location (see Fig. 1 for reference). Atmospheric water and marine water were included in the analysis at all stations.

A one way ANOVA was used to determine significant differences in seawater nitrogen using the sampling location (1–7) as independent variables. Normality (Kolmogorov Smirnov test), and variance homogeneity (Cochran's test) were tested (Zar, 1984). Nitrogen content data was log transformed to satisfy requirements of normality and variance homogeneity and multiple linear regression analysis was used to identify whether nitrates or ammonia explained variations in the nitrogen contents in the tissue of each important macroalgae species. This is assuming that different species preferred one or the other source. Only significant results ($p < 0.05$) are reported (Zar, 1984).

4. Results

Nitrogen concentrations in the water column were variable along the sampling period, with no significant differences among sampling stations ($F = 6, 25; p > 0.05$). The highest mean concentration of nitrates was found in the sampling station SR1 ($11.9 \pm 22.8 \mu\text{M}$), while the lowest was at T1 ($0.6 \pm 0.1 \mu\text{M}$). The highest concentration of ammonia was recorded in the sampling stations AE3 and T1 (11.2 and $11.8 \mu\text{M}$, respectively), while the lowest was recorded at SR2 ($6.2 \pm 2.5 \mu\text{M}$). The highest concentration of DIN was recorded at station SR1 ($18.4 \pm 23.4 \mu\text{M}$), with the lowest observed at SR2 ($3.6 \pm 0.1 \mu\text{M}$) (Table 1).

$\delta^{15}\text{N}$ values found in the N sources of the region, specifically at Altata-Ensenada El Pabellón were variable. Agriculture effluent values of ($\delta^{15}\text{N}-\text{NO}_3^-$ in water were $2.1 \pm 1.0\text{‰}$, in livestock farming $12.0 \pm 1.0\text{‰}$, in poultry farming $13.0 \pm 1.0\text{‰}$, in sewage (or domestic) $16.1 \pm 0.3\text{‰}$, and in shrimp farming $7.0 \pm 1.0\text{‰}$. Atmospheric deposition had a signal of $11.8 \pm 4.3\text{‰}$ and marine coastal waters from the Gulf of California had a signal of $3.7 \pm 0.1\text{‰}$.

Data from the isotopic signature of water were only available for Altata-Ensenada El Pabellón and only ($\delta^{15}\text{N}-\text{NO}_3^-$) was measured (due to low concentrations of NH_4^+). The highest signature was found at the sampling station AE2 ($6.8 \pm 1.8\text{‰}$) and the lowest at sites AE1 and AE2 (3.7‰ for both), no comparisons were made due to the low numbers of data (Table 1).

4.1. Nitrogen content of macroalgae and POM

G. vermiculophylla was the species more frequently registered during the sampling period in the three coastal lagoons. *H. spinella* was observed in Altata-Ensenada El Pabellón and in Teacapán, while *S. filamentosa* was found in Santa María-La Reforma and Altata-Ensenada El Pabellón (SR2 and AE4, respectively). Nitrogen content differed among species at the different stations (Table 2). The highest mean nitrogen content was recorded in *G. vermiculophylla* at stations AE4 and SM1 (2.6% and 2.7% DW, respectively), while the lowest mean concentration was recorded at AE2 ($1.1 \pm 0.1\%$ DW) in the same species. *H. spinella* presented the highest mean nitrogen content at the sampling station AE2 ($3.3 \pm 0.4\%$ DW) and the lowest at T1 ($0.5 \pm 0.1\%$ DW). No significant differences ($p > 0.05$) were found for the mean nitrogen contents between

Table 1
Mean concentration (\pm SD) of nitrates, ammonia, inorganic dissolved nitrogen in waters from selected sampling stations of three coastal lagoons of the coast of Sinaloa. Mean ($\delta^{15}\text{N}-\text{NO}_3^-$) (\pm SD) from Altata-Ensenada El Pabellón (see Fig. 1 for location codes).

