Macroalgae reveal nitrogen enrichment and elevated N:P ratios on the Belize Barrier Reef

Brian E. Lapointe [a,1], Alexander Tewfik [b,*,1], Myles Phillips [b]

[a] *Florida Atlantic University-Harbor Branch Oceanographic Institute, 5600 US 1 North, Ft. Pierce, FL 34946, USA*
[b] *Wildlife Conservation Society, Belize Program, PO Box 768, 1755 Coney Drive, 2nd Floor, Belize City, Belize*

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ABSTRACT

Macroalgal blooms are increasing on the Belize Barrier Reef (BBR) as scleractinian coral cover declines. Although some have attributed this to reduced grazing, the role of land-based nutrient pollution has not been assessed. Nutrient enrichment was quantified through macroalgal tissue analysis from Belize City to the offshore fore reef and at several central BBR lagoon sites. These recent data were compared to baseline data from the 1980s. Significant nearshore-to-offshore gradients of %N, %P and δ^{13}C in macroalgae all indicated land-based sources of these nutrients. Macroalgal δ^{15}N values were generally enriched in nearshore waters where values matched those reported for human sewage. Notably, the N:P ratios of recent macroalgae measurements were elevated at all sites, more than two-fold higher than values from the 1980s (~30: 1 to 70:1). These results support the hypothesis that nitrogen enrichment from land-based sources has increased phosphorus limitation driving macroalgal blooms and coral stress on the BBR.

1. Introduction

Human activities have greatly altered global carbon (C), nitrogen (N) and phosphorus (P) cycles with increases in algal blooms in recent decades related to global increases in N inputs that are now considered above a safe planetary boundary (Glibert, 2017; Steffen et al., 2015). These altered nutrient fluxes, particularly N, are increasingly affecting the health of marine ecosystems through nutrient enrichment and altered stoichiometry of C, N, and P (Hillebrand and Lehmpfuhl, 2011; NRC, 2000; Steffen et al., 2015). Such alterations are often associated with nutrients from land-based freshwater runoff that reflect broader changes in land use, urbanization and agricultural practices across watersheds and airsheds as the human population grows and the demand for crops, livestock, housing and manufacturing facilities increases (Calizza et al., 2020; Rey-Villiers et al., 2020; Thorburn et al., 2013). Nutrient enrichment generally results in increased primary production of algae, including phytoplankton, benthic macroalgae and algal turfs, as overall biodiversity is reduced (Bell et al., 2014; D'Angelo and Wiedenmann, 2014; Duarte, 1995; Foster and Schiel, 2010; Prouty et al., 2017; Tewfik et al., 2007). Unlike some phytoplankton blooms, such as toxic "red tides", macroalgal blooms generally lack direct chemical

toxicity but can still result in harmful environmental effects including changes in habitat, oxygen depletion, production of hydrogen sulfide, alteration of biogeochemical cycles, increased grazing, and die-off of seagrasses and coral reefs (Anderson, 2007; ECOHAB, 1995; Lapointe and Bedford, 2007; McGlathery, 1995). Nutrients from a variety of sources including large river systems (Mississippi, Amazon, Orinoco, Congo) in the Atlantic basin have contributed to increasing macroalgal blooms, most notably the "golden tides" of *Sargassum* spp. from the newly formed Great Atlantic *Sargassum* Belt (GASB); this new feature of the tropical Atlantic Ocean illustrates the varying nature and large scale impact of nutrient enrichment and resulting harmful macroalgal blooms (HAB) that have emerged in recent decades (Anderson, 2007; Lapointe, 1995; Lapointe et al., 2021; Oviatt et al., 2019; Wang et al., 2019).

