

Indicators of nutrient enrichment in coastal ecosystems of the northern Mexican Caribbean



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ABSTRACT

We measured dissolved inorganic nitrogen ($\text{DIN} = \text{NH}_4^+ + \text{NO}_x^- [\text{NO}_3^- + \text{NO}_2^-]$), soluble reactive phosphorous (SRP), and ecological indicators (chlorophyll-*a* concentration, coverage of submerged aquatic vegetation, and %N, C:N and $\delta^{15}\text{N}$ values in the seagrass *Thalassia testudinum*) to evaluate the effects of nutrient enrichment at three shallow coastal sites on the northern part of the Mexican Caribbean (Puerto Morelos, Puerto Juárez, and Isla Blanca) in two contrasting (cold-front or dry) seasons of the year. High nutrient concentrations prevailed in both seasons but were significantly higher during the cold-front season, probably as a result of sediment resuspension and groundwater discharges. The highest $\delta^{15}\text{N}$ values were recorded in Puerto Juárez in both seasons (cold-front, 7.58‰; dry, 7.35‰). The highest macroalgae coverage and NH_4^+ concentration were also recorded in this site. These results, together with the high correlation between $\delta^{15}\text{N}$ and NH_4^+ ($r = 0.94$) and DIN ($r = 0.88$) concentrations, suggest wastewater contamination in Puerto Juárez due to its proximity to the city of Cancún. In contrast, the lowest mean $\delta^{15}\text{N}$ (-1.35 ‰) and nutrient enrichment effects (i.e., highest coverage of *T. testudinum* and lowest macroalgae coverage) were observed in Isla Blanca, a site with no urban settlements. The low $\delta^{15}\text{N}$ values recorded along the Puerto Morelos coastal transect suggest that a significant fraction of the nitrogen assimilated by *T. testudinum* may come from either exudates or the decomposition of drifting *Sargassum* stranded in the coast, as the $\delta^{15}\text{N}$ values recorded in *Sargassum* (-0.64 ‰) were similar to those found in the seagrass (-0.83 ‰). The correlation between the DIN:SRP ratio and chlorophyll-*a* concentration in the study area may indicate N-limitation of phytoplankton growth, despite the high DIN concentrations. Beyond the differences observed between sites – which may indicate site-specific, long-term nutrient inputs – the indicators examined evidence environmental deterioration of the study area, likely due to a shift in the nutrient regime (away from oligotrophic), particularly in Puerto Juárez, the site with the heaviest load of urban and tourism activities.

1. Introduction

Eutrophication is one of the major threats to coastal ecosystems worldwide (Nixon et al., 2001; Nixon, 2009). The effects of eutrophication are more deleterious in oligotrophic ecosystems such as coral reefs and seagrass meadows, two of the most valuable ecosystems in the world (Orth et al., 2006; Duarte et al., 2008), as these are particularly vulnerable to excessive nitrogen and phosphorus inputs (Lapointe and Clark, 1992; Lapointe and Matzie, 1996; Lapointe, 1997;

Deegan et al., 2002; Lapointe et al., 2004; Haynes et al., 2007; Bell et al., 2014). Urban development and tourism activities in coastal areas are associated with their degradation and the ensuing loss of the highly valuable environmental services they provide (Costanza, 1999; Martínez et al., 2007).

The Caribbean coast of the Yucatan Peninsula has experienced an accelerated population growth since the early 1970s, when the population of the State of Quintana Roo was about 88,000 inhabitants, reaching over 1.5 million inhabitants in 2015 (INEGI, 2017). Such

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growth has been closely linked to the tourism industry, the main economic activity in the region. For example, almost 17 million tourists visited the Mexican Caribbean in 2017 (SEDETUR, 2017). Tourism development has expanded throughout the Quintana Roo coast, from the Costa Maya in the south, the Riviera Maya in the central zone, up to the Cancún-Isla Blanca corridor in the north, causing various negative impacts on coastal ecosystems in the region (Murray, 2007; Mc Coy, 2016). Contrasting with the burgeoning development of the tourism industry, municipal wastewater management systems are inadequate. About 31% of the local population uses septic tanks for sewage disposal (INEGI, 2017), with the inherent risk of groundwater pollution by leakage or overflow (Hernández-Terrones et al., 2011, 2015). Such contingencies would increase the input of organic matter and nutrients into aquifer and coastal ecosystems.

The dissolution features of the karst landscape of the Yucatan Peninsula favor rapid water infiltration and transport of groundwater to the coast. These processes favor the formation of both diffused submarine groundwater discharges (SGD) and point-source submarine springs, locally known as “ojos de agua”, which are abundant along the coast (Bauer-Gottwein et al., 2011; Null et al., 2014).

High concentrations of dissolved inorganic nitrogen (DIN) and soluble reactive phosphorous (SRP) have been recorded at various sites along the coast (Carruthers et al., 2005; Mutchler et al., 2007, 2010; Hernández-Terrones et al., 2011, 2015; Null et al., 2014; Camacho-Cruz et al., 2019). Elevated nutrient concentrations seem to have negative impacts on coastal communities. Based on the correlation between *Enterococcus* abundance and $\delta^{15}\text{N}$ values in *Gorgonia ventalina* tissues, Baker et al. (2010) showed that nutrient input to coral reefs is related to sewage pollution from anthropogenic sources. Changes in the trophic status and structure of autotrophic communities have also been related to nutrient-enriched SGD. For example, Mutchler et al. (2007); Mutchler et al. (2010) reported thick mats of fleshy macroalgae in a coastal lagoon, likely associated with sewage inputs. An increase in the coverage of fleshy macroalgae, likely associated with SGD, has been reported for seagrass meadows in the Puerto Morelos reef lagoon (Rodríguez-Martínez et al., 2010). Martínez-Rendis et al. (2015) showed evidence of a phase shift (i.e., increase in the coverage of macroalgae relative to coral cover) along the southern coast of Quintana Roo. Recent studies reported high %N values in tissues of *Thalassia testudinum* and macroalgae, likely associated with nutrient loads in coastal systems in the region (Carruthers et al., 2005; Mutchler et al., 2007, 2010; Sánchez et al., 2013). The $\delta^{15}\text{N}$ values found in *T. testudinum* and fleshy macroalgae suggest that these nutrient inputs might be related to sewage pollution (Carruthers et al., 2005; Mutchler et al., 2007, 2010; Sánchez et al., 2013; Camacho-Cruz et al., 2019).

