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Nitrogen isotopic characterisation of macroalgae blooms from different sites within a subtropical bay in the Gulf of California

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ABSTRACT

In La Paz Bay conspicuous macroalgal blooms of *Ulva* spp. are recurrent in the waterfront of the city; studies relate its growth to seasonality and nutrient enrichment but their relative influence is not known. We use the δ^{15}N to discern amongst nitrogen sources at three sites with different substratum and anthropogenic activities. *Ulva* blooms were monitored monthly at San Juan de la Costa (SJC), Casa del Marino (CM) and El Tecolote (TE). Species presence varied between sites and months. At SJC *Ulva* displayed the highest signal of δ^{15}N associated with fishing products and local mining wastewaters (19.5‰). CM showed isotopic values related to sewage waters (13‰). The δ^{15}N of species at TE were the lowest. We found higher isotopic signatures than in other tropical/subtropical regions, associated with nutrient rich water masses. *Ulva* species can be used to trace and discern amongst different sources of nitrogen from natural or anthropogenic sources.

1. Introduction

Macroalgal blooms are primary symptoms of eutrophication in shallow coastal ecosystems experiencing increased nitrogen (N) and phosphorus (P) loadings (Fletcher, 1996; Valiela et al., 1997; Bricker et al., 2008). The increased nutrients fuel the growth of opportunistic macroalgae and phytoplankton (Lapointe et al., 2004; Morris and Virnstein, 2004). High biomass macroalgae accumulations are usually non-toxic; however, they can cause major ecosystem disruption, such as habitat destruction, oxygen depletion and alterations in nutrient/biogeochemical cycling (McGlathery, 2001; Valiela et al., 1997; Lapointe and Bedford, 2007).

In tropical and subtropical coastal waters, sources of dissolved inorganic nitrogen (dissolved inorganic nitrogen = DIN; as ammonia, nitrate, or nitrite) typically limit the distribution, productivity, and abundance of primary producers such as macroalgae (Nelson et al., 2003; Thornber et al., 2008; Teichberg et al., 2010). Globally, many studies use stable nitrogen isotope ratios (δ^{15}N) as a tool to discriminate between natural and anthropogenic nitrogen sources that could support excessive macroalgal growth (e.g., Costanzo et al., 2001; Umezawa et al., 2002; Gartner et al., 2002; Savage and Elmgren, 2004; Bacchus et al., 2014). The δ^{15}N in macroalgae can be used to assess land-based nutrient enrichment in coastal waters by "fingerprinting" the source of nitrogen because several sources have δ^{15}N signatures that are between known ranges (Heaton, 1986; Lapointe and Bedford, 2007). Some macroalgae can reflect the N isotopic signatures of their source with little fractionation, making these species potential indicators of anthropogenic nutrient inputs (Savage and Elmgren, 2004; Deutsch and Voss, 2006; Thornber et al., 2008; Bristow et al., 2013; Viana and Bode, 2015).

In recent years, the world's largest macroalgal blooms are caused by species that belong to the genus *Ulva* (Liu et al., 2013; Van Alstyne et al., 2015). This genus was found to be especially sensitive in reflecting wastewater inputs to coastal waters and might help predict the potential for prominent and nuisance future macroalgal blooms (Teichberg et al., 2010). As demonstrated in other studies *Ulva* spp. are more competitive in nutrient rich waters where their growth rate is increased and also have higher surface-area to volume ratios compared to other species (Whitehouse and Lapointe, 2015). In subtropical waters around the world studies focus on the influence of anthropogenic activities on macroalgae blooms. For example, in the coral reefs of Tobago the macroalgae *Ulva lactuca*, *Gracilaria thikvahiae* and *Agardhiella subulata* presented the highest δ^{15}N values (~12‰) that were associated to a sewage outfall from Buccoo Point. Waters from the nearby communities undergo secondary treatment that does not remove nitrates and therefore the surroundings are nutrient rich (DIN concentrations 2.4 ± 0.7 μM) (Lapointe et al., 2010). Studies at sites where point-source sewage inputs were eliminated but where nonpoint-source sewage pollution prevail, macroalgae proved to be useful in assessing land-based nutrient sources, since a high δ^{15}N isotopic signature (6.3‰) clearly reflects sewage pollution (Lapointe et al., 2015). Understanding how nitrogen enters lagoons or bays and the way it is subsequently used by

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primary producers is of great importance in assessing the impacts of anthropogenic versus "natural" sources of nutrients in marine systems (Rogers, 2003; Savage and Elmgren, 2004; Piñón-Gimate et al., 2009; Teichberg et al., 2010).

