Ciencias Marinas Journal/Revista

Use of intertidal macroalgae as bioindicators of anthropogenic nutrient disturbance in the rocky coasts of the tropical central Mexican Pacific

Careli Vergara-Carranza[1], Norma López[1,2]*, Alejandra Piñón-Gimate[3]

ARTICLE INFO

Article history:
Received 30 April 2023
Accepted 07 September 2023
Published 22 March 2024

LEER EN ESPAÑOL:
https://doi.org/10.7773/cm.y2024.3416

CORRESPONDING AUTHOR
* E-mail: nlopez@enesmorelia.unam.mx

[1] Unidad Multidisciplinaria de Docencia e Investigación-Zihuatanejo, Escuela Nacional de Estudios Superiores Unidad Morelia and Facultad de Ciencias, Universidad Nacional Autónoma de México, 40880 Zihuatanejo, Guerrero, México.
[2] Departamento de Biología Comparada, Facultad de Ciencias, Universidad Nacional Autónoma de México, 04510 Ciudad de México, México
[3] Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, 23096 La Paz, México

ABSTRACT. Bahía de Zihuatanejo, Mexico, exhibits disturbances in its rocky shores due to large amounts of nutrients that enter the sea due to urbanization and mass tourism. These nutrients are traced in macroalgae using stable isotopes. The objective of this study was to use 4 species of macroalgae as bioindicators to infer nutrient sources in the system through $\delta^{15}N$ and the elemental nitrogen (N) content. Samples were collected in the rainy and dry seasons of 2021 at 3 sites in the Zihuatanejo region: La Madera, Las Salinas, and La Majahua. Water samples were taken at each site and season to determine nitrate (NO_3) and phosphate (PO_4) concentrations. Isotopic signals and N content were evaluated in *Hypnea spinella*, *Padina gymnospora*, *Ulva intestinalis*, and *Ulva lactuca* for each site and season. The concentrations of NO_3 and PO_4, which were highest at Las Salinas and showed no differences between La Madera and La Majahua, were similar to those previously reported for the region. The highest $\delta^{15}N$ signals were found in *U. intestinalis* and *H. spinella* at Las Salinas (16.12‰ and 15.38‰, respectively) and La Madera (15.12‰ and 13.58‰, respectively) and were close to the isotopic wastewater signal. In La Majahua, low signals were obtained for *H. spinella* (13.54‰), *P. gymnospora* (9.24‰), and *U. lactuca* (8.24‰), with values that were close to the isotopic signal of nutrient-rich oceanic waters. The N content varied depending on the species and site, although it was generally higher at Las Salinas. The isotopic signals agreed with those reported for anthropogenic and natural nutrient-enriched coasts. The species *U. intestinalis* and *H. spinella* can be used as bioindicators of anthropogenic disturbance due to sewage discharge in the region.

Key words: macroalgae, bioindicators, stable isotopes, contamination, tropical Mexican Pacific.

INTRODUCTION

Marine ecosystems, particularly coastal areas, have been subject to constant disturbance due to activities such as urbanization, intensive agriculture, industrialization, and mass tourism (Orlando-Bonaca et al. 2008, Benedetti and Trussell 2014, Portugal et al. 2017). Consequently, a large amount of nutrients of anthropogenic origin, mainly organic and inorganic nitrogen (N) compounds that come from agricultural runoff and wastewater, have been deposited in the sea (Martins et al. 2012, Worm and Lenihan 2013, Kowalewski et al. 2015, Zhai et al. 2020). This nutrient flux has seriously compromised flora and fauna communities in the coastal zone (Adams 2005, López et al. 2017, Francescangeli et al. 2020).

Ixtapa-Zihuatanejo, Guerrero, is an important international tourist destination in the tropical central Mexican Pacific region. In fact, this destination had more than 2,088,610 tourists per year until 2018 and 84,566 inhabitants in 2020 (SECTUR 2014, INEGI 2020, GMZ 2022). Urban development in Ixtapa-Zihuatanejo has been accelerated and unplanned; this caused multiple impacts on the coastal zone, including nutrient pollution in Bahía de Zihuatanejo due to wastewater discharge (Nava and Ramírez-Herrera 2011, Inda and Gómez 2015, López et al. 2017, García et al. 2018). This bay receives direct discharges from “La Marina” (flux of 132.8 $L \cdot s^{-1}$) and “La Ropa” (flux of 18 $L \cdot s^{-1}$) treatment plants and a flux of 30 $L \cdot s^{-1}$ indirectly discharged (Planea Tropical S. de R.L. de C.V. 2015, CONAGUA and SEMARNAT 2020).

Open Access
Online ISSN: 2395-9053
Screened via Similarity Check powered by iThenticate
https://doi.org/10.7773/cm.y2024.3416

The discharge of wastewater in Bahía de Zihuatanejo can be classified as a disturbance, taking into account that a disturbance is the set of natural or anthropogenic events that change the desired state of a system, including its structure, composition, and diversity (Battisti et al. 2016, Newman 2019). In particular, nutrient enrichment is an anthropogenic disturbance that leads to changes in the abundance of primary producers, which causes macroalgae to dominate benthic communities in N-rich environments (Bricker et al. 2008, Lapointe et al. 2015).

The use of bioindicators can be a valuable tool to acquire knowledge on the disturbances caused by human activities in ecosystems (Bonanno and Orlando-Bonaca 2018). Characteristically, a bioindicator is highly frequent and abundant, easily identified and sampled, moderately tolerant to disturbances, and widely distributed with respect to the range of exposure to a contaminant (Bonanno et al. 2020). Due to their sessile nature, permanence and abundance on rocky coasts, and their moderate tolerance to disturbances, macroalgae can be used as bioindicators of anthropogenic disturbance in marine environments (Veiga et al. 2013, Bonanno and Orlando-Bonaca 2018). In macroalgae, N forms proteins and participates in the catalysis of enzymes; therefore, it is considered a key and limiting nutrient for the metabolism and growth of these important primary producers (Pedersen and Borum 1996), although phosphorus (P) can also be a limiting nutrient (Valiela et al. 1997). N is found naturally in the water column; however, excess N from wastewater discharges (mainly those derived from domestic urban waters) and fertilizers often leads to increased biomass of primary producers (Thornber et al. 2008).

In recent decades, stable isotope analysis of N in macroalgae has been highly useful to determine the origin and flux of elements in ecosystems (Guerrero and Berlanga 2000, Alquezar et al. 2013, Bergamino et al. 2017). N exists as 1 of 2 non-radioactive stable isotopes. The common isotope is ^{14}N (99.6%) and the rarest is ^{15}N (0.4%); the isotopic signal is the ratio of these 2 N isotopes and is referred to as the $\delta^{15}N$ (expressed in parts per thousand or ‰). The ratio between these 2 isotopes can be used to assess what source of N macroalgae are incorporating. Since a wastewater effluent has a higher proportion of ^{15}N than ^{14}N, the isotopic signal of the effluent is high compared to that of ocean water (Costanzo et al. 2001, Fry 2006). Thus, anthropogenic wastewater will have $\delta^{15}N$ values in a range of +10‰ to +20‰ (McClelland et al. 1997, Kendall 1998), whereas nitrogen fertilizers will have a signal that varies from –4‰ to 4‰, which can be distinguished from atmospheric N as the basis of the isotopic signal (Dailer et al. 2010, Calizza et al. 2015, Bergamino et al. 2017).

Several studies of stable isotopes in macroalgae in coastal systems from different regions of the world show how useful macroalgae are as bioindicators of different sources of N, including effluents of wastewater (Gartner et al. 2002, Rogers 2003, Costanzo et al. 2005), shrimp farming (Lin and Fong 2008), nutrient-rich waters from upwelling (Viana and Bode 2013), and organic matter from macroalgae stranded on the beach or from multiple sources such as agriculture and industrial wastewater, which can have very light isotopic signals that can even be negative (Lemesle et al. 2015, Wang et al. 2016). In addition, some seasonal variations have been observed, as there are point sources that can fluctuate depending on the time of year (Lemesle et al. 2016). In Mexico, research with stable isotopes in macroalgae on the coasts of the Gulf of California has shown that the isotopic signal adequately reflects the source of N, in particular, wastewater, shrimp farming, and upwellings sources, with the study of $\delta^{15}N$ (e.g., Piñón-Gimate et al. 2009, 2017; Ochoa-Izaguirre and Soto-Jiménez 2013).

Although macroalgae from the tropical central Mexican Pacific have been widely studied with various objectives (e.g., Taylor 1945, Dawson 1960, Mateo-Cid and Mendoza-González 1997, Mateo-Cid and Mendoza-González 2012, López et al. 2017, Nava et al. 2021, Sandoval-Coronado and López-Gómez 2021, López et al. 2022), there is only one study in which the isotopic signal in macroalgae was used in the tourist area of Ixtapa-Zihuatanejo. Using $\delta^{15}N$ signals in 5 species of coralline macroalgae, Nava et al. (2014) determined that the reefs closest to the tourist area and least conserved were impacted by wastewater. Furthermore, macroalgae showed isotopic signals of up to 9.5‰, which was considered an enriched signal (Nava et al. 2014).

The aim of this work was to determine the sources of nutrients and their spatial and temporal variation through the N content and the isotopic signal in the tissues of 4 macroalgae species (*Ulva intestinalis*, *Ulva lactuca*, *Hypnea spinella*, and *Padina gymnospora*) in sites with and without anthropogenic influence at 2 times of the year. The use of macroalgae as bioindicators of anthropogenic N sources in the Ixtapa-Zihuatanejo region through $\delta^{15}N$ will complement and provide relevant information on the environmental conditions of the bay.