Sampling station (n)	Nitrate (μM)	Ammonia (μM)	DIN (μM)	$\delta^{15}\text{N}-\text{NO}_3^-$ (‰)
SR1 (3)	11.9 ± 22.8	6.2 ± 2.5	18.4 ± 23.4	–
SR2 (3)	1.5 ± 0.1	2.0 ± 0.1	3.6 ± 0.1	–
AE1 (4)	6.5 ± 8.5	9.2 ± 3.0	16.6 ± 9.8	3.7 ± 1.8
AE2 (4)	6.3 ± 6.4	6.2 ± 6.0	14.6 ± 12.5	6.8 ± 1.8
AE3 (6)	1.8 ± 1.5	11.2 ± 5.9	13.1 ± 7.5	3.7 ± 1.6
AE4 (3)	2.1 ± 1.7	7.2 ± 4.6	9.6 ± 6.1	4.4 ± 2.1
T1 (6)	0.6 ± 0.1	11.8 ± 6.5	16.3 ± 10.2	–

(–): Not determined by insufficient level found; n = number of samples.

Table 2

Triplicate mean (\pm SD) values of nitrogen content and $\delta^{15}\text{N}$ values in the tissue of three species of bloom forming macroalgae in three coastal lagoons of Sinaloa (Gv = *Gracilaria vermiculophylla*, Hs = *Hypnea spinella*, Sf = *Spyridia filamentosa*, DW = dry weight) (see Fig. 1 for location codes).

Station	Biomass (g m^{-2} DW) ^a			N (%)			$\delta^{15}\text{N}$ (‰)		
	Gv	Hs	Sf	Gv	Hs	Sf	Gv	Hs	Sf
SR1	72.4 \pm 126.2	–	–	2.7 \pm 0.1	–	–	13.6 \pm 0.1	–	–
SR2	23.2 \pm 34.4	–	0.1 \pm 0.1	2.1 \pm 0.7	–	2.1 \pm 0.1	5.3 \pm 1.6	–	5.6 \pm 0.1
AE1	–	–	–	–	–	–	–	–	–
AE2	0.3 \pm 0.5	1.4 \pm 2.9	–	1.1 \pm 0.1	3.3 \pm 0.4	–	8.0 \pm 0.1	11.4 \pm 6.0	–
AE3	68.6 \pm 118.4	–	–	1.6 \pm 0.1	–	–	7.8 \pm 0.1	–	–
AE4	2.1 \pm 1.1 ^b	2.5 \pm 2.0 ^b	0.8 \pm 1.7 ^b	2.6 \pm 2.5	2.1 \pm 0.5	2.5 \pm 0.8	8.7 \pm 2.5	8.5 \pm 0.5	7.7 \pm 2.3
T1	56.7 \pm 93.0	1.1 \pm 2.2	–	2.5 \pm 0.5	0.5 \pm 0.1	–	6.0 \pm 1.4	4.3 \pm 0.1	–

(–): No macroalgae samples available for such stations see Fig. 1 for macroalgae species present at sampling stations.

^a Piñón-Gimate et al. (2008) (mean annual values).

^b This study.

the three species at the different sampling stations (Table 2). Nitrogen content of each one of the three species was associated individually with nitrate concentrations in the water column, with no significant correlations with any of the species. However, the nitrogen content in *S. filamentosa* did show an increase when the ammonia concentrations of the water column were high ($p < 0.05$) (Fig. 2). Mean Chl *a* concentrations differed among sampling stations. The highest mean concentration was found at station AE1 ($5.1 \pm 2.3 \text{ mg m}^{-3}$), while the lowest was found in sampling station AE4 ($1.4 \pm 0.7 \text{ mg m}^{-3}$) (Table 3). The mean highest nitrogen contents of POM were found at stations SR1 and SR2 (75.6 ± 40.1 and $25.7 \pm 5.0 \mu\text{g L}^{-1}$), while the lowest were obtained at station AE1 ($6.5 \pm 2.5 \mu\text{g L}^{-1}$) (Table 3), and no evident tendencies were observed with the nutrient concentrations of the water column.

4.2. Isotopic signature of macroalgae and POM

There was a significant difference in $\delta^{15}\text{N}$ values among species at different sites ($p < 0.05$). The highest mean $\delta^{15}\text{N}$ value was that of *G. vermiculophylla* at sampling station SR1 ($13.6 \pm 0.1\text{‰}$) and it was higher than the rest of the stations where it was present (Table 3). This later value was also higher than the mean $\delta^{15}\text{N}$ values in *S. filamentosa* and *H. spinella* at sampling stations SR2, AE4 and T1 (Table 2). Concerning POM (it was not measured at station T1 be-

Table 3

Triplicate mean (\pm SD) values of chlorophyll *a* (Chl *a*), nitrogen content (N) and $\delta^{15}\text{N}$ values in POM (particulate organic matter) in three coastal lagoons of Sinaloa (see Fig. 1 for location codes).