These findings are clear signs of incipient eutrophication caused by nutrient enrichment in some areas of the Mexican Caribbean coast. However, seasonal variations in water quality and ecological indicators of nutrient enrichment on the northern coast of Quintana Roo have not yet been characterized. In this study, we evaluated ecological indicators (%N, C:N and $\delta^{15}\text{N}$ in *T. testudinum*, coverage of submerged aquatic vegetation and chlorophyll-*a* concentration) and nutrient concentrations (SRP and $\text{DIN} = \text{NH}_4^+ + \text{NO}_x [= \text{NO}_3^- + \text{NO}_2^-]$) in marine water at three locations along the northern coast of Quintana Roo, with the aim of gathering an updated diagnosis of the environmental status of these coastal ecosystems.

2. Materials and methods

2.1. Study area

The study was conducted at three sites located on the northern Mexican Caribbean coast (Fig. 1). Isla Blanca (IB) is located at the northern end of the continental portion of the municipality of Isla Mujeres (19,495 inhabitants in 2015; INEGI, 2017), where only scarce urban development and tourism activities take place. IB is near the

mouth of the Chacmochuch lagoon, 25 km north of the city of Cancún. Puerto Juárez (PJ) is located some 25 km south of IB, on the northern part of Cancún city, in the municipality of Benito Juárez. There were 743,626 inhabitants in this municipality in 2015, i.e., half of the total population of the state of Quintana Roo (INEGI, 2017). The rapid population growth in the municipality has been driven by its tourism-based economic development, which has also entailed environmental impacts such as extensive loss of mangrove coverage and beach erosion (Mc Coy, 2016). Puerto Morelos (PM) is located some 30 km south of Cancún city and is the newest municipality of the State of Quintana Roo. PM had 375,502 inhabitants in 2015 (INEGI, 2017), and a low-to-intermediate socioeconomic development level (Sánchez et al., 2013). This municipality adjoins the Puerto Morelos Reef Marine National Park. The main recreational activities carried out in the 2-to-8 m deep reef lagoon include sport fishing, scuba diving and snorkeling tours in the barrier reef (Hernández-Terrones et al., 2011).

The regional climate encompasses three seasons: (1) the cold-front season lasts from November to February and is rainy (321 mm average precipitation) and windy, with sporadic high-speed (up to 90 km/h) winds locally known as *nortes*; (2) the dry season lasts from March to May with 124 mm mean precipitation; and (3) the rainy season, which lasts from June to October with 656 mm mean precipitation (Schmitter-Soto et al., 2002; Hernández-Terrones et al., 2011). Surface runoff is almost inexistent due to the karst landscape of the Yucatan Peninsula. Groundwater discharges into coastal environments are the main source of nutrients (Null et al., 2014). The SGD flux depends on precipitation and, therefore, varies markedly between seasons. Thus, we evaluated indicators of nutrient enrichment at the three study sites in two contrasting seasons, the cold-front and the dry seasons of the year.

2.2. Sampling design

Sampling at IB and PM consisted of six 50 m transects running parallel to the coastline in four zones (Fig. 1): three parallel transects at 10 m, 100 m, and 200 m from the coastline in Zone A (nearshore), and one transect each in Zones B (middle-shore), C (offshore), and D (reef). The distance between transects B, C, and D and the coastline varied between sites. Sampling at the PJ site only encompassed Zones A and B. This sampling scheme was set to detect gradients in the variables measured (nutrient and chlorophyll-*a* concentrations, coverage of submerged aquatic vegetation, and %N, C:N and $\delta^{15}\text{N}$ values in *T. testudinum*) that might be related to groundwater discharges from the coast. Thus, we expected the nutrient enrichment indicators to attain higher values nearshore and decrease farther offshore (Lapointe and Clark, 1992; Arévalo et al., 2007; Herbeck et al., 2014). Samples were taken in February (cold-front season) and May (dry season) 2018.

2.3. Nutrient concentrations in porewater and the water column

Water column concentrations of nitrate + nitrite (NO_x), ammonium (NH_4^+), and soluble reactive phosphorus (SRP) were measured at each sampling site. Triplicate samples were collected in all cases; 60 mL of water was collected with a clean syringe about 10 cm above the seagrass canopy. Samples of sediment porewater (sediment interstitial water) were collected from transects A, B, and C (except for the 100 m transect in zone A; Fig. 1) using a 60 mL plastic syringe fitted with a plastic tip to extract porewater. Water column and sediment porewater samples were immediately filtered through nitrocellulose Millipore® filters 0.22 μm pore size. Filtered samples were stored in acid-washed HDPE bottles, then placed into a cooler (4 °C) for transportation, frozen at -40 °C upon arrival to the laboratory, and kept frozen until analysis. Nutrient concentrations were measured using colorimetric methods (Strickland and Parsons, 1972; Grasshoff et al., 1983) with a Skalar San ++ autoanalyzer. Detection limits for NO_x , NH_4^+ , and SRP were 0.01 μM . These analyses were carried out in the Water Quality Laboratory at Centro Universitario de Investigaciones Oceanológicas,