Materials and methods

Study site

The study was carried out in the Ixtapa-Zihuatanejo region (17°40′39″ N, 101°39′25″ W to 17°36′47″ N, 101°33′28″ W) (Fig. 1). According to the Köppen–Geiger climate classification, the region has the Aw_0 climate, characterized as warm subtropical with rain in summer (García 2004). The average annual temperature is 27.5 °C and average precipitation from 2011 to 2018 was 1,225 mm (CONAGUA 2022). The rainy season occurs from June to October and the dry season from November to May. The tidal regime is a mixed semidiurnal type, with 2 high tides and 2 low tides per day and a variation of –0.56 m to 1.10 m (SEMAR 2021). The hydrodynamics of Bahía de Zihuatanejo includes thermohaline circulation (Morales et al. 2008), which consists of a circular current that runs completely through the bay from the northwest to the southwest (López et al. 2017).

The study sites were La Madera (17°38′14″ N, 101°33′03″ W) and Las Salinas lagoon (17°38′12.2″ N, 101°33′35.4″ W) (sites with anthropogenic influence), in Bahía de Zihuatanejo, and La Majahua (17°38′14″ N, 101°33′03″ W), outside the bay; this site was considered to have no anthropogenic influence (Fig. 1).

La Madera is made up of a 0.2-km long sandy beach section and a rocky area composed of boulders, a rock platform (where the macroalgae collection was carried out), and a cliff at the eastern end of the beach (Escalante-Vargas 2003, Mateo-Cid and Mendoza-González 2012, Google 2021a). This site is surrounded by the urban area of Zihuatanejo, which covers approximately 7.5 km^2 (Google 2021a), has residential and commercial land use, and is also considered an important site for tourism (INEGI 2009).

Las Salinas lagoon is located 1 km away from La Madera and has been identified as a discharge site for wastewater from “La Marina” treatment plant (Planea Tropical S. de R.L. de C.V. 2015). Las Salinas has a floodable area of 7 hectares and is surrounded by the urban area of Zihuatanejo. Its origin is semi-artificial and the predominant soil is sandy and low sand clay, rocks, and gravel. Mangroves constitute the predominant vegetation, particularly the species *Avicenia germinarns*, *Laguncularia racemosa*, and *Conocarpus erectus*. Las Salinas has a permanent connection with the sea through a channel (0.035 km wide and 0.168 km long) located in the southern part (SEMAR 2003, Córdova-Tapia et al. 2014, Izurieta et al. 2014, Senate of la República 2015, Ramírez-López 2020). Along the channel, some rocky areas can be distinguished on the banks (where the macroalgae collection was carried out); depth increases towards the center and the soil becomes sandy (per. obs.).

La Majahua is delimited by 2 rocky points with large cliffs, promontories, and boulders. This beach is mixed and has a 2.8 km extension with a 0.4 km long sandy beach. Sampling was carried out on the southwestern rocky point (Google 2021b). The surrounding vegetation is predominantly medium-sized subdeciduous forest (~282.5 ha) (CONANP 2023), and some individuals of the mangrove *C. erectus* can occasionally be found on the coast (INEGI 2009, Planea Tropical S. de R.L. de C.V. 2015, Google 2021b). To date, no sources of pollution have been identified on this beach because it is

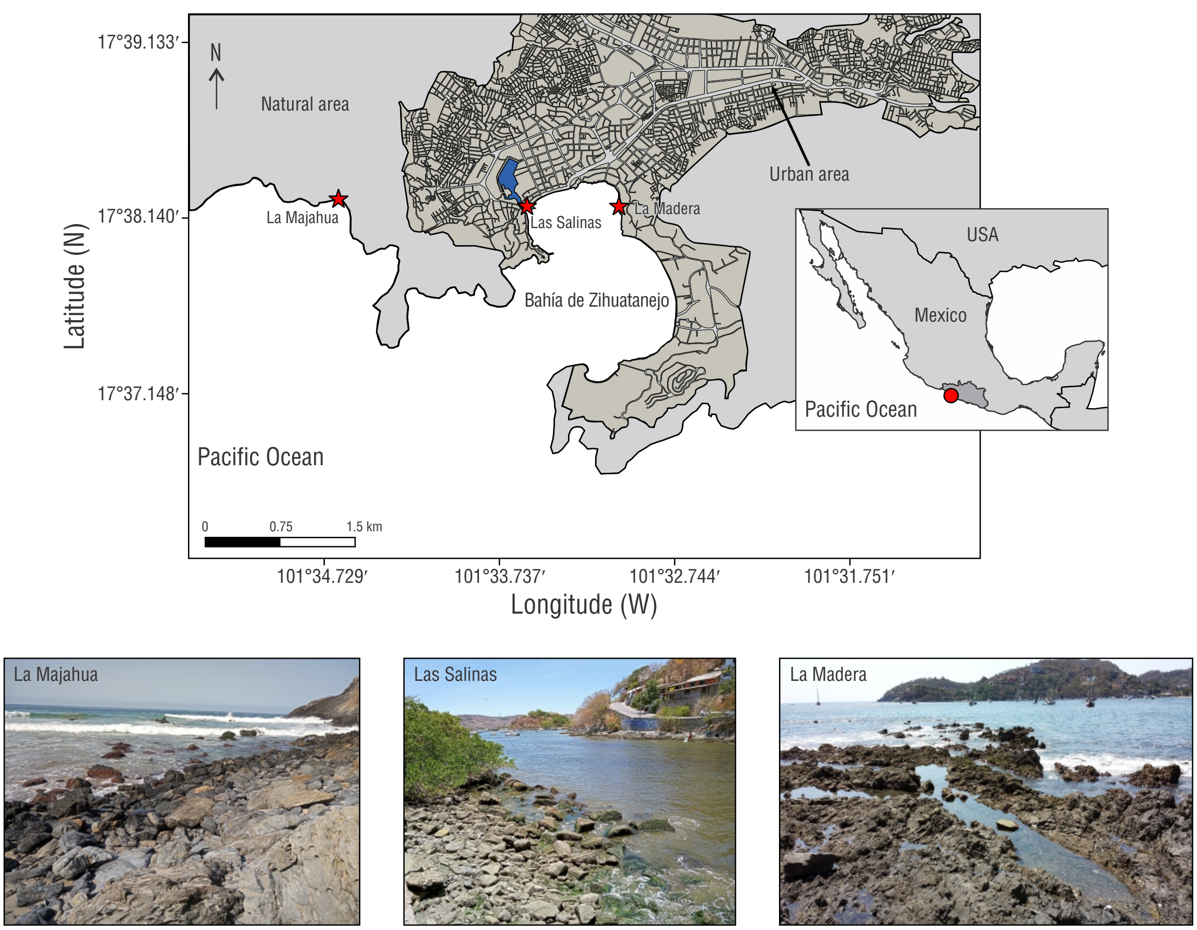

Figure 1. Map of the study area.

located more than 3.5 km from the urban area of Bahía de Zihuatanejo, free of human settlements and surrounded by vegetation. Therefore, in this work, La Majahua was considered the site with the least anthropogenic influence (Google 2021b).

Sample collection

In 2021, 2 field trips were carried out, the first during the dry season (May) and the second in the rainy season (October). Four water samples were taken per site with the exception of Las Salinas, where 3 samples were taken in the dry season, to obtain a total of 23 samples. Each sample was filtered with a PRF syringe and Whatman GF/F filters (0.45 µm) (Maidstone, UK). They were then contained in 125-mL Nalgene bottles to be transferred to the laboratory in thermal bags with cooling gel and stored in a refrigerator at –20 °C until subsequent analysis. A DR/890 colorimeter (Hach, Loveland, USA) was used to determine the concentration of N and P in water samples. The NitriVer3, NitraVer 6, and PhosVer 3 reagents were used to determine nitrates and phosphates.

On the rocky shores of Bahía de Zihuatanejo, filamentous, lamellar, branched-coarse, and leathery macroalgae are notable for their abundance and coverage (Nava et al. 2014, López et al. 2017). In the present work, 4 species of macroalgae belonging to these functional groups were selected, *U. lactuca* (lamellar), *U. intestinalis* (filamentous), *H. spinella* (branched-thick), and *P. gymnospora* (leathery), using the criteria of high coverage, ease of collection, and presence of conspicuous, monospecific growths greater than 10 cm in length. At least 5 thalli per species were taken at each site and during each season. Since no senescent thallus was found, it was possible to use the entire thallus. The species *P. gymnospora* was not present at Las Salinas, and *U. lactuca* was collected in the rainy season due to the absence of *U. intestinalis*. The macroalgae samples were extracted by hand using chisel and hammer, rinsed with seawater, and placed in plastic bags for transport to the laboratory. Throughout the study, 85 samples were obtained. These samples were transferred, while kept cold, to the laboratory and, subsequently, rinsed with fresh water to remove epibionts.

Sample processing

Each sample was dried in a DHG-9145 Eco-Shell convection oven (Corning, USA) at a temperature of 60 °C for 5 to 7 days until a constant dry weight was obtained. The dried material was pulverized with an agate mortar and stored in sterile polypropylene tubes. Between 1 and 2 mg of the sample were weighed and packaged in Costech Analytical (Valencia, USA) 5 × 9 mm tin capsules. The samples were analyzed in the Laboratorio de Análisis de Isótopos Estables of the Facultad de Química de la Universidad Nacional Autónoma de México, Yucatán Unit, to obtain the $^{15}N/^{14}N$ ratio relative to atmospheric N_2 (reference material). An ECS4010 elemental analyzer (Costech Analytical) with a Zero Blank autosampler coupled to a DELTA V Plus isotopic ratio mass spectrometer (Thermo Fisher Scientific, Waltham, USA) was used. The $\delta^{15}N$ values in mL (‰) were calculated with Eq. (1):

$$\delta^{15}N(‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1{,}000 \quad ,$$

where R is equal to $^{15}N/^{14}N$, corresponding to the isotopic signature (Peterson and Fry 1987).