Station	Chl <i>a</i> (mg m^{-3})	N ($\mu\text{g L}^{-1}$)	$\delta^{15}\text{N}$ (‰)
SR1	1.7 \pm 2.1	75.6 \pm 40.1	10.3 \pm 3.1
SR2	2.0 \pm 1.6	25.7 \pm 5.0	1.4 \pm 0.3
AE1	5.1 \pm 2.3	6.5 \pm 2.5	6.5 \pm 0.2
AE2	4.2 \pm 0.2	18.1 \pm 9.7	7.3 \pm 1.1
AE3	1.5 \pm 0.9	14.8 \pm 0.2	6.5 \pm 0.3
AE4	1.4 \pm 0.7	8.1 \pm 0.7	3.8 \pm 3.4
T1	–	–	–

(–): No samples available for such station.

cause of the low concentrations of organic matter), there was a significant difference in the mean $\delta^{15}\text{N}$ values among the sampling stations ($p < 0.05$). The highest mean value was found at sampling station SR1 ($10.3 \pm 3.1\text{‰}$), while the lowest was found at SR2 ($1.4 \pm 0.3\text{‰}$) (Table 3).

A positive correlation was found between the mean $\delta^{15}\text{N}$ values of macroalgae and the mean ($\delta^{15}\text{N}-\text{NO}_3^-$) values of the water column (Fig. 3a). A 1:1 relationship is expected when $\delta^{15}\text{N}$ values in any given organism mirror those of the water column. For macroalgae the correlation was found above the 1:1 expected. On the other hand, no correlation was found between the mean $\delta^{15}\text{N}$ values of POM and the mean ($\delta^{15}\text{N}-\text{NO}_3^-$) values of the water column (Fig. 3b).

The relative contributions of nitrogen from the different sources were variable depending on the sampling location and macroalgae species (Fig. 4). Based on the results obtained from the Isosource model, the most important contribution corresponded to sewage in sampling station SR1 and AE2 for the species *G. vermiculophylla* and *H. spinella* (70% and 39%, respectively). Atmospheric contribution was most important in AE4 for *G. vermiculophylla* and *H. spinella* (50% for both). Agriculture had the major contribution in T1 (40%) and it was best reflected in *H. spinella*. Shrimp farming was the most important nitrogen contributor in T1 for *G. vermiculophylla* (33%).

5. Discussion

Values of DIN concentrations in the water column within the sampling stations were variable in a range of 3.6–24.1 μM . These values have been considered by Pedersen and Borum (1996) as high, indicating that nitrogen is not a limiting nutrient for macroalgal growth. Piñón-Gimate et al. (2008) found that when temperature was optimal for macroalgal growth a high nutrient availability in the water column corresponded with an increase of the biomass of the important species that formed the macroalgal

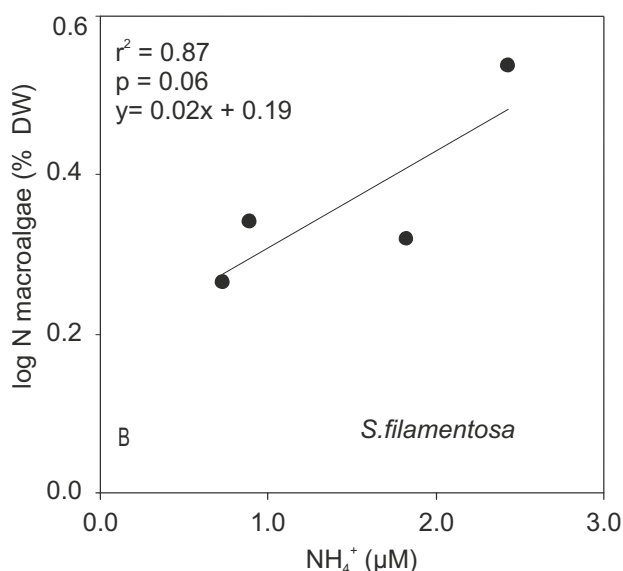


Fig. 2. Significant ($p < 0.05$) correlations between ammonia and log transformed macroalgae nitrogen content of *Spyridia filamentosa*.