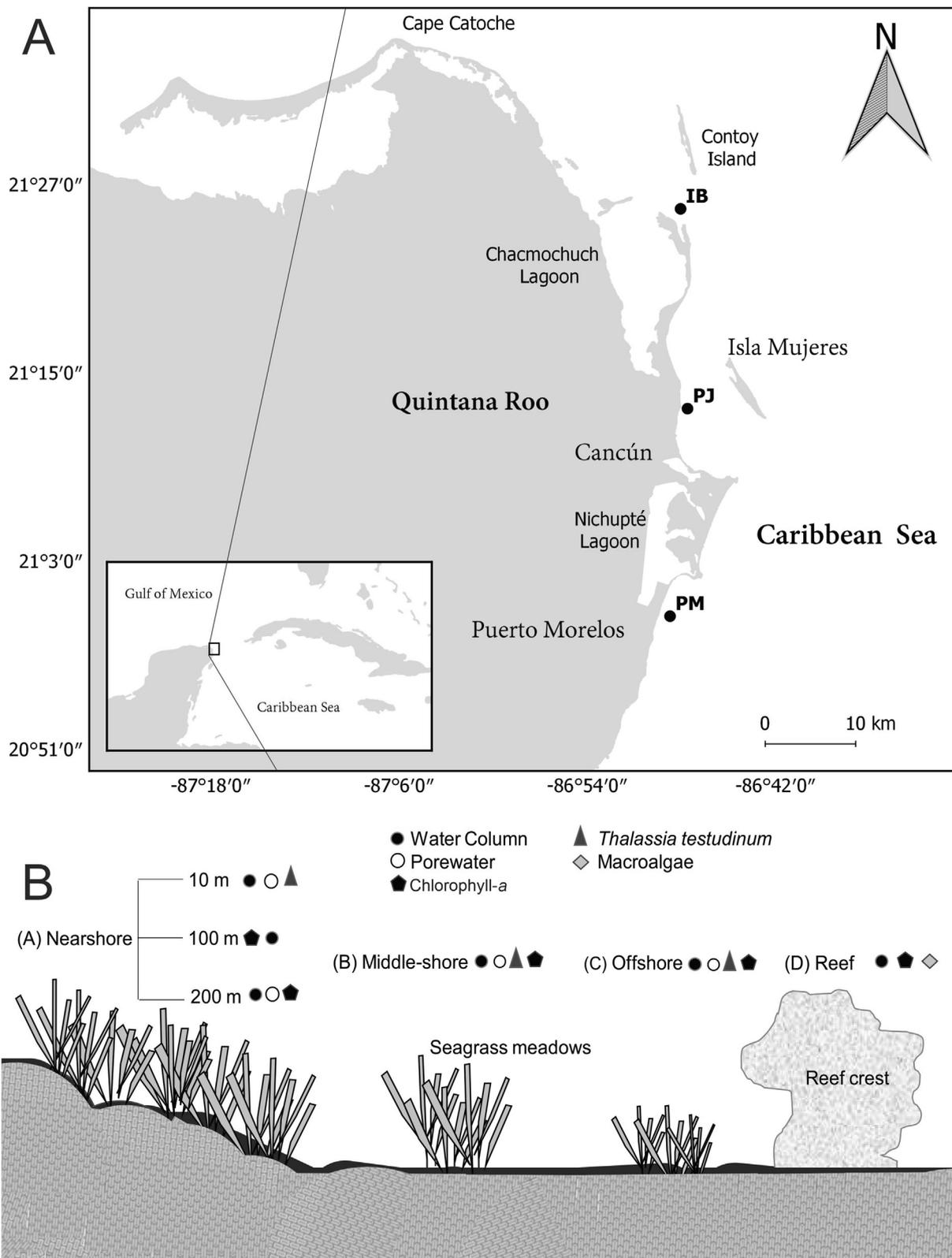


Fig. 1. (A) Location of the three study sites on the northern Mexican Caribbean coast: Isla Blanca (IB), Puerto Morelos (PM) and Puerto Juárez (PJ). (B) Schematic sampling design. Symbols represent the samples taken at each site. Samples were taken along three nearshore transects (Zone A), one middle-shore transect (Zone B), and one offshore transect (Zone C) in seagrass meadows, and along one transect on the reef barrier (Zone D). All samples were collected in triplicate.

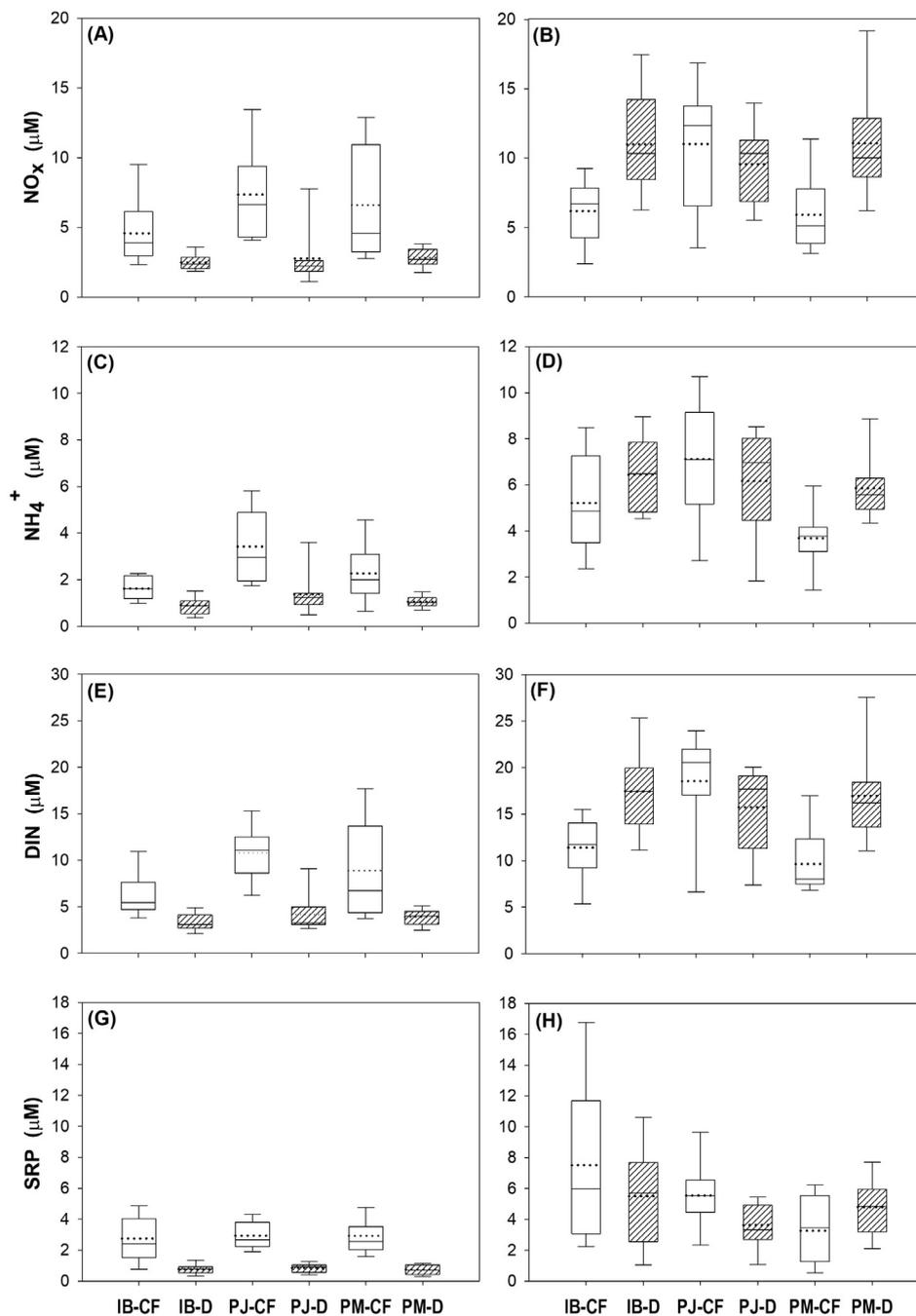


Fig. 2. Concentrations of NO_x, NH₄⁺, DIN, and SRP in the water column (A, C, E and G), and in porewater (B, D, F, and H) during the cold-front (CF) and dry (D) seasons of the year at Isla Blanca (IB), Puerto Juárez (PJ), and Puerto Morelos (PM). The horizontal lines inside each box denote the median (solid line) and the mean (dotted line) of the variable.