Coral reefs support much of the world's marine biodiversity, provide significant livelihoods (e.g. fisheries, tourism) as well as coastal protection, but have been in significant decline since the 1970s due to a growing number of recognized stressors (Bellwood et al., 2004; Gardner et al., 2003; Hughes et al., 2003; Wear and Thurber, 2015). They are now considered one of the most threatened ecosystems on our planet (Birkeland, 2004; Hughes et al., 2017) with significant losses of scleractinian (i.e. hard) coral cover having been observed even along the

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iconic Great Barrier Reef, Australia (Bellwood et al., 2004; De'ath et al., 2012; Ortiz et al., 2018). Scleractinian corals have evolved to thrive in oligotrophic waters of the tropics through a polytrophic feeding capacity including a critical exchange of benefits with symbiotic *Symbiodinium* (i. e. zooxanthellae) algae, traditional heterotrophy and various mechanisms to assimilate, sequester and recycle nutrients, especially N, from dissolved compounds in pulsed oceanic upwellings and local sources such as fish excretion (Muscatine and Porter, 1977; Shantz and Burkepile, 2014; Trench, 1974). However, in watersheds impacted by increasing urbanization and expanding agricultural lands, associated anthropogenic nutrient enrichment has been shown to alter freshwater food webs and effect overall ecological integrity of lakes, rivers, and springs (Anderson and Cabana, 2005; Cabana and Rasmussen, 1996; Winemiller et al., 2011). Marine ecosystems, including coral reefs, also show significant alterations including greater susceptibility to bleaching, diseases, and mortality at an ever-increasing scale (Burkepile et al., 2020; D'Angelo and Wiedenmann, 2014; Geeraert et al., 2020; Lapointe et al., 2019; Lapointe et al., 2010; Oviatt et al., 2019; Tomascik and Sander, 1987) in addition to ongoing impacts from overfishing, tourism development and climate change (Baker et al., 2013; Gardner et al., 2003; Norstrom et al., 2016; Ortiz et al., 2018; Roberts, 1995).

In the Caribbean basin, scleractinian coral cover has declined from ~50% to ~10% over the last 50 years with increasing dominance by macroalgae and turf algae (Gardner et al., 2003; Jackson et al., 2014; Lapointe et al., 2019). While early studies on scleractinian coral losses focused largely on the effects and management of land based nutrient pollution and eutrophication (Banner, 1974; Smith et al., 1981; Tomascik and Sander, 1987), more recent work has focused on top-down explanations for the coral-algae phase shifts (Hughes, 1994; Jackson, 1997; McClanahan et al., 2011; Mumby et al., 2012). However, mounting evidence indicates that the coral-algae phase shift is not driven simply by declines in herbivores, which have been increasing or stable given the use of marine protected areas and fishing bans, but due to human development on land and resulting elevated nutrient enrichment in coastal waters (Arias-González et al., 2017; Gonzalez-De Zayas et al., 2020; Lapointe et al., 2019; Suchley et al., 2016; Tewfik et al., 2017). As nutrient availability increases to coral reefs and associated coastal ecosystems beyond thresholds (i.e. tipping point), a positive feedback loop may be established that make the altered, damaged and simplified conditions persistent and difficult to reverse without addressing the driving nutrient source (El-Khaled et al., 2021; Lapointe, 1997; Scheffer and Carpenter, 2003; Tewfik et al., 2005).

In Belize, Central America, increases in human population (6-fold in the last 70 years, 58,000 to 404,000) and environmental impacts (deforestation, overfishing, algal overgrowth, and coral bleaching) have followed and sometimes exceeded global trends (Babcock et al., 2018; Cherrington et al., 2012; Jackson et al., 2014). This includes unsustainable development activities along rivers (e.g., deforestation, rangeland expansion, water extraction), within and adjacent to the Belize Barrier Reef (BBR) lagoon and offshore atolls (e.g. coral dredging, mangrove cutting, aquaculture, tourism infrastructure) resulting in losses of ecosystem services (Auil-Gomez, 2014; Boles and Requena, 2011; Byron and Osipova, 2013; Ledwin, 2010). The GASB has also caused the influx of tons of *Sargassum* biomass to Caribbean coastal waters and beaches, including those of Belize, which adds to the negative impacts on nearshore environments through reduced sunlight, decay, nutrient release and hypoxia/anoxia (Lapointe et al., 2021; Wang et al., 2019). Live scleractinian coral cover within the South Water Caye Marine Reserve (SWCMR) and Glover's Reef Marine Reserve (GRMR), parts of a UNESCO World Heritage Site (Byron and Osipova, 2013), has been reduced from >80% in the 1970s (Wallace and Scheferman, 1977) to ~5% on patch reefs and ~17% on fore reefs, while algae of various morphologies constitute >45% of total benthic cover on both reef types (Tewfik, 2020).