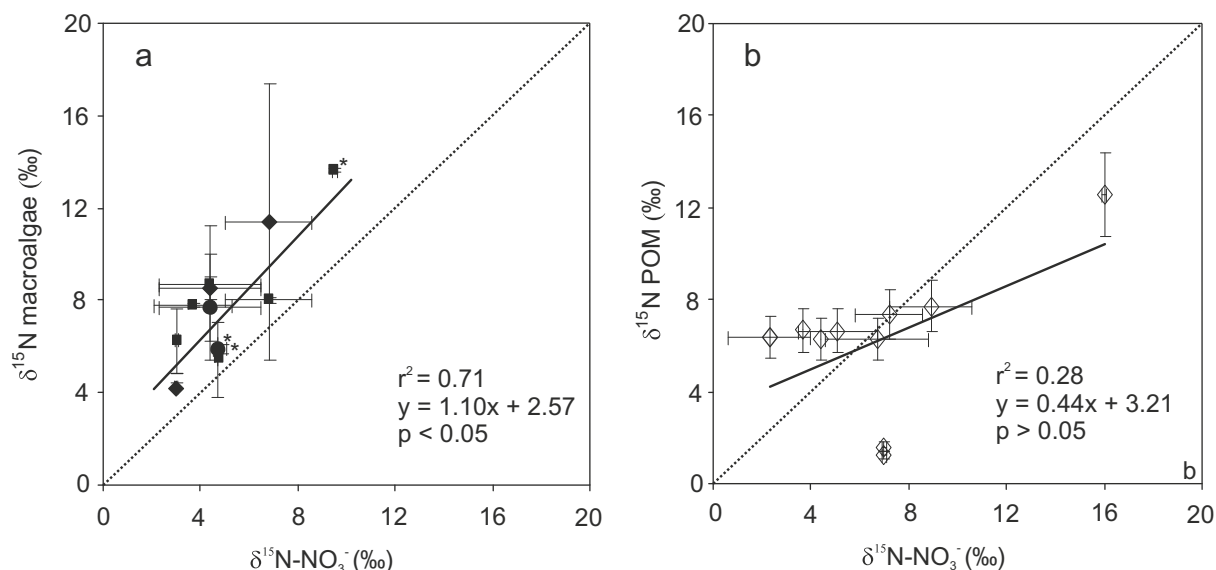


Fig. 3. ($\delta^{15}\text{N}-\text{NO}_3^-$ values vs $\delta^{15}\text{N}$ values of (a) macroalgae and (b) POM. The 1:1 line indicates the theoretical $\delta^{15}\text{N}$ value of the macroalgae and POM, if the $\delta^{15}\text{N}$ value of the nitrate was mirrored exactly. ■: *Gracilaria vermiculophylla*, ♦: *Hypnea spinella* and ●: *Spyridia filamentosa*, ◇: POM. *Indicate theoretical values of ($\delta^{15}\text{N}-\text{NO}_3^-$ for the particular influence at sampling stations SR1, SR2 and T1 (from this study in Altata-Ensenada El Pabellón).