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2.4. Chlorophyll-a concentration

Triplicate samples of surface water for chlorophyll-a analysis were collected from transects A, B, C, and D (except for the 10 m transect in Zone A), using opaque 1 L plastic bottles. Samples were placed into a cooler (4 °C) for transport. In the laboratory, samples were filtered through GF/F Whatman® filters under low vacuum pressure (< 5 mm Hg); the filters were packed in aluminum foil and frozen in liquid nitrogen until analysis. To extract pigments, the GF/F filters were placed into conic-bottom 2 mL tubes containing 0.1 mm diameter zirconia/glass beads and 1.5 mL of 100% acetone (HPLC grade), ground with a

mini bead-beater (BioSpec Products Inc), and kept at -20 °C for 24 h. Then, samples were centrifuged and the supernatant transferred to amber vials for HPLC analysis using the method described by van Heukelem and Thomas (2001); the detection limit of this method is ~ 0.07 μg L⁻¹ of the injected extract. The analyses were carried out in the FICOTOX Laboratory at Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California (CICESE).

2.5. Coverage of *Thalassia testudinum* and macroalgae

Twelve 0.25 m² quadrats were randomly placed along each transect in Zones A, B, C, and D (Fig. 1) at IB and PM, and along Zones A and B at PJ. The percent coverage of *T. testudinum* and macroalgae in each

quadrat were estimated visually, and photographs were captured to corroborate field estimates.

2.6. N content and $\delta^{15}N$ in tissues of *Thalassia testudinum* and macroalgae

Five *T. testudinum* shoots were randomly taken in triplicate along each of transects A (10 m), B, and C in IB and PM; and along transects A (10 m) and B in PJ, keeping a distance of at least 10 m between samples. Macroalgae specimens were collected from the reef transects (Zone D); specimens of *Dictyota* sp. were collected at IB and specimens of *Styopodium zonale* at PM. Samples of drifting *Sargassum* were also collected nearshore (200 m) at the PM site. The macroalgae and *T. testudinum* leaves were placed in plastic bags, stored in a cooler for transport, and frozen upon arrival at the laboratory. Vegetal samples were rinsed with Milli-Q water for initial removal of epiphytes and sediment, submerged into a 10% HCl solution for 60 s, and gently scrubbed with a brush and Milli-Q water for the complete removal of epiphytes and sediment. Non-senescent, entire leaves with no evident herbivory damage were selected for analysis. Clean samples were dried (at 60 °C for 48 h), ground in a porcelain mortar, and stored in 1.5 mL Eppendorf tubes until analysis. Subsamples of approximately 3 mg were placed in tin capsules for %C, %N, C:N and $\delta^{15}N$ analyses using a CE NC2500 elemental analyzer, coupled to a Thermo Finnigan Delta plus XP iSRM spectrometer, via Thermo-Finnigan Conflo III. These analyses were carried out in the Stable Isotope Laboratory at University of California, Santa Cruz.

2.7. Data analyses

Non-parametric Kruskal-Wallis H tests were used to test for significant ($\alpha = 0.05$) differences in nutrient enrichment indicators between sites, separately for each season; *post hoc* multiple comparisons were carried out using the Dunn's test. Non-parametric Mann-Whitney U tests were used to test for significant ($\alpha = 0.05$) differences between seasons (cold-front vs. dry), separately for each site. The Spearman's rank correlation coefficient ($\alpha = 0.05$) was used to examine the correlation between variables.

3. Results

3.1. Nutrients

Nutrient concentrations were highly variable in the three study sites. No clear gradients could be discerned either in the water column or in porewater. However, we found a marked seasonal variation in

NO_x , NH_4^+ , SRP, and DIN concentrations in the water column, which were significantly higher during the cold-front season (Fig. 2, Table 1). Concentrations of nitrogen species and SRP in the cold-front season were almost two-fold and three-fold higher, respectively, than those in the dry season. The pronounced decrease in SRP concentration during the dry season was also associated with a higher DIN:SRP ratio.

Significant differences in nutrient concentrations between sites were found only in the cold-front season: the PJ site had higher NH_4^+ concentration in the water column than IB ($p < 0.001$) and PM ($p < 0.05$), and its NO_x ($p < 0.01$) and DIN ($p < 0.001$) values were significantly higher than those recorded at the IB site (Table 1).

At IB and PM, NO_x and DIN porewater concentrations were significantly higher in the dry than in the cold-front season. The nutrients in the dry season, particularly NH_4^+ , DIN and SRP, were significantly higher in sediment porewater than in the water column (Table 1).

3.2. Chlorophyll-a

Mean chlorophyll-a concentrations were low ($\leq 0.33 \mu g L^{-1}$) at the three sites in both seasons. Chlorophyll-a concentrations were somewhat higher in the dry season but only attained statistical significance in the PM site. The highest mean chlorophyll-a concentrations were recorded at the IB site in both seasons (Table 1). In addition, chlorophyll-a concentration was positively correlated with the DIN:SRP ratio in the study area (Fig. 3B).