The Belize River is the largest river in Belize and part of a bi-national freshwater system reaching from the eastern Peten District of Guatemala to the eastern Caribbean coast of Belize (Fig. 1a). It is fed by the Greater Mopan/Belize River Catchment (GMBRC), which spans the breadth of Belize with about 40% of its total area extending into Guatemala (i.e. the Mopan River sub-basin). A 17.8% decline in overall forest cover has occurred across the Belizean portion of the watershed over the past three decades (Boles and Requena, 2011; Cherrington et al., 2012). This watershed is one of 16 in Belize and the largest in the country (Fig. 1a), draining 25% of Belize, containing 45% of the human population and experiencing considerable pressure from unsustainable logging, agriculture and urban development resulting in deforestation, land degradation and pollution (Auil-Gomez, 2014; Karper and Boles, 2004).

Together, C, N and P are major elements supporting primary production in the oceans and are present in the relative molar proportions of 106:16:1 ("Redfield Ratio") (Redfield, 1958), which is remarkably consistent across shallow waters (<500 m) of ocean basins (Fanning, 1992; Redfield, 1958). An extensive analysis of nutrient contents within aquatic plants has shown a greater degree of variation in nutrient limitation of growth and C:N:P ratios for coastal macroalgae populations, rather than an intrinsic difference in their chemical composition relative to that of phytoplankton (Duarte, 1992). Accordingly, elemental analysis of macroalgal tissue for %C, %N and %P is commonly used to assess availability and growth limitation by N and/or P through determination of C:N, C:P, and N:P molar ratios (Atkinson and Smith, 1983; Lapointe et al., 1992b). As C:N ratios in macroalgae increase above 13:1, N becomes increasingly important as a growth-limiting nutrient (Lapointe and Bedford, 2010; Lapointe et al., 1987; Ochoa-Izaguirre and Soto-Jimenez, 2015). Similarly, increases in the C:P ratio above 350:1 are indicative of increasing P limitation (Atkinson and Smith, 1983; Lapointe et al., 1992b). The N:P ratio is particularly useful in gauging the relative growth limitation by N vs. P, where N:P ratios >30:1 indicates P-limitation and lower values N-limitation (Atkinson and Smith, 1983; Lapointe et al., 1992b).

Stable isotope analysis of macroalgal tissue for C ($\delta^{13}C$) and N ($\delta^{15}N$) is an established technique to identify the origin and source(s) of these elements (Abaya et al., 2018; Fry, 2006; Lachs et al., 2019; Lapointe et al., 2010). The $\delta^{13}C$ values can help determine where the algae and associated habitat lies across a terrestrial to oceanic influenced gradient (Fry, 2006; Peterson and Fry, 1987). This is based on differences in the assimilation of CO_2 in C3 and C4 plant photosynthetic pathways, where C3 plants dominate most terrestrial ecosystems and have a much lighter (i.e. more negative) $\delta^{13}C$ signature than C4 plants (Peterson and Fry, 1987; Philp, 2007). The $\delta^{15}N$ values are especially useful because N is generally the primary limiting nutrient in marine waters (Howarth and Marino, 2006; Ryther and Dunstan, 1971), including coral reef ecosystems (Lapointe et al., 2019). The $\delta^{15}N$ method is based on the fact that organisms are enriched in $\delta^{15}N$ relative to their sources of nitrogen (e.g. food, dissolved constituents) and therefore various natural (N_2 fixation) and anthropogenic (sewage, fertilizers) N sources have distinct $\delta^{15}N$ values that allow for discrimination of the N source supporting algal growth (Fry, 2006; Risk et al., 2008). Enrichment (i.e. increased) of natural $\delta^{15}N$ indicates input of human/animal derived sewage and fertilizers and low or depleted (i.e. decreased) $\delta^{15}N$ values indicate synthetic agricultural fertilizers (Haber-Bosch process), atmospheric N deposition, N_2 fixation, or secondary bacterial metabolism and fractionation (Lapointe et al., 2005; McClelland et al., 1997; Ochoa-Izaguirre and Soto-Jimenez, 2015).