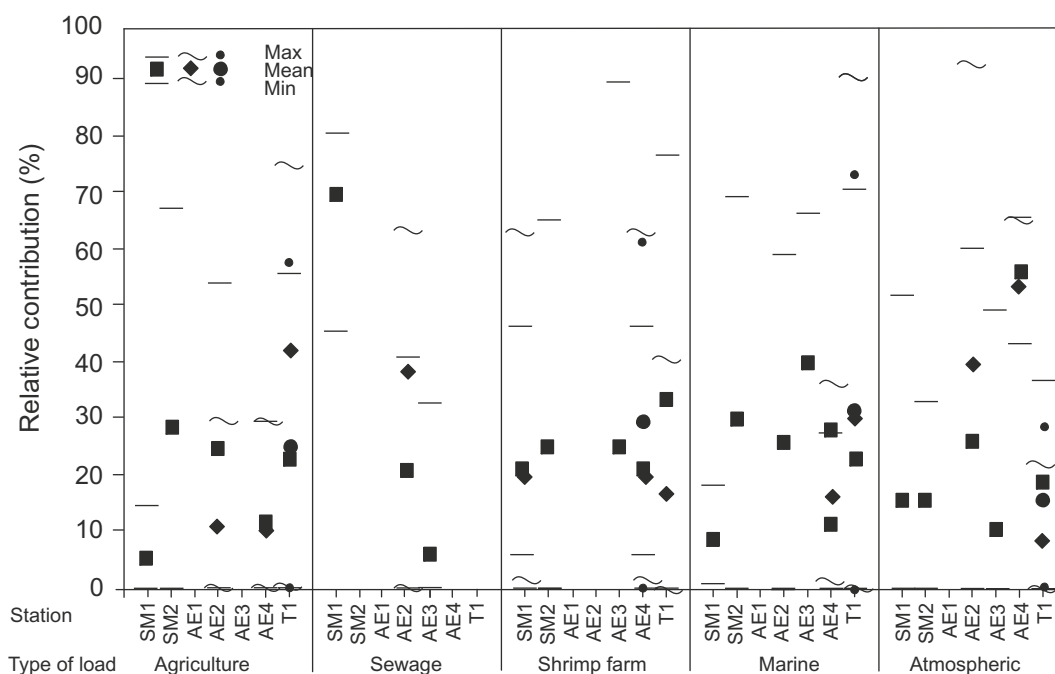


Fig. 4. Mean percentage contribution obtained with the Isosource Model Mixing program for each macroalgae species and sampling station, with maximum and minimum values. ■: *Gracilaria vermiculophylla*, ♦: *Hypnea spinella* and ●: *Spyridia filamentosa*.

blooms. However, no correlation was observed between nitrogen content in seaweeds and DIN concentrations, only the N content in *S. filamentosa* increased with the ammonia concentrations in the water column. This could be a result of a differential uptake of the nitrogen forms available in the water column depending on the species (Hanisak and Harlin, 1978), as has been previously observed in other studies. Deutsch and Voss (2006) found that *Ceramium rubrum* and *Fucus vesiculosus* incorporate seven times more NH_4^+ than NO_3^- . Pedersen and Borum (1996) in Roskilde Fjord (Denmark) reported that the tissue of four macroalgal species reflected the nutrient availability in the water column. In this study, the N content of *G. vermiculophylla* and *H. spinella* showed

no increase or relation with increasing concentrations of DIN (either as ammonia or nitrate) in the water column. In other studies, it was found that the low values of N content were often misleading since it is rapidly diluted during growth (Fong et al., 2001, 2003). Although macroalgae assimilate and store excess bioavailable nutrients in their tissues before responding with growth (Fong et al., 2001, 2003), storage on a longer-term basis occurs when nutrient supply exceeds growth rate (Fujita, 1985; Fong et al., 1994, 2004). Therefore, it seems that *G. vermiculophylla* at sampling stations with high concentrations of nitrogen is not reflected in the tissue likely because it used the excess of N primarily for growth (Lin and Fong, 2008). This can be observed in the high biomass

values at the sampling stations where it was found (Table 2). Thus, macroalgae from sites with high DIN concentrations will show lower N content values but high biomass. For example, *H. spinella* biomass that increased with nitrogen concentrations in relation to the phosphorus concentrations. In the region, the occurrence of *G. vermiculophylla* blooms have been related to phosphorus increases (Piñón-Gimate et al., 2008), which can also explain why the tissue of the macroalgae is not reflecting the DIN concentrations of the water column.

Results of $\delta^{15}\text{N}$ values in macroalgae species were variable depending on the site. At station SR1 at Santa María-La Reforma lagoon system, *G. vermiculophylla* showed the highest values ($13.6 \pm 0.1\text{‰}$). It was similar to the signal reported in macroalgae ($9\text{--}14\text{‰}$) influenced by sewage discharges (Costanzo et al., 2001) (Table 4). Macroalgae that utilizes naturally fixed N have $\delta^{15}\text{N}$ values of 0‰ (France et al., 1998), in contrast to those using sewage N, which become more enriched from 3‰ to 16‰ (Lapointe, 1997; Costanzo et al., 2001). Sampling station SR1 has a high influence of municipal waste waters from El Tule estuary (Piñón-Gimate et al., 2008), located at about 15 km from the direct source. Even when the whole system is highly influenced by agricultural sources (Fig. 1), *G. vermiculophylla* and POM best reflect the signal of located sewage inputs (with some fractioning depending on the distance from the source) as it has been observed by Costanzo et al. (2001). For example, it was confirmed by the results of the mixing model obtained for the macroalgae that showed a contribution of more than 70% for N sewage. The $\delta^{15}\text{N}$ values of *G. vermiculophylla*, *S. filamentosa* and POM at SR2 in the lagoon, were lower (5.3‰ , 5.4‰ and 1.4‰ , respectively) and consistent with $\delta^{15}\text{N}$ values of fertilizer-rich agricultural run-off. In the region, agricultural loads have a signature of 2.1‰ , a figure that is comparable to those previously reported by Kendall and Caldwell (1998) and Costanzo et al. (2001) ($\delta^{15}\text{N}$ signatures close to 0‰). Macroalgae at this site (SR2) is probably fractionating the signal from the agricultural loads, typically fractioning in macroalgae occur in a range from 2‰ to 4‰ in nutrient rich waters (Peterson and Fry, 1987). It is worth noting that at SR2 station shrimp farming also contributes with more than 20% of the nitrogen load (Fig. 4). Although Santa