Statistical analysis

Shapiro–Wilk normality tests were performed for nitrate and phosphate data and Kolmogorov–Smirnov tests for stable isotopes and elemental N values to determine whether the data were homogeneous and homoscedastic. As they were not normal for nutrients, a non-parametric Kruskal–Wallis test was performed to demonstrate significant differences between sites and seasons for nitrates, phosphates, and macroalgae (stable isotopes and elemental N). When there were significant a posteriori differences, a multiple comparisons test was performed to identify different groups.

Results

The average values of nitrates and phosphates were statistically higher ($KW\text{-}H_{(2,\ 23)} = 14.49$, $P < 0.05$ and $KW\text{-}H_{(2,\ 23)} = 14.03$, $P < 0.05$, respectively) at Las Salinas (0.481 ± 0.07 $mg \cdot L^{-1}$ and 2.34 ± 0.86 $mg \cdot L^{-1}$, respectively) with respect to La Madera (0.032 ± 0.03 $mg \cdot L^{-1}$ and 0.246 ± 0.08 $mg \cdot L^{-1}$, respectively) and La Majahua (0.018 ± 0.006 $mg \cdot L^{-1}$ and 0.266 ± 0.12 $mg \cdot L^{-1}$, respectively). Average values of nitrates and phosphates, although not statistically significant, were higher in the rainy season (0.18 ± 0.2 $mg \cdot L^{-1}$ and 0.2 ± 1.2 $mg \cdot L^{-1}$, respectively) than in the dry season (0.14 ± 0.2 $mg \cdot L^{-1}$ and 0.74 ± 0.9 $mg \cdot L^{-1}$, respectively). At Las Salinas, nitrates and phosphates were considerably higher than in the other 2 sites in both seasons, and nutrients were higher during the rainy season in all 3 sites than in the dry season (Table 1).

Ulva intestinalis had the highest average value in the isotopic signal for the whole study (13.02 ± 2.48‰), followed by *H. spinella* (12.22 ± 2.27‰), *P. gymnospora* (9.36 ± 1.03‰), and *U. lactuca* (8.24 ± 0.42‰) (Fig. 2a). The isotopic signals of the 4 species showed significant differences ($KW\text{-}H_{(3,81)} = 38.3881$, $P < 0.05$). An a posteriori test of multiple comparisons showed significant differences ($P < 0.05$) between *U. intestinalis* and *P. gymnospora*, *U. intestinalis* and *U. lactuca*, *H. spinella* and *P. gymnospora*, and *H. spinella* and *U. lactuca*. Las Salinas had the highest average value of $\delta^{15}N$ (13 ± 3.02‰), followed by La Madera (12.18 ± 1.89‰) and La Majahua (9.8 ± 2.01‰). Differences in the isotopic signals of the 3 sites were significant ($KW\text{-}H_{(2,81)} = 20.6276$, $P < 0.05$). The a posteriori test of multiple comparisons showed significant differences ($P < 0.05$) between La

Table 1. Values of nitrates (NO_3) and phosphates (PO_4) by site and season.

Site	Season	NO_3 (mg·L^{-1})	PO_4 (mg·L^{-1})
Las Salinas	Dry	0.476 ± 0.01	1.98 ± 1.3
	Rainy	0.485 ± 0.08	2.61 ± 0.3
La Madera	Dry	0.022 ± 0.005	0.287 ± 0.07
	Rainy	0.042 ± 0.04	0.205 ± 0.07
La Majahua	Dry	0.02 ± 0.0	0.377 ± 0.05
	Rainy	0.017 ± 0.001	0.155 ± 0.03

Majahua and La Madera and La Majahua and Las Salinas (Fig. 2b). The average value of δ^{15}N was higher in the dry season (12.97 ± 2.54‰) than in the rainy season (10.04 ± 1.7) (Fig. 2c). Significant differences in δ^{15}N were found between the dry and rainy seasons (KW-$H_{(1,81)}$ = 20.9449, $P < 0.05$).

Significant differences in the isotopic signal of *U. intestinalis* were found between sites (KW-$H_{(2,25)}$ = 5.9871, $P < 0.05$). The signals for La Madera and Las Salinas were higher than the signal from La Majahua ($P < 0.05$). No significant differences were found between seasons for *U. intestinalis* (KW-$H_{(1,25)}$ = 0.1571, $P = 0.6919$). No significant differences were observed for *H. spinella* between sites (KW-$H_{(2,30)}$ = 1.2877, $P = 0.5253$), but were observed between seasons (KW-$H_{(1,30)}$ = 21.7742, $P < 0.05$). The highest signal was found in the dry season ($P < 0.05$). For *P. gymnospora*, significant differences were found between sites (KW-$H_{(1,21)}$ = 11.4248, $P < 0.05$), with the lowest isotopic signal at La Majahua ($P < 0.05$). Significant differences were found for seasons (KW-$H_{(1,21)}$ = 6.4341, $P < 0.05$). The highest isotopic signal occurred during the dry season ($P < 0.05$).

We observed variations in N content that depended on the species, site, and season (Table 2). Significant differences were found in elemental N concentrations between species (KW-$H_{(3,81)}$ = 9.5314, $P < 0.05$). We demonstrated significant differences ($P < 0.05$) between *U. intestinalis* and *P. gymnospora* with the multiple comparisons test (Fig. 3a). Significant differences were found for sites (KW-$H_{(2,81)}$ = 21.2793, $P < 0.05$), with the lowest value ($P < 0.05$) at La Majahua compared to the values of Las Salinas and La Madera (Fig. 3b). In addition, there were significant differences between seasons (KW-$H_{(1,81)}$ = 36.7625, $P < 0.05$), with the highest average value in the rainy season compared to the dry season (Fig. 3c).

For *U. intestinalis*, significant differences in N content were observed between sites (KW-$H_{(2,25)}$ = 9.4578, $P < 0.05$). The multiple comparisons test showed that the content of N was significantly lower at La Majahua ($P < 0.05$) compared to the values of Las Salinas and La Madera. There were significant differences in N content between seasons (KW-$H_{(1,25)}$ = 16.1571, $P < 0.05$). The highest N concentration occurred in the rainy season ($P < 0.05$) in *U. intestinalis*. No significant differences were observed in the N content of *H. spinella* between sites (KW-$H_{(2,30)}$ = 3.3581, $P = 0.2$), but there were differences between seasons (KW-$H_{(1,30)}$ = 21.7742, $P < 0.05$). Furthermore, the N concentration was significantly higher in the rainy season ($P < 0.05$). No significant differences in the N content of *P. gymnospora* were observed between sites (KW-$H_{(1,21)}$ = 1.6066, $P = 0.2$). There were significant differences between seasons (KW-$H_{(1,21)}$ = 8.9054, $P < 0.05$), and the highest N concentration was found in the rainy season ($P < 0.05$).

The pattern observed in the variations of δ^{15}N showed that, in both seasons in Las Salinas and La Madera, *U. intestinalis* had a higher signal than *H. spinella* and *P. gymnospora*. Only at La Majahua did *U. intestinalis* and *U. lactuca* show signals lower than those of *H. spinella* in both seasons. The δ^{15}N decreased in the rainy season for the species present at each site (Fig. 4a). Regarding N content, *H. spinella* and *U. intestinalis* had the highest concentrations at the 3 sites and in both seasons. Regardless of the species, La Madera and Las Salinas had the highest values of N content in the rainy season, in contrast to La Majahua, which had the lowest values recorded (Fig. 4b).

Discussion

According to the results obtained in this study for the Ixtapa-Zihuatanejo region, the concentration of nitrates and phosphates in the water was higher at Las Salinas than at La Madera and La Majahua. The nutrient values found in this work (Table 1) were lower than those reported by Ramírez-López (2020) and higher than those reported by IMTA (2010) (Table 3). However, the patterns of the highest and lowest concentrations reported during the dry and rainy seasons, respectively, were maintained. Other studies of coastal areas describe that nitrate and phosphate values above 0.1 mg·L^{-1} indicate anthropogenic nutrient enrichment (Valiela et al. 1997). Based on the values obtained and those that have been reported, Las Salinas has greater anthropogenic pressure as it is influenced by "La Marina" treatment plant. The values obtained for La Madera are within the range of the values reported for Las Salinas. Due to the proximity of both sites, it is likely that wastewater values, both N and P, are being reflected. On the contrary, the values found at La Majahua were low and also coincide with those reported for the same region in areas with little anthropogenic influence (Table 3). Although the N:P ratio could not be obtained, the concentrations of both nutrients could be indicating that these are not limited for primary producers; we recommend subsequent studies to obtain dissolved inorganic N, total N, total P in water, and P in the macroalgal tissue to confirm this observation.