The global significance of the BBR ecosystem, the core of the longest coral reef system in the western hemisphere (i.e. Mesoamerican Reef), has been formally recognized through a UNESCO World Heritage Site designation (Belize Barrier Reef Reserve System including SWCMR and GRMR) in 1996 (Byron and Osipova, 2013). However, assessing the level of nutrient enrichment and eutrophication in the coastal waters of the BBR due to human impacts on watersheds and subsequent management of these nutrients has received inadequate attention despite the availability of some early baseline work in the central lagoon (Lapointe et al., 1987, 1992a; Tomasko and Lapointe, 1991). The lack of effective
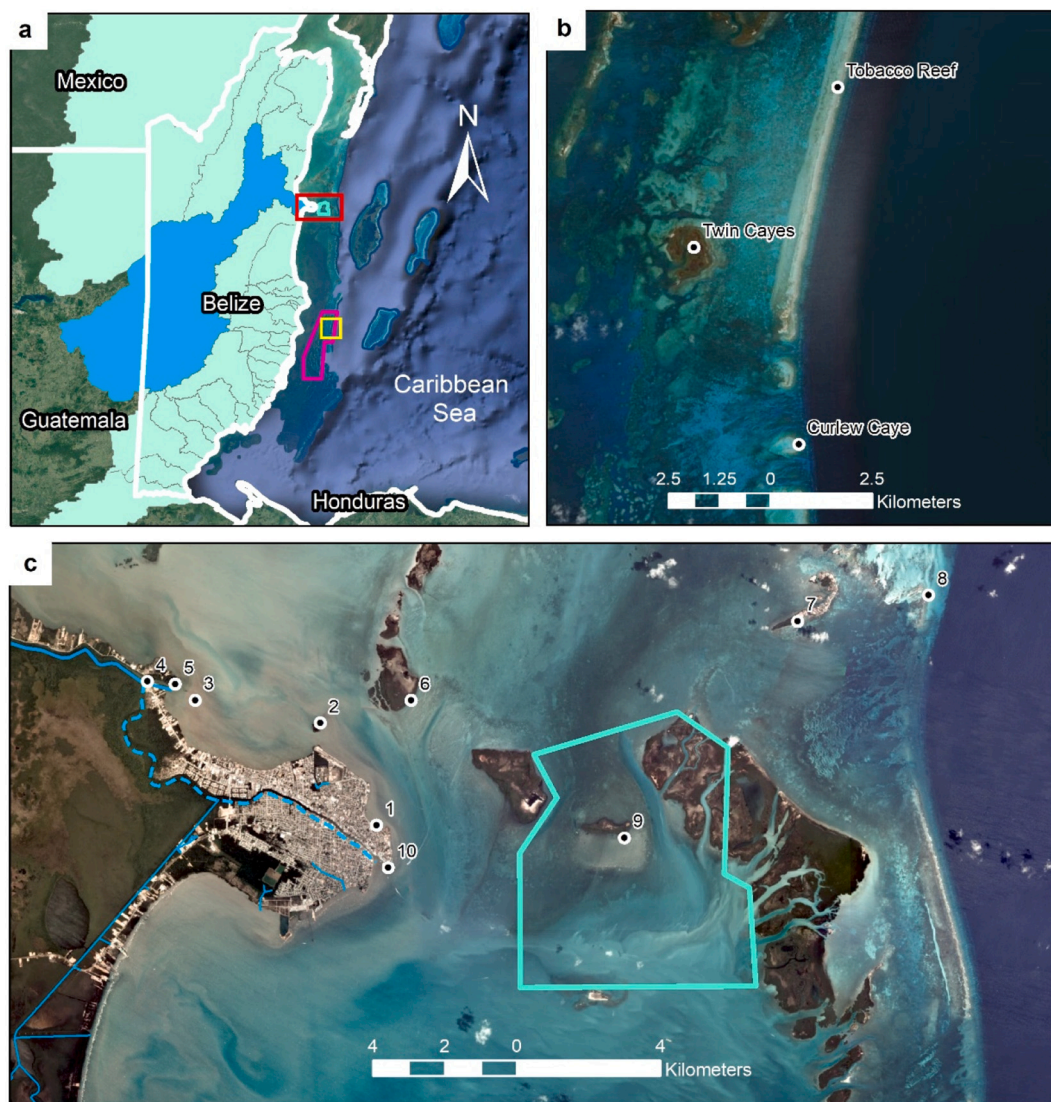
Fig. 1. Study area and sampling sites (Tables 1 and 2): (a) outlines of all 16 watersheds in Belize and the Greater Mopan/Belize River Catchment (GMBRC) (blue shade) straddling the border between Guatemala and Belize, Central America (red rectangle = area of image c, pink polygon = area of South Water Caye Marine Reserve (SWCMR), yellow square = area of image b); (b) North-eastern section of SWCMR and locations of Tobacco Reef, Twin Cayes and Curlew Caye sampled in the 1980s and 2017; (c) Belize City, the Belize River (thick blue line), Haulover Creek (dashed blue line), major canals (thin blue lines), area of the Swallow Caye Wildlife Sanctuary (SCWS, aqua polygon) and out to the barrier reef, March 2019. Note brown sediment plume surrounding the peninsula of Belize City. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ecosystem monitoring or a focused ridge-to-reef conservation strategy and specific concerns over nutrient enrichment and sedimentation resulting in declining water quality and impacted habitats have also been highlighted as ongoing issues on the BBR (Fig. 2c, d) (Auil-Gomez, 2014; Byron and Osipova, 2013; Murry, 2020; Winemiller et al., 2011).