3.3. Coverage of *Thalassia testudinum* and Macroalgae

The coverage of *T. testudinum* did not change significantly between seasons (Table 2). The highest coverage of *T. testudinum* (53–65%) and the lowest coverage of fleshy (2–5%) and calcareous macroalgae (0.2–4%) were found at the IB site in both seasons of the year. By contrast, the highest coverage of calcareous (~13%) and fleshy macroalgae (~25%) were found at the PJ site. In the seagrass meadows transects (transects A to C), *Laurencia cf. poitei* and *Caulerpa* sp were the dominant fleshy macroalgae species, whereas *Penicillus capitatus* and *Halimeda opuntia* were the most important calcareous algae species. The coverage of *T. testudinum* was negatively correlated ($r = -0.80$, $p < 0.001$) with the coverage of fleshy macroalgae in the cold-front season, and with the coverage of fleshy ($r = -0.76$, $p < 0.001$) and calcareous ($r = -0.71$, $p < 0.001$) macroalgae during the dry season in the PJ site.

Macroalgae coverage in the reef transects at PM and IB ranged between 50 and 60% and did not vary significantly between seasons.

Table 1

Mean nutrient concentrations (μM) in the water column and sediment porewater, and mean chlorophyll-a concentration ($\mu g L^{-1}$) in the water column, during the cold-front (CF) and dry (D) seasons of the year at Isla Blanca (IB), Puerto Juárez (PJ), and Puerto Morelos (PM). Standard errors are given in parenthesis. Figures in bold case identify the higher value in those cases where significant differences between seasons were found.

Site	n	NO_x	NH_4^+	SRP	DIN	DIN:SRP	Chl-a
Water Column							
IB _{CF}	18	4.57 (0.54)**	1.62 (0.11)*	2.74 (0.4)*	6.19 (0.54)*	4.02 (1.32)	0.23 (0.02)
IB _D	18	2.48 (0.14)	0.9 (0.12)	0.76 (0.09)	3.38 (0.66)	6.42 (1.61)**	0.33 (0.04)
PJ _{CF}	12	7.35 (0.94)*	3.42 (0.44)*	2.92 (0.28)*	10.78 (0.84)*	3.76 (0.24)	0.12 (0.019)
PJ _D	12	2.77 (0.63)	1.38 (0.29)	0.84 (0.08)	4.88 (0.93)	4.96 (0.55)	0.16 (0.01)
PM _{CF}	15	6.60 (1.06)**	2.27 (0.34)**	2.9 (0.28)*	8.87 (1.34)***	3.05 (0.37)	0.10 (0.006)
PM _D	18	2.84 (0.21)	1.05 (0.06)	0.74 (0.08)	3.89 (0.22)	6.31 (0.74)*	0.21 (0.03)*
Sediment Porewater							
IB _{CF}	12	6.19 (0.64)	5.21 (0.6)	7.51 (1.46)	11.40 (0.93)	7.6 (3.07)	
IB _D	12	11 (1.05)*	6.45 (0.45)	5.49 (0.93)	17.45 (1.33)**	6.09 (1.2)	
PJ _{CF}	12	11 (1.31)	7.12 (0.74)	5.54 (0.63)***	18.54 (1.65)	4.32 (0.62)	
PJ _D	9	9.56 (0.9)	6.17 (0.74)	3.62 (0.47)	15.74 (1.51)	9.46 (2.65)	
PM _{CF}	12	5.93 (0.8)	3.69 (0.37)	3.26 (0.61)	9.62 (1.03)	13.61 (5.03)	
PM _D	12	11.08 (1.16)**	5.86 (0.41)*	4.8 (0.53)	16.94 (1.48)**	9.62 (3.12)	

n = number of samples. For Chlorophyll-a (Chl-a), n = 15 in IB and PM and n = 9 in PJ for each season. * $p < 0.001$, ** $p < 0.01$, *** $p < 0.05$.

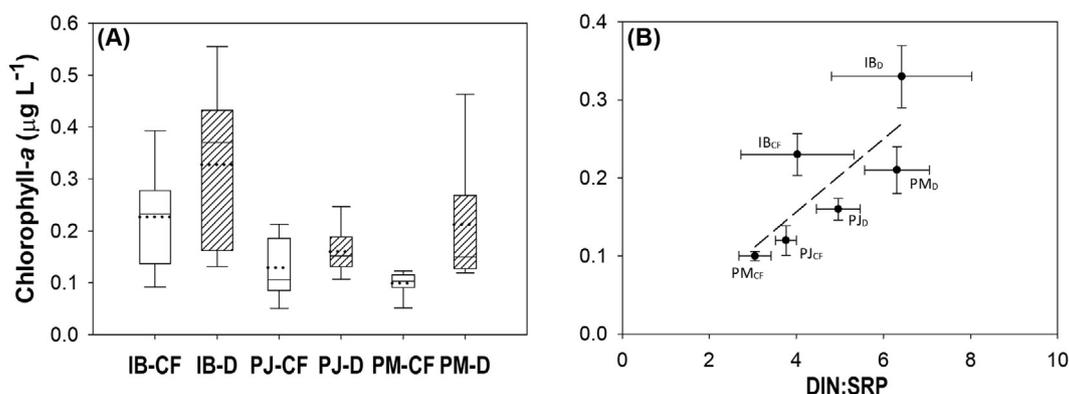


Fig. 3. Chlorophyll-*a* concentrations in the study sites in the cold-front and dry seasons (A). Correlation plot between mean DIN:SRP ratio and chlorophyll-*a* mean concentration ($r = 0.829$, $p < 0.05$) at IB, PJ, and PM during the cold-front and dry seasons (B).

3.4. N content in *Thalassia testudinum* leaves

Elemental N content in *T. testudinum* leaves did not vary significantly between seasons in the study area. However, significant between-site differences were found, with the highest %N recorded at IB in the dry season (Table 2). We found a positive correlation ($r = 0.84$, Fig. 4A) between NH_4^+ levels in the water column and %N levels at the PJ site during the cold-front season. Nitrogen contents in macroalgae varied between species. *Dictyota* sp and *Stypopodium zonale* showed higher values than drifting *Sargassum* collected near the shore (Table 3).

3.5. $\delta^{15}\text{N}$ in *Thalassia testudinum* leaf tissue and macroalgae

Overall, $\delta^{15}\text{N}$ in *T. testudinum* ranged between -4.33 and 8.25‰ , with no significant differences between seasons in any of the three sites studied. However, there were some significant differences between sites in $\delta^{15}\text{N}$ values. The highest $\delta^{15}\text{N}$ values in the cold-front season were recorded at the PJ site ($p < 0.05$), but $\delta^{15}\text{N}$ at PJ was significantly higher vs IB only in the dry season ($p < 0.05$, Table 2). Moreover, in the cold-front season, $\delta^{15}\text{N}$ values at PJ were positively correlated with %N ($r = 0.92$, Fig. 4B), NH_4^+ ($r = 0.94$, Fig. 4C), and DIN ($r = 0.88$, Fig. 4D) concentrations in the water column.