As expected, δ^{15}N isotopic signals in *U. intestinalis*, *U. lactuca*, *H. spinella*, and *P. gymnospora* showed variations depending on the species, site, and season of the year. High

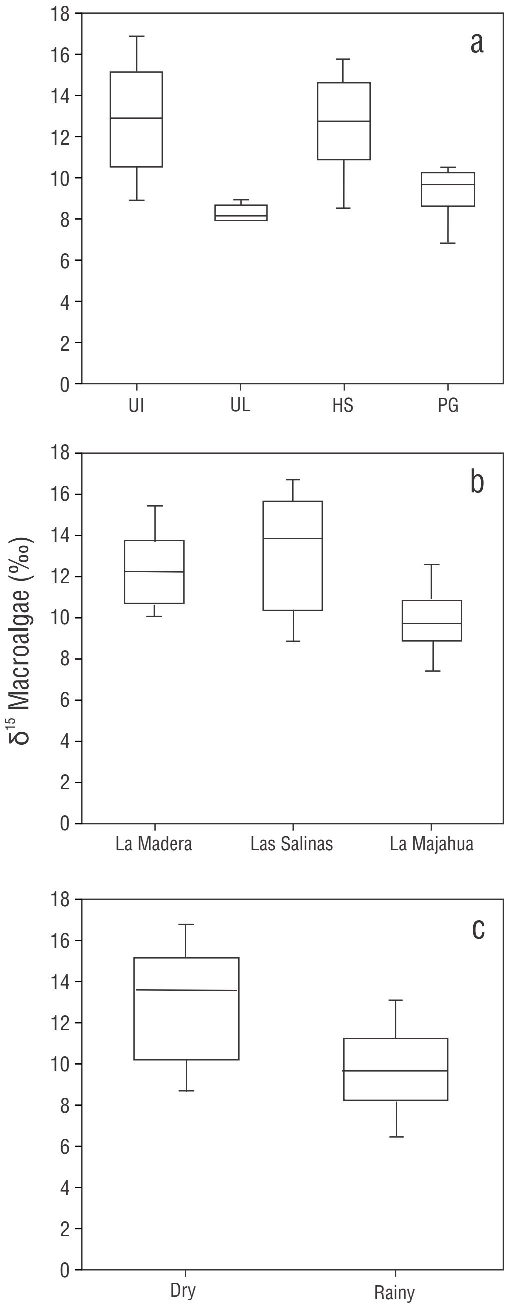

Figure 2. $\delta^{15}N$ (‰) by species, site, and season throughout the study. UI = *Ulva intestinalis*, UL = *Ulva lactuca*, HS = *Hypnea spinella*, and PG = *Padina gymnospora*.

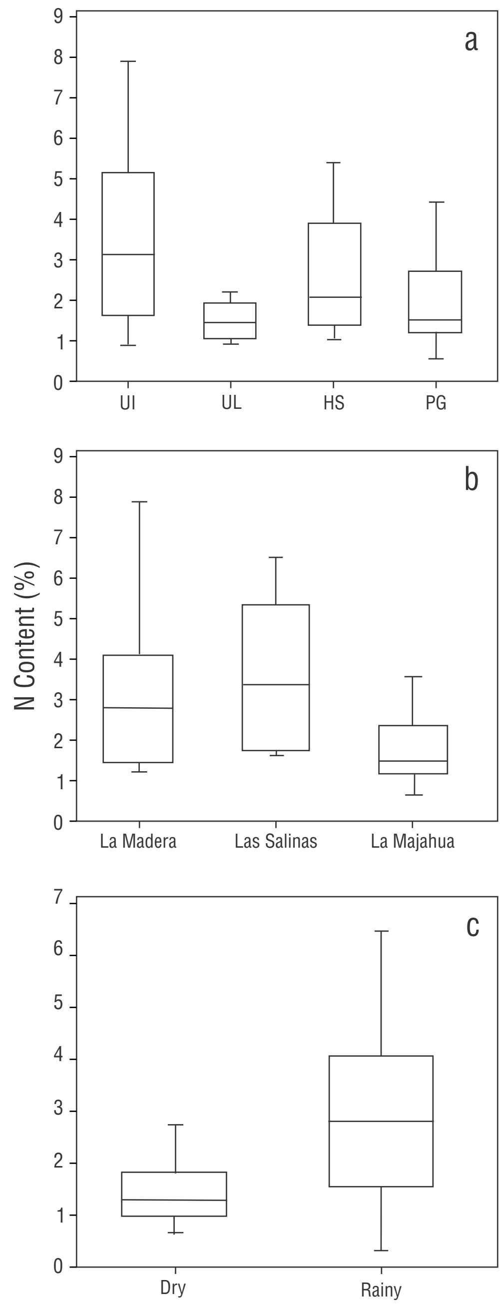

Figure 3. Elemental nitrogen (N) (%) by species, site, and season throughout the study. UI = *Ulva intestinalis*, UL = *Ulva lactuca*, HS = *Hypnea spinella*, and PG = *Padina gymnospora.*

values were found in the isotopic signal of *U. intestinalis* with respect to *H. spinella* and *P. gymnospora*, which could be related to its filamentous form. This shape allows the rapid absorption of nutrients from the water column, so this group of algae reflects N sources with high efficiency (Dailer et al. 2010). Regardless of their form, the physiological responses of algae to environmental conditions have been observed to influence isotopic fractionation. That is, when going through different biological or enzymatic processes, the proportion of the heavy isotope over the light one varies (Bergamino et al. 2017) in such a way that there will be variations in the $\delta^{15}N$ values with respect to the nutrient sources (Ochoa-Izaguirre and Soto-Jiménez 2015). However, macroalgae are expected to be sensitive enough to record changes in concentrations of dissolved N and its stable isotope in the water column and, in this way, integrate and reflect N sources in coastal systems (Ochoa-Izaguirre and Soto-Jiménez 2015).

In the present study, the isotopic signal of the species varied between sites. The highest isotopic signals were detected at Las Salinas and La Madera whereas the lowest were detected at La Majahua, which indicates that the macroalgae reflect the different sources of N (Fig. 4). For example, although *P. gymnospora* had a $\delta^{15}N$ isotopic signal of 9.92–10.4‰ at La Madera, this signal was lower than the signals of the other species and coincided with the values reported for *Padina* spp. on Ishigaki Island, Japan ($\delta^{15}N$ values greater than 8‰), which were related to an enrichment of the isotopic signal due to anthropogenic influence (Umezawa et al. 2002). Although these authors did not mention the point source of enrichment for *Padina* spp., it coincides with the values of enriched waters. It is likely that this particular species differentially assimilates N and, therefore, reflects a lower isotopic signal than the other species. Even so, an isotopic signal close to wastewater was observed, as has been reported in other studies (Table 4).

The highest values of the isotopic signal were observed at Las Salinas. In previous studies, elevated isotopic signals have been linked to wastewater (Table 3, Fig. 4a). For example, in La Paz, Baja California Sur, Mexico, $\delta^{15}N$ isotopic signal values of 16.5‰ in *U. intestinalis* were recorded for a site influenced by untreated wastewater (Piñón-Gimate et al. 2017). This coincides with the highest $\delta^{15}N$ of *U. intestinalis* recorded at Las Salinas, which was 16.2‰. Furthermore, this value is within the range of the isotopic signal shown by untreated wastewater. Therefore, the high values found at Las Salinas could reflect the enrichment of N from the discharge of "La Marina" treatment plant and the clandestine discharge of untreated wastewater from urban settlements (IMTA 2010, Ramírez-López 2020).

At La Madera, *U. intestinalis*, *H. spinella*, and *P. gymnospora* had average signals of 13.9‰, 12.4‰, and 10.2‰, respectively. Because these are high isotopic signals, they also reflect the influence of wastewater discharges (Table 3). Reports indicate that Las Salinas is influenced by wastewater and highly affected by these discharges (IMTA 2010). Hotels and restaurants have established around Las Salinas; therefore, it is possible that untreated wastewater from these businesses is discharged into the sea (per. obs.). Nava et al. (2014) collected samples of *Jania* genus species at Las Gatas reef in the southeastern end of Bahía de Zihuatanejo and obtained an isotopic signal of 9.23‰. This value, which was lower than that observed in the present study, was considered by the authors as coming from wastewaters that influence the bay. As at La Madera, Las Gatas has restaurants that indirectly discharge their untreated wastewater into the sea (Instituto de Ingeniería 2011), which causes high isotopic signals.

The isotopic signal of the 4 species studied at La Majahua (7.88–13.54‰) is within the range of nutrient-rich waters. However, this town is far from the urban area of Zihuatanejo and no point sources of pollution have been identified. In addition, no information was found about any specific source of nutrient enrichment on this beach. However, the natural disposition of nutrients in oceanic currents can also explain the values found at La Majahua. For example, Aguiñiga et al. (2010) found isotopic signals between 10‰ and 12‰ in Alfonso Basin, Bahía de La Paz, which were related to the advection of equatorial subsurface waters enriched in $\delta^{15}N$ in some periods. Subsequently, Piñón-Gimate et al. (2017) reported $\delta^{15}N$ values of 9.3‰ for *U. lactuca* and 10.4‰ for *U. rigida* at a site with conditions similar to those of La Majahua. This site was free of urban settlements and without point sources of anthropogenic pollution, but influenced by enriched oceanic waters. Therefore, these authors concluded that the isotopic signal in *Ulva* spp. reflects that of the surrounding sea (Table 4). Nava et al. (2014) found isotopic

Table 2. Ranges of $\delta^{15}N$ (‰) and nitrogen (N) content (%) of 4 species of macroalgae and the N sources observed in the 3 locations of the present study. RW = wastewater, AD = advection waters, WFP = waste fishing products, and OW = oceanic waters.