For this study, we hypothesized that patterns of land-based nutrient runoff from rivers (i.e. Belize River) and urban centres (i.e. Belize City) would be evident in macroalgal tissue nutrient content across adjacent areas of the BBR. To test this hypothesis, we examined the relationships between the C, N, P contents, stoichiometry (C:N:P) and stable isotopes of C and N (i.e. $\delta^{13}C$, $\delta^{15}N$) found within the tissue of a variety of common macroalgae species sampled in the lower riverine, estuarine and shallow marine environments from around Belize City to the offshore fore reef habitats of the BBR (Figs. 1a, 2a, b). This information was used to determine the source (s) and relative levels of nutrient enrichment and potential alterations, specifically in the N:P ratio that can directly stress and ultimately cause die-off of scleractinian corals (Lapointe et al., 2019; Rosset et al., 2017). We also compared our Belize

City transect data from 2019 to similar elemental content (N, P) and stoichiometry data collected from macroalgae in the SWCMR in 2017 and during the 1980s. This provided both an expanded geographic scope of potential nutrient impact while also using a unique and local historical baseline to gauge the change in nutrient availability across the BBR over the past four decades.

2. Methods

To assess cross-shelf patterns in % C, %N, %P, C:N:P molar ratios and $\delta^{13}C$ and $\delta^{15}N$ values in macroalgal tissue within the BBR ecosystem, populations of macroalgae were sampled at ten sites along a transect extending offshore from Belize City (population: 61,000+), the largest human settlement in the country (Fig. 1b). This included the lower reaches and estuary of the Belize River, which runs through the largest watershed in the country, as well as Haulover Creek, a modified tidal channel that runs from a junction with the Belize River near its outfall, through mangroves and the commercial centre of Belize City (Figs. 1c,

Fig. 2. (a) Lower reaches and mouth of Belize River (sites 3, 4, 5) with mangroves and bridge/northern highway (March 2019); (b) Downtown Belize City at mouth of Haulover Creek (near site 10) with ferry docks and moored fishing sloops that supply two major seafood processing facilities on Haulover Creek (May 2019); (c) bloom of the green filamentous macroalga *Chaetomorpha* sp. on reef flats near Curlew Caye, South Water Caye Marine Reserve (SWCMR), April 2018; (d) lower reaches of the New River, Northern Belize with signs of hyper-eutrophication (May 2021); Photos: B. Lapointe (a), A. Tewfik (b, c), J. Maaz/WCS (d). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2a, b). The lower portion of Haulover Creek is the mooring area for fleets of coastal ferries and small-scale commercial fishing vessels (Fig. 2b). The outfall of Haulover Creek is also the location of a tourist village servicing passengers from as many as four visiting cruise ships per day moored southeast of the city within the BBR lagoon and the potential source of prop-driven re-suspended sediments and illegally dumped pollutants (Diedrich, 2010). Two