In La Paz Bay macroalgal blooms of *Ulva* had been previously reported. These blooms were associated with pulses of nutrients derived from hurricane events through increased runoff, although nutrients were not measured at the time of the study (Águila-Ramírez et al., 2005). In a recent study by Chávez-Sánchez (2012), the presence of *Ulva* was associated with the presence of high nitrogen concentrations (~27.2 μM of total nitrogen); however, nutrient sources are not currently identified in the area. Even do macroalgae blooms in the bay are conspicuous and the municipal authorities have to pay for the removal of drifted macroalgae to the shore (Aguilera-Morales et al., 2005), these are not yet considered nuisance blooms. In this present study, we investigate the $\delta^{15}N$ signatures of *Ulva* species at three sites in La Paz Bay. These sites have diverse associated substrate, anthropogenic land uses and water characteristics. In order to determine the primary source of DIN for bloom forming macroalgae we compare the $\delta^{15}N$ signatures of *Ulva* species (derived from the literature) to that of known sources that directly influence these sites and are considered to be the primary nitrogen source supporting macroalgal growth in an annual cycle.

2. Material and methods

2.1. Study area and sampling collection

La Paz Bay is a semi-protected water body located in the Western littoral area of the Gulf of California, between 24°06′ N and 24°47′ N and between 110°18′ W and 110°45′ W. It has an approximate surface of 1200 km^2 and is bounded by the Baja California peninsula and by the insular complex of Espiritu Santo. The connection with the gulf is through the San Lorenzo Channel located on its western side (Obeso-Nieblas, 2003). The climate is arid dry (BWh): annual evaporation (215 mm) exceeds annual precipitation (180 mm); the maximum rainfall in the bay occurs between July and October and is associated with southeast winds, tropical storms and hurricanes; dominant winds come from the northwest from November to March. The water masses are predominantly equatorial surface water (ESW) that flow from the Gulf to the bay. Once there, due to evaporation processes, this water salinity increases to above 35 ppt, thus becoming Gulf of California Water (GCW) mass (Monreal-Gómez et al., 2001).

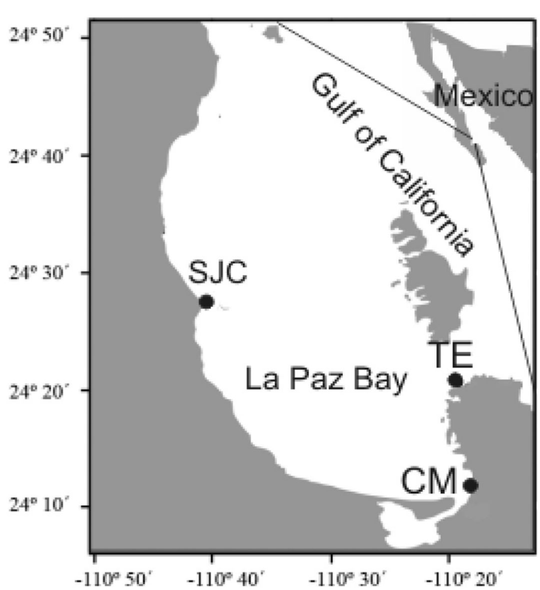

Fig. 1. Selected sites at La Paz Bay. SJC = San Juan de la Costa, CM = Casa del Marino, TE = Tecolote.

Recorded air temperature ranges (March to June: 17.4–27.1 °C; July to October: 24.4–30.1 °C; November to February: 15.2–19.3 °C) and precipitation patterns (rainiest months recorded July to October: 2.1–102.3 mm/month) were used to describe three seasons as follows (INEGI, 2010): dry season (March to June), rainy season (July to October) and cold season (November to February).