Species	Site	$\delta^{15}N$ (‰)	N (%)	Known source of N
Ulva intestinalis	La Madera	12.8–15.12	1.11–7.8	RW
	Las Salinas	10.58–16.12	1.48–6.4	RW and WFP
	La Majahua	10.4	0.76–1.97	OW
Hypnea spinella	La Madera	11.2–13.58	1.07–5.2	RW
	Las Salinas	9.98–15.38	1.41–5.3	RW and WFP
	La Majahua	9.68–13.54	0.88–3.4	OW
Padina gymnospora	La Madera	9.92–10.4	0.99–4.3	RW
	La Majahua	7.88–9.24	0.4–3.3	AD
Ulva lactuca	La Majahua	8.24	0.8–2.1	AD

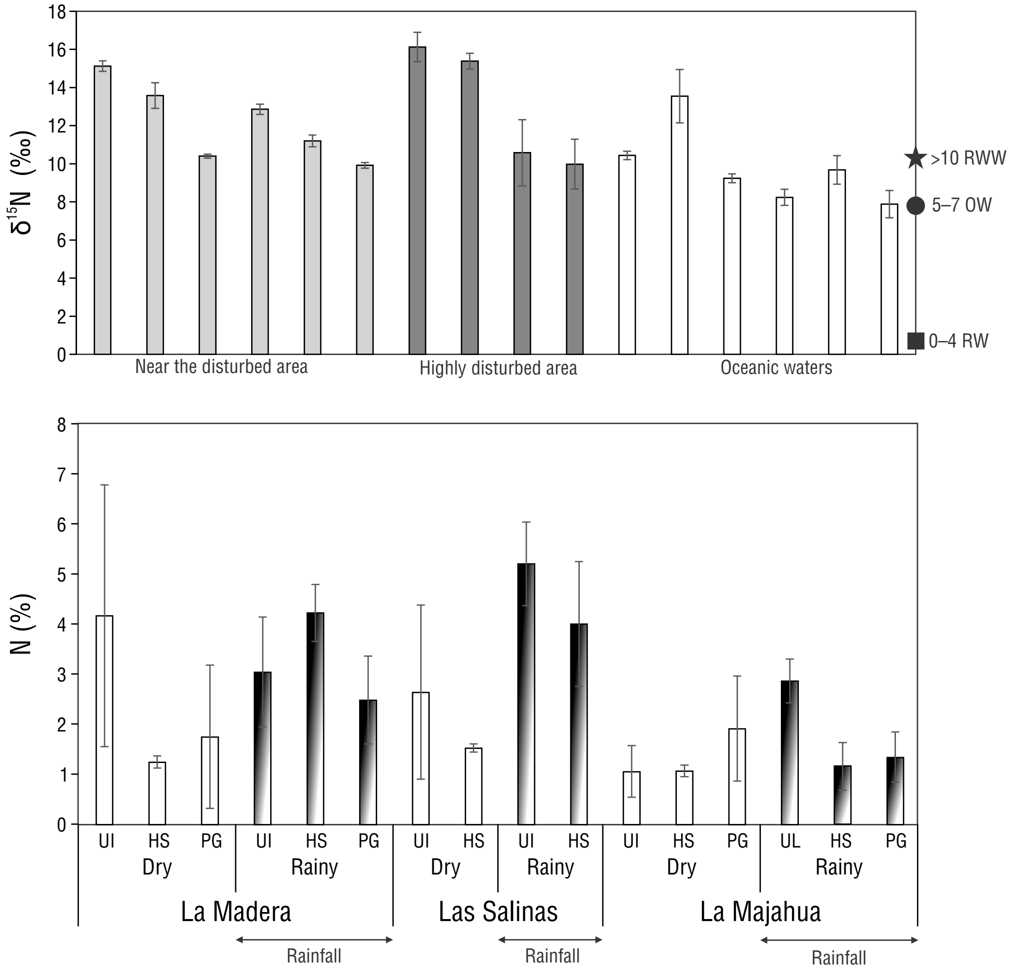

Figure 4. $\delta^{15}N$ and elemental nitrogen (N). (**a**) Average (±SD) of $\delta^{15}N$ (‰) in the 4 species of macroalgae from the 3 sites in the 2 study seasons. RWW = wastewater, OW = oceanic water, RW = rain water. (**b**) Average (±SD) of the N content (%) in the 4 species of macroalgae from the 3 sites in the 2 study seasons of Bahía de Zihuatanejo. UI = *Ulva intestinalis*, UL = *Ulva lactuca*, HS = *Hypnea spinella*, and PG = *Padina gymnospora*. Rainfall: 251 mm (September)–56.5 mm (October) (CONAGUA 2023).

signals of 8‰ in reefs located outside Bahía de Zihuatanejo and indicated that these environments do not have anthropogenic influence, which coincides with what was found in this study for La Majahua. Therefore, the isotopic signals of $\delta^{15}N$ at our site could be reflecting nutrient-rich oceanic waters. The typical isotopic signal of oceanic nitrate, a potential source of N for macroalgae, is around 5.5‰ for deep-sea nitrate and becomes enriched in surface waters (e.g., >5‰) due to preferential uptake of ^{14}N nitrate by phytoplankton over ^{15}N nitrate (Granger et al. 2004). In Hanalei Bay in Hawaii, the isotopic signal of nitrate dissolved in surface waters was close to 7‰ (Derse et al. 2007), so the isotopic signal values of the macroalgae in the present study would reflect the signal of surface oceanic waters. The coastal area of Ixtapa-Zihuatanejo is influenced by the Mexican Coastal Current (Rodríguez 2016) and the Western Mexican Current (Kessler 2006).

The highest values of the isotopic signal were found during the dry season (12.97‰ ± 2.54‰) compared to the rainy season (10.04‰ ± 1.7‰). Ochoa-Izaguirre and Soto-Jiménez (2015) previously reported this pattern for macroalgae species from an estuary in the state of Sinaloa. The values reported in Bahía de Zihuatanejo may be due to mixing processes during the rainy season. The deposition of atmospheric N, which has an isotopic signal close to 0‰, is reflected in the decrease in $\delta^{15}N$, since rainwater (3‰) has an isotopic signal close to atmospheric N (Heaton 1986). Mixing processes have been observed in different studies (e.g., Piñón-Gimate et al. 2017). However, when the mix has multiple sources of nutrients, it is difficult to identify the source with the greatest contribution to the ecosystem (Ochoa-Izaguirre et al. 2017). In Bahía de Zihuatanejo, there is considerable precipitation in the rainy season (Fig. 4) and point sources are maintained (treatment plant). Therefore, it can be inferred that there is mixing with rainwater due to the decrease in the isotopic signal from 12.9‰ to 10‰, which is similar to what was reported in previous studies (Ochoa-Izaguirre and Soto-Jiménez 2013, 2015). Nevertheless, more studies on the isotopic signal of sources inside and outside the bay must be carried out to help

Table 3. Nutrient concentrations reported for the studied sites and surrounding sites of Bahía de Zihuatanejo.

	NO_2		NO_3		NH_4		Organic N		NT		PT		
Site	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Reference
La Madera	<0.204	<0.021	0.004	<0.204	<0.126*	0.21*	0.793	<0.714	0.793		0.082	0.07	IMTA (2010)
Las Salinas	<0.204	0.13	0.066	1.42	3.44*	1.58*	5.81	1.42	9.25		0.346	0.16	
	14.72		6.07								1.0		Ramírez-López (2020)
Outside of the bay	<0.204	<0.021	<0.0022*	<0.204*	<0.126*	<0.126*	<0.774	<0.714	0.774		0.053*	0.04*	IMTA (2010)

* Average value

NO_2: nitrites; NO_3: nitrates; NH_4: ammonium; N: Nitrogen; NT: total N; PT: total phosphorus

Table 4. $\delta^{15}N$ (‰) values of 4 species of macroalgae and the associated nitrogen (N) sources in other studies in different parts of the world.

Species	Site	$\delta^{15}N$ (‰)	Nitrogen source	Reference
Ulva intestinalis (as *Enteromorpha*)	Dakwan, Taiwan	8.0–8.6	Sewage water effluent from wastewater treatment plant	Lin et al. (2007)
	Nanwan, Taiwan	13.1–14.9	Sewage water effluent from wastewater treatment plant	
Ulva intestinalis	Narragansett Bay, Rhode Island, USA	15	Sewage water effluent	Thornber et al. (2008)
	Urías Estuary, Sinaloa, Mexico	13.9	Seafood processing industry and wastewater	Ochoa-Izaguirre and Soto-Jiménez (2015)
	Casa del Marino, Bahía de La Paz, Mexico	16.5	Non-treated wastewater	Piñón-Gimate et al. (2017)
Ulva lactuca	Southern Florida Bay Region, USA	>8	Sewage water effluent from wastewater treatment plant	Lapointe et al. (2004)
	East coast of central Florida, USA	5.0–13.0	Wastewater and residual groundwater	Barile (2004)
	Green Island, Taiwan	1.9–3.9	Natural	Lin et al. (2007)
	Urías Estuary, Sinaloa, Mexico	13.4	Seafood processing industry and non-treated wastewater	Ochoa-Izaguirre and Soto-Jiménez (2015)
Hypnea spinella	Southeast region of the Gulf of California	8.5–11.4	Agriculture, wastewater, shrimp farm effluent	Piñón-Gimate et al. (2009)
Padina spp.	Ishigaki Island, Japan	>8	Anthropogenic	Umezawa et al. (2002)

monitor the environment of the Ixtapa-Zihuatanejo region. The isotopic signal ranges obtained in this study coincided with those recorded in other studies around the world that reported the same N sources in the same genera and species of macroalgae (Table 4).

Macroalgae from La Madera and Las Salinas had a higher proportion of elemental N in their tissues in both seasons, which was similar to what was observed in the highest nutrient concentrations of the water column at these sites. This supports the conclusion of nutrient enrichment by wastewater. In Majahua, the N concentration was lower, which coincides with a lower concentration of nutrients in the water compared to the other sites. This is consistent with other studies, which showed that the concentration of elemental N in the tissues of macroalgae varied and adequately reflected the nutrient concentrations of the water column. The content and proportion of N and P in macroalgal tissues can be used as indicators of the availability of these nutrients in the ecosystem (Fong et al. 1994, Kim et al. 2014). Ochoa-Izaguirre et al. (2017) found that the N concentration in macroalgal tissue ranged from 1.66% to 2.37% in highly impacted sites. Our results are consistent with these values. However, some of the N content values were high in La Majahua. Considering that this site has no anthropogenic influence, this may be a result of enriched surface oceanic waters, as discussed above.