María-La Reforma lagoon system is more influenced by agriculture loads (Fig. 1), different sites can be receiving more influence from different sources of nitrogen from the associated watershed. This is reflected in the different $\delta^{15}\text{N}$ values of the macroalgae of the same system and in the proportional contributions of the sources. The same occurred at station AE2, where the values of ($\delta^{15}\text{N}\text{--NO}_3^-$) in water and phytoplankton were intermediate (6.8 ± 1.8 and $7.3 \pm 1.1\text{‰}$, respectively), but the $\delta^{15}\text{N}$ signatures of macroalgae were higher ($8.0 \pm 0.1\text{‰}$ and $11.4 \pm 6.0\text{‰}$ for *G. vermiculophylla* and *H. spinella*, respectively). This station is highly influenced by the discharges of the Culiacán River, which receives loads from agriculture, domestic waste waters from Navolato, and shrimp farm effluents (Fig. 1). Values of the ($\delta^{15}\text{N}\text{--NO}_3^-$) from residual waste waters of the CRW of Altata-Ensenada El Pabellón lagoon system were close to 16‰ . The signal of ($\delta^{15}\text{N}\text{--NO}_3^-$) of the water column at AE2 was 6.8‰ , lower than that of sewage. However, the model showed that the major contributor in this station is sewage, which is also reflected in the two macroalgae species present. On the other hand, this site is also close to AE1, in the mouth of the lagoon with marine water influence, with a signal of 4.0‰ , slightly higher than tropical oceanic water that is typically low in $\delta^{15}\text{N}$, usually less than 3‰ (Costanzo et al., 2001). Our results for the waters from AE1 and the modeling proportions (20%) confirm that continental waters can still be influencing as far as this sampling station, when samples were collected and tidal flux occurs.

At stations AE3 and AE4, the ($\delta^{15}\text{N}\text{--NO}_3^-$) values from the water column were 3.7‰ and 4.4‰ , respectively, lower than that of shrimp farms (7‰) and sewage (16.1‰). Macroalgae values at these stations appeared to be fractionating the values of the water column (range from 7.7‰ to 8.7‰ depending on the species and site), while the values of $\delta^{15}\text{N}$ in POM (from 6.5‰ to 7.3‰) varied within the range of ($\delta^{15}\text{N}\text{--NO}_3^-$) values of water and $\delta^{15}\text{N}$ from macroalgae. At these stations the major contribution corresponded to shrimp farm effluents ($>20\%$; Fig. 4).

G. vermiculophylla and *H. spinella* were found in sampling station T1. They showed similar $\delta^{15}\text{N}$ values (6.0‰ and 4.3‰ , respectively). Such figures were comparable to the values obtained from agricultural loads in the CRW of Altata Ensenada El Pabellón lagoon

Table 4
 $\delta^{15}\text{N}$ values for macroalgae species influenced by various N sources with different $\delta^{15}\text{N}$ values.