Dictyota sp. showed markedly higher $\delta^{15}\text{N}$ values (2.67‰ and 1.87‰) vs. *Stypopodium zonale* (0.07‰ and 0.28‰) in both seasons. Drifting *Sargassum* samples had negative $\delta^{15}\text{N}$ values (Table 3).

4. Discussion

4.1. Seasonal differences

The noticeable seasonal differences in nutrient levels in the water column, with higher concentrations and higher variability during the cold-front season at the three study sites, might be due to differences in

precipitation and wind energy between seasons. Strong winds (80–90 km/h) and abundant rains typically occur during the cold-front season in the study area (Herrera-Silveira, 1998; Hernández-Terrones et al., 2011). Rain leads to increased diffuse SGD of continental water along the coast. Nutrient inputs to the water column in coastal ecosystems are associated with the influx of continental water (Hernández-Terrones et al., 2015). In addition, wind stress in these shallow coastal ecosystems induces the resuspension of sediment and interstitial water, which increases the flux of nutrients from sediments to the water column.

Nutrient concentrations in sediment porewater were higher than in the water column during the dry season. However, DIN and SRP showed contrasting patterns between seasons. During the cold-front season, we found 1.12–2.96 times more SRP, and 1.46–1.91 times more DIN, in porewater than in the water column; these proportions increased to 3.37–8.19 for SRP and 3.22–5.16 for DIN in the dry season. Hence, during the dry season SRP was probably removed from the water column and retained in sediments in a higher proportion than DIN, leading to proportionally higher levels of DIN relative to SRP in the water column in this season.

Chlorophyll-*a* concentrations in the study area were low in general and suggest no nutrient enrichment. Our chlorophyll-*a* values are lower than those (0.37–0.62 $\mu\text{g L}^{-1}$) reported by Brodie et al. (2007) for sites in the Great Barrier Reef affected by nutrient inputs, and are similar to the levels reported by Mutchler et al. (2010) for the Mexican Caribbean coast. The slight increase in chlorophyll-*a* in the dry season may be related to the small change in DIN:SRP ratios observed in this season, when these approached the Redfield ratio (16:1). Although the DIN:SRP ratios were always lower than 16:1 (Table 1), small increases in this ratio can trigger phytoplankton growth in N-limited environments (Furnas et al., 1990; Schaffelke et al., 2012). The positive correlation between the DIN:SRP ratio and chlorophyll-*a* concentration suggests that phytoplankton growth in the study area is N-limited.

Table 2

Mean %N, C:N, $\delta^{15}\text{N}$ (‰) values in *T. testudinum* leaf tissues, and percent coverage of *T. testudinum* and macroalgae during the cold-front (CF) and dry (D) seasons of the year at Isla Blanca (IB), Puerto Juárez (PJ), and Puerto Morelos (PM). Standard errors are given in parenthesis. Figures in bold case identify the higher value in those cases where significant differences between seasons were found.

Site	n	<i>Thalassia testudinum</i> indicators			Macroalgae coverage (%)		
		%N	C:N	$\delta^{15}\text{N}$	Cover (%)	Calcareous	Fleshy
IB _{CF}	9	2.02 (0.10)	20.27 (1.06)***	1.04 (0.55)	53.66 (4.3)	4.43 (1.18)*	2.02 (0.48)
IB _D	9	2.16 (0.02)	17.1 (0.39)	0.87 (0.72)	65.7 (4.81)	0.23 (0.14)	5.19 (1.58)
PJ _{CF}	6	1.81 (0.06)	20.95 (0.39)	4.85 (1.24)	39.33 (5.01)	12.57 (1.74)	24.87 (3.8)
PJ _D	6	1.80 (0.1)	21.7 (0.97)	4.27 (1.40)	50.0 (4.87)	13.27 (0.01)	19.10 (3.39)
PM _{CF}	9	1.88 (0.06)	21.9 (0.66)	1.74 (0.24)	34.41 (2.99)	8.22 (0.8)**	4.27 (0.73)
PM _D	9	1.83 (0.04)	21.3 (0.57)	1.08 (0.63)	40.67 (2.94)	3.53 (0.64)	7.91 (1.11)**

n = number of samples (only for %N, C:N, and $\delta^{15}\text{N}$). * $p < 0.001$, ** $p < 0.01$, *** $p < 0.05$