By obtaining the isotopic signal and the elemental N content in the tissue of the macroalgae in this study, we concluded that the selected macroalgae species were good bioindicators of nutrient enrichment by natural and anthropogenic sources, as has been observed in other studies. In particular, species of the genus *Ulva* seem to best reflect the isotopic signals of their sources, followed by *H. spinella* and *P. gymnospora*. Likewise, we can conclude that Bahía de Zihuatanejo is influenced by wastewater from the human settlements that surround it, as shown by stable isotopes. We recommend subsequent studies to obtain the isotopic signal of sources of nutrients to the system and the particulate organic matter to develop mixture models and understand the contributing proportion of each source to the system. Macroalgae are good bioindicators because they are easy to collect, present all year round, and grow in almost all coastal environments. Stable isotope analyses have been characterized as accessible in terms of costs and macroalgae sample obtention and processing. Thus, it is recommended to monitor the isotopic signal in macroalgae to detect natural and anthropogenic nutrient sources in the Ixtapa-Zihuatanejo region at the sites analyzed in this study and others in the region over several years. The characterization of the possible sources of anthropogenic nutrients that impact the sites, including the analysis of the content of nutrients (N and P) in water and macroalgae, will facilitate the identification of contamination sources of N and the magnitude of its impact. Considering that the development plan of the municipality contemplates growth in infrastructure, local authorities can use this study as a starting point to carry out informed efforts for the conservation and management of resources in the coastal zone of the Ixtapa-Zihuatanejo region, one of the most important tourist destinations in the country.

Acknowledgments

This research was funded by the PAPIIT Apoyo a Proyectos de Investigación e Innovación Tecnológica Program (project IN219421). Alejandra Piñón-Gimate appreciates the support of the Instituto Politécnico Nacional for the scholarship from the Programa de Estímulos al Desempeño de los Investigadores and the Sistema Nacional de Investigadores of the Consejo Nacional de Ciencia y Tecnología. We appreciate the support of MSc. Pedro Ramírez-García Armora, MSc. Carlos Candelaria, Admid Núñez, Alejandra Sandoval, and Jessica Caro in the field work; the support of Jessica Caro, Lourdes Alejandre, Mahyra Castillo, and Alejandra Peña in the laboratory work; the support of MSc. Pedro Ramírez in the statistical analyses; and the support of MSc. Korynthia López Aguiar and Q. Jessica Olivares Amador in the stable isotope analyses.

English translation by Claudia Michel-Villalobos.

References

Adams SM. 2005. Assessing cause and effect of multiple stressors on marine systems. Mar Pollut Bull. 51(8–12):649-657. https://doi.org/10.1016/j.marpolbul.2004.11.040

Aguiñiga S, Sanchez A, Silverberg N. 2010. Temporal variations of C, N, $\delta^{13}C$, and $\delta^{15}N$ in organic matter collected by a sediment trap at Cuenca Alfonso, Bahía de La Paz, SW Gulf of California. Cont Shelf Res. 30(15):1692-1700. https://doi.org/10.1016/j.csr.2010.07.005

Alquezar R, Glendenning L, Costanzo S. 2013. The use of the brown macroalgae, *Sargassum flavicans*, as a potential bioindicator of industrial nutrient enrichment. Mar Pollut Bull. 77(1–2):140-146. http://doi.org/10.1016/j.marpolbul.2013.10.013

Barile J. 2004. Evidence of anthropogenic nitrogen enrichment of the littoral waters of East Central Florida. J Coast Res. 20(4):1237-1245. https://doi.org/10.2112/04-0212.1

Battisti C, Poeta G, Fanelli G. 2016. Heterogeneity, dynamism, and diversity of natural systems. In: Battisti C, Poeta G, Fanelli G (eds.), An Introduction to Disturbance Ecology. Switzerland: Springer Cham. p. 1-6. https://doi.org/10.1007/978-3-319-32476-0

Benedetti CL, Trussell G. 2014. Intertidal rocky shores. In: Bertness M, Bruno J, Silliman B, Stachowicz J (eds.), Marine Community Ecology and Conservation. Sunderland (USA): Sinauer Associates, Inc. p. 203-225.

Bergamino L, Tudurí A, Bueno C, Brugnoli E, Valenzuela L, Martínez A, García-Rodríguez F. 2017. Aplicación de isótopos estables como indicadores de flujos de energía en ambientes costeros de Uruguay [Application of stable isotopes as indicators of energy flows at Uruguay coastal environments]. INNOTEC. 2017(13):9-18. https://doi.org/10.26461/13.01

Bonanno G, Orlando-Bonaca M. 2018. Perspectives on using marine species as bioindicators of plastic pollution. Mar Pollut Bull. 137:209-221. https://doi.org/10.1016/j.marpolbul.2018.10.018

Bonanno G, Veneziano V, Piccione V. 2020. The alga *Ulva lactuca* (Ulvaceae, Chlorophyta) as a bioindicator of trace element contamination along the coast of Sicily, Italy. Sci Total Environ. 699:134329. https://doi.org/10.1016/j.scitotenv.2019.134329

Bricker SB, Longstaff B, Dennison W, Jones A, Boicourt K, Wicks C, Woerner J. 2008. Effects of nutrient enrichment in the nation's estuaries: A decade of change. Harmful Algae. 8(1):21-32. https://doi.org/10.1016/j.hal.2008.08.028

Calizza E, Aktan Y, Costantini ML, Rossi L. 2015. Stable isotope variations in benthic primary producers along the Bosphorus (Turkey): a preliminary study. Mar Pollut Bull. 97(1–2):535-538. https://doi.org/10.1016/j.marpolbul.2015.05.016

[CONAGUA] Comisión Nacional del Agua. 2022. Normales climatológicas por estado [Normal weather conditions by state]: CONAGUA; [accessed 2022 Apr 19]. https://smn.conagua.gob.mx/es/climatologia/informacion-climatologica/normales-climatologicas-por-estado?estado=gro.

[CONAGUA, SEMARNAT] Comisión Nacional del Agua, Secretaría de Medio Ambiente y Recursos Naturales. 2020. Inventario nacional de plantas municipales de potabilización y de tratamiento de aguas residuales en operación [National inventory of municipal water purification and wastewater treatment plants in operation]: Gobierno de México; [accessed 2020 Mar 20]. https://www.gob.mx/conagua/documentos/inventario-de-plantas-municipales-de-potabilizacion-y-de-tratamiento-de-aguas-residuales-en-operacion.

[CONANP] Comisión Nacional de Áreas Naturales Protegidas. 2023. Estudio Previo Justificativo para el establecimiento del Área Natural Protegida Área de Protección de Flora y Fauna APFF Hermenegildo Galeana, en el estado de Guerrero, México [Preliminary Justification Study for the implementation of the Protected Natural Area Hermenegildo Galeana Flora and Fauna Protection Area, in the state of Guerrero, Mexico]. Guerrero, (Mexico): CONANP.

Córdova-Tapia F, Zambrano-González L, Ayala-Azcárraga C, Orozco-Martínez C, López-Guijosa A, Ortiz H, Levy-Gálvez K. 2014. Análisis de la Manifestación de Impacto Ambiental del Proyecto "Dragado de la Laguna de Salinas, Zihuatanejo de Azueta, Guerrero" [Analysis of the Environmental Impact Demonstration of the Project "Dredging the Salinas Lagoon, Zihuatanejo de Azueta, Guerrero"]. Mexico City (Mexico): Grupo de Análisis de Manifestaciones de Impacto Ambiental, Unión de Científicos Comprometidos con la Sociedad. Technical Report, MIA-12GE2014HD02. 14 p.

Costanzo SD, O'Donohue MJ, Dennison WC, Loneragan NR, Thomas M. 2001. A new approach for detecting and mapping sewage impacts. Mar Pollut Bull. 42(2):149-156. https://doi.org/10.1016/S0025-326X(00)00125-9

Costanzo SD, Udy J, Longstaff B, Jones A. 2005. Using nitrogen stable isotope ratios ($\delta^{15}N$) of macroalgae to determine the effectiveness of sewage upgrades: changes in the extent of sewage plumes over four years in Moreton Bay, Australia. Mar Pollut Bull. 51(1–4):212-217. https://doi.org/10.1016/j.marpolbul.2004.10.018

Dailer M, Knox S, Smith E, Napier M, Smith M. 2010. Using $\delta^{15}N$ values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Maui, Hawai'i, USA. Mar Pollut Bull. 60(5):655-671. https://doi.org/10.1016/j.marpolbul.2009.12.021

Dawson E. 1960. Marine Red Algae of Pacific Mexico. Allan Hancock Pacific Expeditions. Los Angeles (USA): U.C. Press. 17(1):1-409.

Derse E, Knee KL, Wankel SD, Kendall C, Berg CJ, Paytan A. 2007. Identifying sources of nitrogen to Hanalei Bay, Kauai,

utilizing the nitrogen isotope signature of macroalgae. Environ Sci Technol. 41(15):5217-5223.
https://doi.org/10.1021/es0700449

Escalante-Vargas I. 2003. Variación fenológica de la ficoflora durante un ciclo anual en playa la madera, Zihuatanejo, Guerrero [Phenological variation of phycoflora during an annual cycle on La Madera beach, Zihuatanejo, Guerrero] [BSc thesis]. [Mexico]: Universidad Nacional Autónoma de México. 199 p.

Fong P, Donohoe M, Zedler B. 1994. Nutrient concentration in the tissue of the macroalga *Enteromorpha* as a function of nutrient history: an experimental evaluation using field microcosm. Mar Ecol Prog Ser. 106:273-281.