In the bay, following a survey of all shallow areas, three sampling locations were chosen based on the obvious presence of some benthic macroalgae species. Each site presented different characteristics: (1) San Juan de la Costa (24°22′30″ N, 110°42′00″ W) has a substrate that consists of boulders and patches of sandy bottom. There is an important mining company (Roca Fosfórica Mexicana II -ROFOMEX) that extracts phosphorite at this site. The phosphorite is then transported to other sites for processing (Mesa-Zavala, 2013). A temporary fishing camp (with only in house hosting fishing activities) is located in the area; (2) Casa del Marino (24°10′20.8″ N, 110°18′33.3″ W) is located at the south of the bay and is a protected and shallow area. This location was affected by anthropogenic activities and subsequently the natural substratum is modified through added inorganic and organic material. The sandy bottoms are covered in part by some boulders, shells and coral remains (Chávez-Sánchez, 2012). It is right on the waterfront of the city of La Paz and thus is subject to major touristic activities; (3) El Tecolote (24°20′9″ N, 110°19′00″ W) is located near the San Lorenzo Channel. The southern portion is uninhabited and is characterised by a hard rock platform with sandy patches (Fig. 1). Nutrient concentrations (Dissolved nitrogen -DIN- and total nitrogen -TN-) at each site and for each season during the study period were taken from a technical report (unpublished data; Table 1).

At each site, thalli of each *Ulva* species were collected monthly between February and December 2013 producing three to five composite samples; in all cases, macroalgal specimens were attached to the substratum ($n = 214$). In order to determine species level, a subsample of each species was taken and identified using adequate taxonomic keys from the region (Abbott and Hollenberg, 1976; Hayden et al., 2003; Norris, 2010). Samples were washed in the field, with species separated by hand when more than one was identified. In the laboratory, individual thalli samples were oven dried at 60 °C for two to three days prior to grinding into a fine powder using a mortar and pestle. A 1 mg sample was weighed and packed in tin capsules for isotopic analysis. Samples were analysed in the Laboratory of Mass Spectrometry (Laboratorio de Espectrometría de Masas, LEsMa-Centro Interdisciplinario de Ciencias Marinas, CICIMAR); the proportion of $^{15}N/^{14}N$ relative to the atmospheric N_2 was obtained using an elemental combustion COSTECH ECS4010® analyser and later using a THERMO SCIENTIFIC DELTA V PLUS® mass spectrometer for isotopic ratios. $\delta^{15}N$ values, in per ml (‰) concentration, were calculated as $[(R_{sample} / R_{standard}) - 1] \times 10^3$, with R equal to $^{15}N/^{14}N$, corresponding to the isotopic signature.

Temperature, salinity and turbidity were measured monthly (during low tide at one-meter depth, always at morning between 8 and 9 am, three measurements of each parameter were recorded) using a HORIBA-50U multiparameter probe (accuracy ±0.3 °C; ±3 ppt and ±5 g/L, respectively), at each site at the same time of day.

Table 1
Mean DIN (Dissolved inorganic nitrogen) and total nitrogen (NT) concentrations in the water column at the study sites during 2013 (Unpublished data).

Site	Nutrient	Cold season μM L^{-1}	Dry season μM L^{-1}	Rainy season μM L^{-1}	Cold season μM L^{-1}
SJC	DIN	21.8	20.8 ± 3.9	18.8 ± 31.7	8.3 ± 6.9
	NT	39.9	22.9 ± 3.8	36.7 ± 34.1	14.3 ± 2.3
CM	DIN	1.5	5.7 ± 1.9	6.1 ± 2.0	15.6 ± 20.0
	NT	38.5	34.2 ± 2.9	22.9 ± 9.1	19.8 ± 14.0
TE	DIN	6.9 ±	4.8 ± 0.1	5.9 ± 1.3	1.2 ± 0.9
	NT	11.9 ±	15.2 ± 0.1	16.7 ± 8.9	21.2 ± 14.6

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