seafood processing facilities, Northern and National Fishermen Cooperatives, which handle most of the country's queen conch (*Lobatus gigas*) and spiny lobster (*Panulirus argus*) exports, operate on the banks of Haulover Creek <500 m from its outfall (Fig. 2b). Sampling sites included dock and bridge pilings, seawalls, submerged tree logs, mangrove prop roots, seagrass/algae soft sediments and hard bottom coral reefs along the mainland, around uninhabited and populated mid-shelf cayes and the shallow fore reef outside of the reef crest and main lagoon spanning a distance of approximately 22 km (Fig. 1c). Sampling also occurred within the Swallow Caye Wildlife Sanctuary (SCWS), declared in 2002 as a 36 km^2 protected area primarily for the endangered West Indian manatee, *Trichechus manutus*, and foraging grounds for a discrete population of bottlenose dolphin (*Tursiops truncatus*) lying just 3 km directly east of Belize City (Garcia et al., 2017) (Fig. 1). All macroalgae field collections were performed from a small boat on March 25, 2019 (dry season) with a team from the Belize office of the Wildlife Conservation Society. The sampling was performed by wading or snorkelling with whole macroalgae plants initially collected into nylon mesh bags and returned to the boat. The macroalgae were gently cleaned of sediment and debris by washing with

seawater in a plastic colander, then placed in plastic Whirl-Pak baggies in a dark cooler for transport back to land for identification and initial processing.

Upon return to the field laboratory in Belize City, the macroalgae were rinsed briefly in deionized water to remove any remaining surface debris and salt and identified to species (Littler and Littler, 2000). The cleaned, composite macroalgae samples were dried in aluminum dishes in a lab oven at 60 °C for 48 h and then placed in new Whirl-Pak baggies. After transport back to the United States, the dried macroalgae were randomly separated into two replicate samples per species from each site and subsequently powdered using a mortar and pestle. The dried samples were stored in plastic screw-top scintillation vials until shipment to the University of Georgia's Center for Applied Isotope Studies Stable Isotope Ecology Laboratory (UGA-SIEL) for analysis. At UGA-SIEL, samples were analyzed for δ^{13}C and δ^{15}N (‰), as well as %C and %N (based on dry weight) on a Thermo Delta V IRMS coupled to a Carlo Erba NA1500 CHN-Combustion Analyzer via a Thermo Conflo III Interface (see http://sisbl.uga.edu/ratio.html#top;) ‰ (ThermoScientific, 2007). National Institute of Standards and Technology reference materials 8549, 8558, 8568, and 8569 were used to routinely calibrate working standards prepared in the laboratory. QA/QC results were incorporated into the raw data reports received by UGA-SIEL. Each sample was also analyzed for %P (based on dry weight), where approximately 2 mg of dried tissue was weighed into crucibles, ashed at 500 °C for 4 h, and extracted with 0.2 mL of Aqua Regia acid. The acid extracts were then diluted 41:1 with deionized water for TP (as PO4-P) analysis on an