Francescangeli F, Quijada M, Armynot du Châtelet E, Frontalini F, Trentesaux A, Billon G, Bouchet VMP. 2020. Multidisciplinary study to monitor consequences of pollution on intertidal benthic ecosystems (Hauts de France, English Channel, France): Comparison with natural areas. Mar Env Res. 160:105034.
https://doi.org/10.1016/j.marenvres.2020.105034

Fry B. 2006. Stable isotope ecology. New York: Springer. 318 p.

García E. 2004. Modificaciones al sistema de clasificación climática de Köppen [Modifications to the Köppen climate classification system]. Mexico: Universidad Nacional Autónoma de México, Instituto de Geografía. 90 p.

García O, Arias-Villalón V, Barquín Serrano R, Ontiveros-Mendoza M, Ortega-Saldana O. 2018. La percepción de los impactos del turismo en la comunidad de Ixtapa-Zihuatanejo, México, desde el modelo de mapas causales [Perception of tourism impacts in the Ixtapa-Zihuatanejo community, Mexico, from the causal maps model]. Rio Grande del Sur (Brazil): Universidad de Caxias do Sul. 10(3):441-463.
https://doi.org/10.18226/21789061.v10i3p441

Gartner A, Lavery P, Smit J. 2002. Use of $\delta^{15}N$ signatures of different functional forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage dispersal. Mar Ecol Prog Ser. 235:63-73.

[GMZ] Gobierno Municipal de Zihuatanejo. 2022. Plan municipal de desarrollo 2021-2024 Zihuatanejo De Azueta [Municipal development plan 2021-2024 Zihuatanejo de Azueta]: GMZ; [accessed 2022 Feb 7]. https://zihuatanejodeazueta.gob.mx/plan-de-desarrollo-municipal.

Google. 2021a. Bahía de Zihuatanejo (17°38′12.5″ N, 101°33′03.1″ W) [map]: Google; [accessed 2021 Sep 5]. https://maps.app.goo.gl/rwK2BphbxoYGF46r7.

Google. 2021b. Playa Majahua, Zihuatanejo (17°38′15.9″ N, 101°34′39.2″ W) [map]: Google; [accessed 2021 Sep 5]. https://maps.app.goo.gl/2orMHvJEC5UzWg3FA.

Granger J, Sigman M, Needoba A, Harrison J. 2004. Coupled nitrogen and oxygen isotope fractionation of nitrate during assimilation by cultures of marine phytoplankton. Limnol Oceanogr. 49(5):1763-1773.
https://doi.org/10.4319/lo.2004.49.5.1763

Guerrero R, Berlanga M. 2000. Isótopos estables: fundamento y aplicaciones [Stable isotopes: basis and applications]. Act Soc Esp Micro. 30:17-23.

Heaton E. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chem Geol. 59:87-102.

[IMTA] Instituto Mexicano de Tecnología del Agua. 2010. Estudio de Clasificación de la Bahía de Ixtapa-Zihuatanejo, Gro. [Ixtapa-Zihuatanejo Bay Classification Study, Gro.]. Guerrero (Mexico): IMTA. Technical Report: FON-CNA-2004-02-016.

Inda T, Gómez S. 2015. Los centros integralmente planeados (CIP'S) en México = The integrally planed centers (IPC's) in Mexico. Rev Latino-Am Turismologia. 1(1):36-53.

[INEGI] Instituto Nacional de Estadística y Geografía. 2009. Compendio de información geográfica municipal de los Estados Unidos Mexicanos, José Azueta, Guerrero [Municipal Geographic Information Report of the United Mexican States, José Azueta, Guerrero]: INEGI; [accessed 2022 Dec]. http://www3.inegi.org.mx/contenidos/app/mexicocifras/datos_geograficos/12/12038.pdf.

[INEGI] Instituto Nacional de Estadística y Geografía. 2020. Panorama sociodemográfico de Guerrero: Censo de Población y Vivienda 2020 [Sociodemographic panorama of Guerrero: Population and Housing Census 2020]: INEGI; [accessed 2022 Dec]. https://www.inegi.org.mx/contenidos/productos/prod_serv/contenidos/espanol/bvinegi/productos/nueva_estruc/702825197858.pdf.

Instituto de Ingeniería. 2011. Problemática Encontrada en Playa las Gatas e Isla Ixtapa en el Municipio de Zihuatanejo de Azueta, Estado de Guerrero = Problems Found in Playa las Gatas and Isla Ixtapa in the Municipality of Zihuatanejo de Azueta, State of Guerrero. Mexico City: UNAM. 86 p.

Izurieta J, Saldaña P, Inclan L, Sánchez J, Ordoñez A, Ruíz L, Mejía T. 2014. Contaminación en la bahía de Zihuatanejo. In: Botello A V, Páez-Osuna F, Méndez-Rodríguez L, Betancourt-Lozano M, Álvarez-Borrego S, Lara-Lara R (eds.), Pacífico Mexicano. Contaminación e Impacto Ambiental: Diagnóstico y Tendencias. Campeche (Mexico): UAC, UNAM-ICMYL, CIAD-Mazatlán, CIBNOR, CICESE. p. 751-788.

Kendall C. 1998. Tracing nitrogen sources and cycling in catchments. Isotope tracers in catchment hydrology. Amsterdam (the Netherlands): Elsevier. p. 519-576.
https://doi.org/10.1016/B978-0-444-81546-0.50023-9

Kessler S. 2006. The circulation of the eastern tropical Pacific: A review. Prog Oceanogr. 69(2–4):181-217.
https://doi.org/10.1016/j.pocean.2006.03.009

Kim S, Park SR, Kang YH, Kim GY, Lee KS, Lee HJ, Won NI, Kil HJ. 2014. Usefulness of tissue nitrogen content and macroalgal community structure as indicators of water eutrophication. J Appl Phycol. 2014(26):1149-1158.
https://doi.org/10.1007/s10811-013-0194-1

Kowalewski M, Wittmer JM, Dexter TA, Amorosi A, Scarponi D. 2015. Differential responses of marine communities to natural and anthropogenic changes. Proc Royal Soc B. 282:1803.
https://doi.org/10.1098/rspb.2014.2990

Lapointe BE, Barile PJ, Matzie WR. 2004. Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: discrimination of local versus regional nitrogen sources. J Exp Mar Biol Ecol. 308(1):23-58.
https://doi.org/10.1016/j.jembe.2004.01.019

Lapointe BE, Herren LW, Debortoli DD, Vogel MA. 2015. Evidence of sewage-driven eutrophication and harmful algal blooms in Florida's Indian River Lagoon. Harmful Algae. 43:82-102.
https://doi.org/10.1016/j.hal.2015.01.004

Lemesle S, Erraud A, Mussio I, Rusig M, Claquin P. 2016. Dynamics of $\delta^{15}N$ isotopic signatures of different intertidal macroalgal species: assessment of bioindicators of N sources in coastal areas. Mar Pollut Bull. 110(1):470-483.
https://doi.org/10.1016/j.marpolbul.2016.06.006

Lemesle S, Mussio I, Rusig AM, Menet-Nédélec F, Claquin P. 2015. Impact of seaweed beachings on dynamics of $\delta^{15}N$ isotopic signatures in marine macroalgae. Mar Pollut Bull. 97(1–2):241-254.
https://doi.org/10.1016/j.marpolbul.2015.06.010

Lin H, Wu C, Kao J, Kao Y, Meng J. 2007. Mapping anthropogenic nitrogen through point sources in coral reefs using $\delta^{15}N$ in macroalgae. Mar Ecol Prog Ser. 335:95-109.

Lin T, Fong P. 2008. Macroalgal bioindicators (growth, tissue N, $\delta^{15}N$) detect nutrient enrichment from shrimp farm effluent entering Opunohu Bay, Moorea, French Polynesia. Mar Pollut Bull. 56(2):245-249. https://doi.org/10.1016/j.marpolbul.2007.09.031

López N, Candelaria C, Ramírez-García P, Rodríguez D. 2017. Structure and temporal dynamic of tropical turf-forming macroalgal assemblages of the western coast of Mexico. Lat Am J Aquat Res. 45(2):329-340. https://doi.org/10.3856/vol45-issue2-fulltext-9

López N, León-Tejera H, González-Reséndiz L, Candelaria C, Ramírez-García P, Rodríguez D. 2022. Macroalgas arrecifales del Pacífico Centro-Sur de México: Estado del arte [Central-South Pacific reef macroalgae: State of the art]. Hidrobiológica. 32(3):285-293.

Martins CDL, Arantes N, Faveri C, Batista MB, Oliveira EC, Pagliosa PR, Fonseca AL, Nunes JMC, Chow F, Pereira SB, et al. 2012. The impact of coastal urbanization on the structure of phytobenthic communities in southern Brazil. Mar Pollut Bull. 64(4):772-778. https://doi.org/10.1016/j.marpolbul.2012.01.031

Mateo-Cid E, Mendoza-González C. 1997. Nuevos registros de algas marinas para Oaxaca, México [New algae records for Oaxaca, Mexico]. Polibotanica. 4:54-74.

Mateo-Cid E, Mendoza-González C. 2012. Algas marinas bentónicas de la costa noroccidental de Guerrero, México [Benthic seaweed from the northwest coast of Guerrero, Mexico]. Rev Mex Biodivers. 83(4):905-928. https://doi.org/10.7550/rmb.28104

McClelland JW, Valiela I, Michener RH. 1997. Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. Limnol Oceanogr. 42(5):930-937. https://doi.org/10.4319/lo.1997.42.5.0930

Morales R, Vélez H, Mejía A, Ramírez I, Izurieta J, Saldaña P. 2008. Hidrodinámica de la Bahía de Zihuatanejo, México [Hydrodynamic of the Bay of Zihuatanejo, Mexico]. XXIII Congreso Latinoamericano de Hidráulica Cartagena de Indias Colombia, Septiembre 2008. Colombia. 15 p.