Macroalgae species	$\delta^{15}\text{N}$ (‰)	Location	N source	$\delta^{15}\text{N}$ (‰)	Reference
<i>Ulva</i> sp.	6.1–9.5	Boston Harbor,	Sewage effluent from	NH_4^+ effluent 6.1–8.3	Tucker et al. (1999)
<i>Enteromorpha</i> sp.	7.3–14.4	Massachusetts, USA	treatment facilities		
<i>Catenella nipae</i>	9.0	Moreton Bay, Australia	Sewage effluent	Not available	Costanzo et al. (2001)
<i>Catenella nipae</i>	>5.0	Northeast Coast of Australia	Shrimp farm effluent	Not available	Costanzo et al. (2004)
<i>Ulva australis</i>	8.8–12.8	Ocean Reef, treatment plant	Sewage effluent	Secondary treated sewage	Gartner et al. (2002)
<i>Vidalia</i> sp.	6.3–10.2	Beenyup, Western Australia		DIN 13.5–23.5	
<i>Ecklonia radiata</i>	8–14				
<i>Ulva lactuca</i>	5.0–13.0	East of Central Florida, USA	Residual water loads and	Not available	Barile (2004)
<i>Chaetomorpha linum</i>			subterranean waste waters		
<i>Gracilaria tikvahiae</i>					
<i>Caulerpa prolifera</i>					
<i>Laurencia intricate</i>	1.0–6.5	South of Florida Bay and	Sewage effluent and	Local sewage nitrogen >3 ;	Lapointe et al. (2004)
<i>Cladophora catenata</i>	1.0–5.5	Lower Florida Keys, USA	agricultural runoff	agricultural nitrogen 1–3	
Other algae	1.0–10				
<i>Fucus vesiculosus</i>	3.0–9.5	Himmerjarden Bay,	Sewage effluent	Not available	Savage and Elmgren (2004)
		Switzerland			
<i>Ulva</i> sp.	7.6–13.5	Warnor System, Baltic Sea,	Sewage effluent	Anthropogenic influence	Deutsch and Voss (2006)
<i>Ceramium</i> sp.	4.7–9.5	NE of Germany		6.2–9.7	
<i>Polysiphonia</i> sp.	6.9–8.6				
<i>canthophora spicifera</i>	5.3–5.9	Opunohu Bay, Moorea,	Shrimp farm effluent	Shrimp farm effluent 4.2–6	Lin and Fong (2008)
		French Polinesia			
<i>Gracilaria vermiculophylla</i>	7.8–8.0	Southeastern Gulf of	Agriculture, sewage and	Agriculture 2.1 Sewage 16.1	This study
		California	shrimp farm effluents	Shrimp farm 7.0	
<i>Hypnea spinella</i>	8.5–11.4	Southeastern Gulf of	Agriculture, sewage and	Agriculture 2.1 Sewage 16.1	This study
		California	shrimp farm effluents	Shrimp farm 7.0	
<i>Spyridia filamentosa</i>	7.7	Southeastern Gulf of	Agriculture, sewage and	Agriculture 2.1 Sewage 16.1	This study
		California	shrimp farm effluents	Shrimp farm 7.0	

system (range 2.1–4‰). Teacapán lagoon system had the lowest global contributions from agriculture, shrimp farms, and sewage compared with the other two lagoons, although it has been reported that agriculture contributes relatively 3 times more nitrogen to the system than shrimp farms and 12 times more than sewage waters (Fig. 1) (Páez-Osuna et al., 2007). This is reflected in the macroalgae from T1, with a $\delta^{15}\text{N}$ value influenced by the contribution of N sources from agriculture, shrimp farming and marine origin (Fig. 4).

Our study shows that $\delta^{15}\text{N}$ of macroalgae in nutrient enriched waters of the coast of Sinaloa is the most sensitive to this and other indicators (i.e. N content, biomass), since this parameter could detect known effluent values better. $\delta^{15}\text{N}$ of the macroalgae showed a positive and significant correlation with the ($\delta^{15}\text{N}-\text{NO}_3^-$) of the water column (Fig. 3). However, a mirror correlation, as it has been found in laboratory studies (Deutsch and Voss, 2006) was not found between these values probably because of the fractioning occurring in macroalgae.

Results revealed that $\delta^{15}\text{N}$ of POM from Altata-Ensenada El Pabellón are reflecting an integrated value of the ($\delta^{15}\text{N}-\text{NO}_3^-$) of the water column from the entire lagoon and not from one particular site. The lack of tracking of the ($\delta^{15}\text{N}-\text{NO}_3^-$) of the water column from POM may be due to the short life history of the phytoplankton (considered the main component of the POM) relative to the macroalgae and/or might have more relation with its planktonic nature, i.e. moving in and out of different sources along the waters of the lagoons. This is contrary to what could occur in sampling sites from Santa María-La Reforma that are inlets with clearly more influence from one given source. Our study suggests that nutrients from different sources are being utilized by macroalgae even when the main source of anthropogenic nitrogen to the system is other. If it is the direct influence of the nitrogen loads or because they are selective with the nitrogen chemical species, is a matter that requires more study. Thus, there is great urgency for more research on developing appropriate methods to monitor the spatial scale of nutrient enrichment and to mitigate its impacts where localized pulses of nitrogen are associated with macroalgal blooms.

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