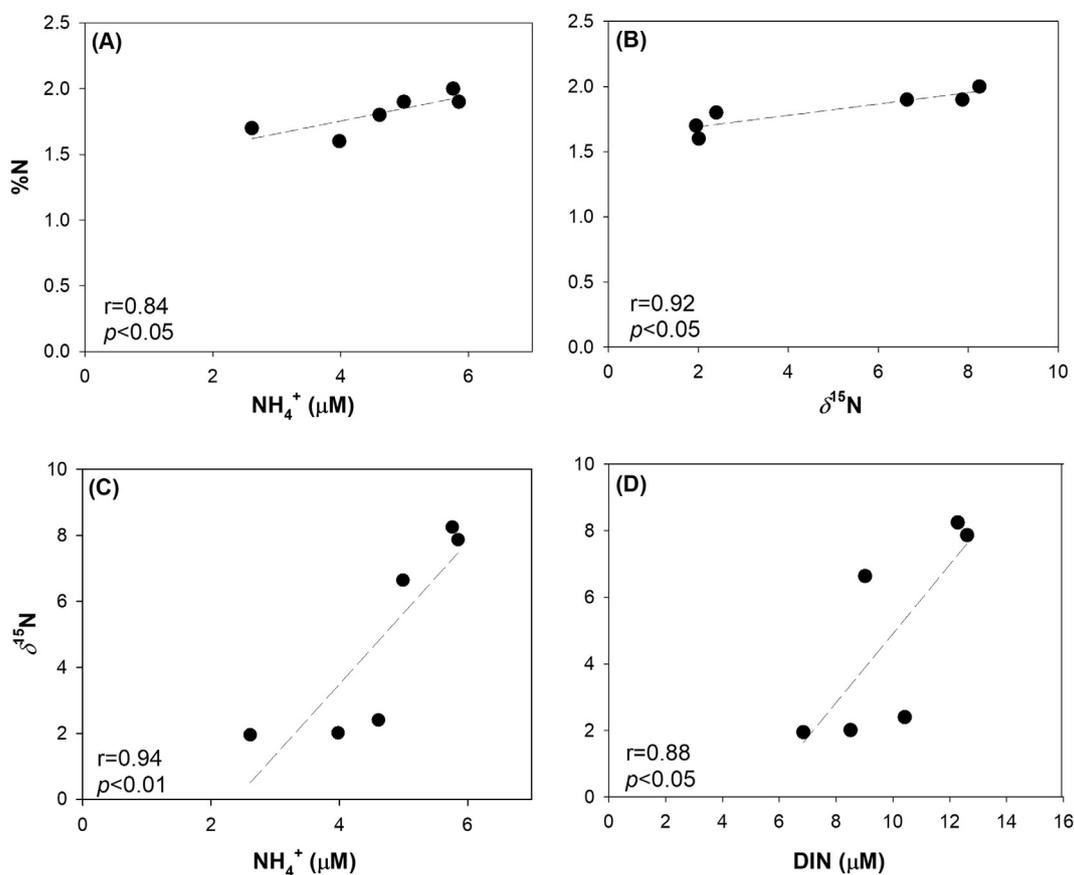


Fig. 4. Correlations between %N and NH_4^+ (A), %N and $\delta^{15}\text{N}$ (B), $\delta^{15}\text{N}$ and NH_4^+ (C), and between $\delta^{15}\text{N}$ and DIN (D), in the water column at the PJ site during the cold-front season.

Table 3

Nitrogen content (%) and $\delta^{15}\text{N}$ (‰) in macroalgae specimens collected at the Puerto Morelos (PM) and Isla Blanca (IB) sites.

Season	Site	Macroalgae species	%N	$\delta^{15}\text{N}$ (‰)
Cold-front	IB	<i>Dictyota</i> sp.	2.46 (0.17)	2.67 (0.08)
		<i>Styopodium zonale</i>	2.0 (0.26)	0.07 (0.40)
	PM	<i>Sargassum</i> sp.	1.1 (0.15)	-0.64 (0.39)
Dry	IB	<i>Dictyota</i> sp.	2.0 (0.01)	1.87 (0.11)
		<i>Styopodium zonale</i>	1.96 (0.23)	0.28 (0.05)
	PM	<i>Sargassum</i> sp.	1.26 (0.12)	-0.95 (0.13)

Standard errors are given in parenthesis, n = 3 in all cases.

The seasonal differences observed in nutrient concentrations in the water column may have induced variations in macroalgae coverage but might not have been sufficient to change %N, $\delta^{15}\text{N}$, or the coverage of *Thalassia testudinum*. The observed values of these indicators may result from site-specific, long-term nutrient inputs. Thus, the differences observed between sites may be indicative of changes in the local dynamics of nutrient fluxes and the response of autotrophic organisms.

4.2. Differences between sites in nutrient enrichment effects

The signs of nutrient enrichment observed at the PJ site can be accounted for by its proximity to the resort city of Cancún and the Puerto Juárez port infrastructure. Numerous studies in karst coastal environments have shown that nutrients in water discharges are higher near urban settlements (Lapointe and Clark, 1992; Lapointe et al., 2004; Álvarez-Góngora and Herrera-Silveira, 2006; Hernández-Terrones et al., 2011). The high NH_4^+ concentrations in the water column at the PJ site evidence anthropogenic N inputs as the presence of this nutrient

Table 4

Highest $\delta^{15}\text{N}$ values recorded in *Thalassia testudinum* leaves in Caribbean coastal ecosystems of Quintana Roo between 2002 and 2018.

Site	Season	$\delta^{15}\text{N}$ (‰)	N	Reference
Nichupté Lagoon	Dry	9.06 (0.73)	4	Carruthers et al. (2005)
Akumal Bay	Dry-Rainy	7 (1)*	3	Mutchler et al. (2007)
Akumal	Dry	6.1 (1.9)	2	Mutchler et al. (2010)
Nichupté Lagoon	Dry	9.8–10.8	4	Sánchez et al. (2013)
Tulum	Dry	7.6–8.8	–	Sánchez et al. (2013)
Tulum	Cold-Front	5.9	–	Camacho-Cruz et al. (2019)
**Puerto Juárez	Cold-Front	7.58 (0.48)	3	This study
**Puerto Juárez	Dry	7.35 (0.37)	3	This study

Values shown are means. Standard errors are given in parenthesis, except when otherwise indicated. *Standard deviation. **Mean values for the 10 m transect.

has been related to recent contamination sources (Lapointe et al., 1990; Camacho-Cruz et al., 2019). In addition, the $\delta^{15}\text{N}$ values recorded in *T. testudinum* nearshore (10 m from the coast) in both seasons (cold-front season, 7.58‰; dry season, 7.35‰) were the highest recorded in our study area and may indicate a large input of wastewater from the continent. The $\delta^{15}\text{N}$ values recorded at this site are similar to those reported by Carruthers et al. (2005), Mutchler et al. (2007), Sánchez et al. (2013), and Camacho-Cruz et al. (2019) for sites heavily impacted by anthropogenic N sources (Table 4), and are among the highest values reported for *T. testudinum* leaves along the northern Mexican Caribbean coast (Table 4). Albeit $\delta^{15}\text{N}$ enrichment can also be caused by denitrification in the water column or in marine sediments (Corbett et al., 1999; Griggs et al., 2003; Fourqurean et al., 2005; Mutchler et al., 2007; Sánchez et al., 2013), the strong correlations observed between $\delta^{15}\text{N}$ and NH_4^+ , DIN, and %N in the cold-front season (Fig. 4) were consistent with a possible anthropogenic origin of the N taken up by *T.*

testudinum. Moreover, the low $\delta^{15}\text{N}$ values recorded 700 m away from the coastline (transect B) in both seasons (cold-front season, 2.12‰; dry season, 1.2‰) suggest a dilution effect of coastal N.