Nava H, Ramírez- Herrera T. 2011. Government conservation policies on Mexican coastal areas: is "top-down" management working. Rev Biol Trop. 59(4):1487-1501.

Nava H, Ramírez-Herrera M, Figueroa-Camacho G, Villegas-Sanchez M. 2014. Habitat characteristics and environmental factors related to boring sponge assemblages on coral reefs near populated coastal areas on the Mexican Eastern Pacific coast. Mar Biodivers. 44:45-54.

Nava H, López N, Ramírez-García P, Garibay-Valladolid E. 2021. Contrasting effects of the El Niño 2015–16 event on coral reefs from the central pacific coast of Mexico. Mar Ecol. 42(2):e12630. https://doi.org/10.1111/maec.12630

Newman EA. 2019. Disturbance Ecology in the Anthropocene. Front Ecol Evol. 7:147. https://doi.org/10.3389/fevo.2019.00147

Ochoa-Izaguirre MJ, Soto-Jiménez MF. 2013. Evaluation of nitrogen sources in the lagoon system of Urias, Gulf of California, based on stable isotopes in macroalgae = Evaluación de las fuentes de nitrógeno en el sistema lagunar de Urías, golfo de California, mediante el uso de isótopos estables en macroalgas. Cienc Mar. 39(4):413-430. http://doi.org/10.7773/cm.v39i4.2285

Ochoa-Izaguirre MJ, Soto-Jiménez MF. 2015. Variability in nitrogen stable isotope ratios of macroalgae: consequences for the identification of nitrogen sources. J Phycol. 51(1):46-65. https://doi.org/10.1111/jpy.12250

Ochoa-Izaguirre M, Voltolina D, Victorino-Sánchez G. 2017. Nitrogen, Phosphorus and N: P ratio in macroalgae of two Lagoon Systems of NW Mexico. Turkish J Fish Aquat Sci. 17(5):911-919.

Orlando-Bonaca M, Lipej L, Orfanidis S. 2008. Benthic macrophytes as a tool for delineating, monitoring and assessing ecological status: The case of Slovenian coastal waters. Mar Pollut Bull. 56(4):666-676. https://doi.org/10.1016/j.marpolbul.2007.12.018

Pedersen F, Borum J. 1996. Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. Mar Ecol Prog Ser. 142:261-272.

Peterson BJ, Fry B. 1987. Stable isotopes in ecosystem studies. Ann Rev Ecol Syst. 18(1):293-320. https://doi.org/10.1146/annurev.es.18.110187.001453

Piñón-Gimate A, Soto-Jiménez MF, Ochoa-Izaguirre MJ, García-Pagés E, Páez-Osuna, F. 2009. Macroalgae blooms and $\delta^{15}N$ in subtropical coastal lagoons from the Southeastern Gulf of California: Discrimination among agricultural, shrimp farm and sewage effluents. Mar Pollut Bull. 58(8):1144-1151. https://doi.org/10.1016/j.marpolbul.2009.04.004

Piñón-Gimate A, Espinosa-Andrade N, Sánchez A, Casas-Valdez M. 2017. Nitrogen isotopic characterization of macroalgae blooms from different sites within a subtropical bay in the Gulf of California. Mar Pollut Bull. 116(1–2):130-136. https://doi.org/10.1016/j.marpolbul.2016.12.075

Planea Tropical S. de R.L. de C.V. 2015. Actualización plan director de desarrollo urbano Zihuatanejo-Ixtapa 2015-2030 [Update Master Plan for Urban Development Zihuatanejo-Ixtapa 2015-2030]. Zihuatanejo (Mexico): Gobierno de Zihuatanejo. Urban Development Plan. 203 p.

Portugal AB, Lopes-Carvalho F, Oliveira SM, Horta PA, Castro-Nunes JM. 2017. Structure of macroalgal communities on tropical rocky shores inside and outside a marine protected area. Mar Environ Res. 130:150-156. https://doi.org/10.1016/j.marenvres.2017.07.019

Ramírez-López P. 2020. Diagnóstico de la problemática de la contaminación del agua en el estero de las Salinas, Zihuatanejo, Guerrero [Diagnosis of the problem of water pollution in the Salinas estuary, Zihuatanejo, Guerrero]. Morelia (Michoacán, Mexico): Universidad Nacional Autónoma de México. 152 p.

Rodríguez E. 2016. Estacionalidad de la hidrografía y la circulación en el Pacífico tropical frente a México y áreas adyacentes con énfasis en la Corriente Costera Mexicana [Seasonality of hydrography and circulation in the tropical Pacific off Mexico and adjacent areas with emphasis on the Mexican Coastal Current]. Ensenada (Baja California, Mexico): CICESE. 98 p.

Rogers K. 2003. Stable carbon and nitrogen isotope signatures indicate recovery of marine biota from sewage pollution at Moa Point, New Zealand. Mar Pollut Bull. 46(7):821-827. https://doi.org/10.1016/S0025-326X(03)00097-3

Sandoval-Coronado BA, López-Gómez NA. 2021. Patrones de abundancia y distribución de macroalgas del arrecife coralino de Ixtapa, Zihuatanejo, Guerrero [Macroalgae abundance and distribution patterns of the coral reef of Isla Ixtapa, Zihuatanejo, Guerrero]. Cien Mar. 25(73):57-71. https://doi.org/10.59673/cym.v25i73

[SECTUR] Secretaría de Turismo. 2014. Estudio de Competitividad Turística del Destino Ixtapa-Zihuatanejo, Gro [Tourist Competitiveness Study of Ixtapa-Zihuatanejo, Gro]. Chilpancingo de los Bravo (Mexico): Universidad de Guerrero, SECTUR. Technical Report. 487 p.

[SEMAR] Secretaría de Marina. 2003. Estudio exploratorio de las condiciones oceanográficas en el espigón de Puerto Mío en la Bahía de Zihuatanejo, Gro. Manzanillo [Exploratory study of the oceanographic conditions in the breakwater of Puerto Mío at the Bay of Zihuatanejo, Gro. Manzanillo]. Colima (Mexico): Instituto Oceanográfico del Pacífico. Technical Report. 69 p.

[SEMAR] Secretaría de Marina. 2021. Información diversa, Nivel de referencia, Planos de mareas referidos al nivel de bajamar media inferior [Diverse information, Reference Level, Tidal planes referring to the lower mean low tide level]: SEMAR; [accessed 2022 Apr 19]. https://oceanografia.semar.gob.mx/telems/CROQUIS%20PACIFICO/37%20ZIHUA24_NBMI.pdf.

Senado de la República. 2015. Punto de acuerdo que exhorta al titular del ejecutivo federal a implementar un proyecto integral de rescate de la Laguna de Las Salinas, zonas aledañas y de la playa principal en Zihuatanejo de Azueta, Guerrero [Resolution urging the leader of the federal executive to implement a comprehensive rescue initiative for Laguna de Las Salinas, its surrounding areas, and the primary beach of Zihuatanejo de Azueta, Guerrero]: Senado de la Republica; [accessed 2022 Apr 19]. https://infosen.senado.gob.mx/sgsp/gaceta/62/3/2015-04-16-1/assets/documentos/DICT_Med_Amb_Laguna_Las_Salinas.pdf.

Taylor WR. 1945. Pacific Marine Algae of the Allan Hancock Expeditions to the Galapagos Islands. Allan Hancock Pacific Expeditions. 12(1):1-528.

Thornber SC, DiMilla P, Nixon SW, McKinney RA. 2008. Natural and anthropogenic nitrogen uptake by bloom-forming macroalgae. Mar Pollut Bull. 56(2):261-269. https://doi.org/10.1016/j.marpolbul.2007.10.031

Umezawa Y, Miyajima T, Yamamuro M, Kayanne H, Koike I. 2002. Fine-scale mapping of land-derived nitrogen in coral reefs by $\delta^{15}N$ in macroalgae. Limnol Oceanogr. 47(5):1405-1416. https://doi.org/10.4319/lo.2002.47.5.1405

Valiela I, McClelland J, Hauxwell J, Behr P, Hersh D, Foreman K. 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnol Oceanogr. 42(5):1105-1118.

Veiga P, Rubal M, Vieira R, Arenas F, Sousa-Pinto I. 2013. Spatial variability in intertidal macroalgal assemblages on the North Portuguese coast: consistence between species and functional group approaches. Helgol Mar Res. 67(1):191-201. https://doi.org/10.1007/s10152-012-0315-2

Viana IG, Bode A. 2013. Stable nitrogen isotopes in coastal macroalgae: Geographic and anthropogenic variability. Sci Total Environ. 443:887-895. https://doi.org/10.1016/j.scitotenv.2012.11.065

Wang Y, Liu D, Richard P, Di B. 2016. Selection of effective macroalgal species and tracing nitrogen sources on the different part of Yantai coast, China indicated by macroalgal $\delta^{15}N$ values. Sci Total Environ. 542(Part A):306-314. https://doi.org/10.1016/j.scitotenv.2015.10.059

Worm B, Lenihan H. 2013. Threats to Marine Ecosystems. Marine Community Ecology and Conservation. Sunderland (USA): Sinauer Associates, Inc. 476 p.

Zhai T, Wang J, Fang Y, Qin Y, Huang L, Chen Y. 2020. Assessing ecological risks caused by human activities in rapid urbanization coastal areas: Towards an integrated approach to determining key areas of terrestrial-oceanic ecosystems preservation and restoration. Sci Total Environ. 708:135153. https://doi.org/10.1016/j.scitotenv.2019.135153