A high N content in *T. testudinum* leaves from the PJ site would be expected, as this indicator is related to nitrogen availability and should reflect the influx of this nutrient from external sources (Sánchez et al., 2013). However, the median %N and C:N ratio ranged between 1.85 and 1.75% and between 21.15 and 22.5, respectively; these values would suggest N limitation (Duarte, 1990), but this would be inconsistent with the high DIN concentration observed. As documented by Davis and Fourqurean (2001), nutrient competition between macroalgae and the seagrass might have caused the low N content found in *T. testudinum* leaves from the PJ site. Those authors noticed a decrease in the *T. testudinum* C:N ratio upon removal of the calcareous algae *Hali-medea incrassata* and deduced a subsequent higher N uptake by *T. testudinum*. The macroalgae coverage and its negative correlation with *T. testudinum* suggest that macroalgae might be taking up the available nutrients and grow at the expense of the seagrass in the PJ site.

By contrast, human activities and urban settlements at the IB site are scarce. In this site we recorded the highest coverage of *T. testudinum* and the lowest coverage of macroalgae, also, *T. testudinum* leaves showed no signs of N limitation (i. e., the highest %N content was recorded at this site), and $\delta^{15}\text{N}$ was noticeably low. For example, the $\delta^{15}\text{N}$ values recorded 3900 m away from the coastline (transect C) changed from -1.35 to 0.72 ‰ between the cold-front and the dry season, and from -2.83 to 0.33 ‰ on transect B (1850 m from the coastline). Therefore, an atmospheric N source is likely being utilized by *T. testudinum* offshore since the $\delta^{15}\text{N}$ value of this source is typically ~ 0 ‰ (Sigman and Casciotti, 2001). By contrast, the mean $\delta^{15}\text{N}$ recorded nearshore (10 m transect) ranged from 1.56 to 2.1‰. The contribution of N sources with higher $\delta^{15}\text{N}$ seems to occur near the shore, being more important during the cold-front season. IB is likely influenced by water masses carrying high concentrations of nutrients and phytoplankton, coming from either the Chacmochuch coastal lagoon (~ 1 km to the west) or the Cape Catoche upwelling zone, located ~ 25 km to the north (Merino, 1997). The influence of these water masses may account for the high chlorophyll-*a* and nutrient concentrations observed at the IB site.

Values of nutrient enrichment indicators (i.e., nutrient concentrations, coverage of submerged aquatic vegetation, and $\delta^{15}\text{N}$) at the PM site were intermediate between the recorded at IB and PJ. However, compared to the values reported by Hernández-Terrones et al. (2011) and, more recently, by Null et al. (2014) and Almazán-Becerril et al. (2015), DIN and SRP concentrations at the PM site have experienced a marked increase over the past 12 years (at least one order of magnitude for DIN, and two orders of magnitude for SRP). These data suggest that the nutrient regime at this site is gradually shifting. The recent massive influx of drifting *Sargassum* represents an additional source of N for *T. testudinum* at the PM seagrass meadows (van Tussenbroek et al. 2017). The mean $\delta^{15}\text{N}$ values recorded in *T. testudinum* samples collected 10 m (0.62‰) or 1300 m (-0.83 ‰) away from the coastline (nearshore transect and transect B, respectively) during the dry season were similar to those measured in nearshore samples of drifting *Sargassum* spp. collected in either season (cold-front season, -0.64 ‰; dry season, -0.95 ‰). By contrast, $\delta^{15}\text{N}$ (3.45‰) in *T. testudinum* samples collected in the dry season 2600 m offshore (transect C) was higher than in those collected closer to the shore. These observations suggest that N uptake from exudates or from the decomposition of *Sargassum* on the beach may explain the differences between transects. Van Tussenbroek et al. (2017) reported a shift in $\delta^{15}\text{N}$ in *T. testudinum* from seagrass meadows at Puerto Morelos after the massive influx of *Sargassum* that took place from mid-2014 to late 2015. Another massive influx of *Sargassum* to the Mexican Caribbean coasts occurred in 2018 (during our study), with a peak in May (Rodríguez-Martínez et al., 2019). Although drifting algae were found at the PM site in both of our sampling campaigns, *Sargassum* breakdown was more intense during the dry

season (May). The variation in the isotopic ratios recorded at this site suggest that *Sargassum* may interfere with the detection of anthropogenic N inputs.

Although the indicators of nutrient enrichment evaluated in this study did not attain critical values in some of our study sites, the region does show signs of environmental deterioration that denote a steady shift in the trophic status of the coastal system that threatens its health status.

5. Conclusions

Seasonal water discharges and sediment resuspension may control nutrient inputs and stoichiometry (DIN:SRP ratio) in the shallow coastal ecosystems of the northern Mexican Caribbean. The positive correlation between the DIN:SRP ratio and chlorophyll-*a* concentration indicates that phytoplankton growth in the study area is likely limited by nitrogen.

Although the spatial differences in the nutrient enrichment indicators evaluated in this study may result from site-specific, long-term nutrient inputs, the study region shows signs of environmental deterioration that suggests a shift in the trophic status of the system (away from oligotrophic).

The large increases (at least one order of magnitude in DIN and two orders of magnitude in SRP) that have occurred over the past ~ 12 years in water-column nutrient concentrations at the Puerto Morelos reef lagoon may be related to anthropogenic inputs and massive arrivals of drifting *Sargassum*. The $\delta^{15}\text{N}$ values recorded in *T. testudinum* leaves suggest that *Sargassum* is an important source of N to the environment.

The IB site showed little signs of nutrient enrichment; our results for this site can be used as a benchmark to evaluate how its environmental conditions change with the implementation of the massive urban and tourism development plans that, according to the Partial Urban Development Plan for the Chacmochuch Peninsula (PPDU 2018–2020), are envisioned for this zone. The environmental conditions in IB after urbanization may approach those in the other sites studied here. By contrast, the PJ site clearly exhibits the impact of massive urban and tourism development on the valuable coastal ecosystems of the northern Mexican Caribbean coast.

CRediT authorship contribution statement

Javier A. Pérez-Gómez: Conceptualization, Formal analysis, Resources, Investigation, Methodology, Visualization, Writing - original draft. **Ernesto García-Mendoza:** Conceptualization, Resources, Writing - review & editing, Funding acquisition. **Aramis Olivos-Ortiz:** Conceptualization, Resources, Writing - review & editing. **A. Paytan:** Conceptualization, Resources, Writing - review & editing. **M. Rebolledo-Vieyra:** Conceptualization, Resources, Writing - review & editing, Funding acquisition. **Benjamín Delgado-Pech:** Conceptualization, Resources, Investigation, Methodology, Writing - review & editing. **Antonio Almazán-Becerril:** Conceptualization, Resources, Formal analysis, Methodology, Visualization, Investigation, Writing - original draft, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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