Alpkem 300 series analyzer. The resulting, %C, %N, %P, C:N:P, δ^{13}C, and δ^{15}N molar ratio data of the macroalgae were used to make inferences regarding nutrient sources and availability.

Identical techniques from above samplings were used for the collection and analysis of N and P contents and molar ratios in macroalgae at three sites in the SWCMR in June 2017, which included two patch reefs (Curlew Caye, Tobacco Reef) and a mangrove island (Twin Cayes) (Figs. 1b, 2c) (Lapointe and Tewfik, 2018). These sites are positioned approximately 70 km south of our Belize City transect, between 16.5 and 21 km east of the outfall of the Sittee River and provide a good mix of lagoon habitats (mangroves, coral reefs) similar to those of the Belize City samples (Fig. 1). The SWCMR sites are identical to those where macroalgae N and P contents and molar ratios are available from the 1980s (Lapointe et al., 1987, 1992a) (Fig. 1c), which provide a unique baseline for assessing changes in nutrient status in the BBR that could have occurred over the last 35 years.

Salinity measurements were collected in May 2019, November 2019 and May 2020 using a calibrated YSI ProPlus multi-parameter meter adjacent to macroalgae sampling sites extending from Belize City and the Belize River to the offshore fore reef (Fig. 1c). All measurements were taken at the surface (0.5–1.0 m depth) to assess the offshore extent of the buoyant freshwater plume of the Belize River and associated waterways along the sampling transect.

All statistical analyses were performed using routines with Sigmaplot 13.0 (SYSTAT, 2014). These analyses used means (+/-SE) of paired replicates ($N = 21$) of macroalgae species for the Belize City transect (Table 1) and one to three replicates for the 2017 collections at SWCMR (Table 2). Analyses included One-Way Analysis of Variance and regression. Comparisons to patterns from the 1980s at SWCMR were based on published results (Lapointe et al., 1987, 1992a). Effects of statistical tests were considered significant when *P*-values were equal to or <5% for all procedures ($P \leq 0.05$). Non-parametric approaches were used with failures of normality or equal variance (SYSTAT, 2014).

3. Results

3.1. Macroalgal tissue analysis

The mean tissue values (+/− SE) for %N, %P, C:N:P ratios, δ^{13}C and δ^{15}N from the analysis of 42 macroalgae samples across 10 sites of the

Table 2
Elemental contents (%N, %P) and molar ratios (N:P) of macroalgae (n = samples, mean (SE)) collected at 3 sites within the South Water Caye Marine Reserve (SWCMR), Curlew Caye, Twin Cayes, Tobacco Reef (Fig. 1b), June 2017.

Species	Site	n	%N	%P	N:P
Acanthophora spicifera	Curlew Caye, SWCMR, Belize	1	0.88	0.031	63.4
Dictyota menstrualis	Curlew Caye, SWCMR, Belize	3	0.98 (0.15)	0.048 (0.003)	44.9 (4.9)
Halimeda opuntia	Curlew Caye, SWCMR, Belize	3	0.05 (0.022)	0.02 (0.004)	54.8 (7.1)
Acanthophora spicifera	Twin Cayes, SWCMR, Belize	2	1.67 (0.018)	0.04 (0.001)	88.5 (43.2)
Dictyota menstrualis	Twin Cayes, SWCMR, Belize	3	1.42 (0.008)	0.07 (0.002)	45.3 (21.7)
Halimeda opuntia	Twin Cayes, SWCMR, Belize	3	0.60 (0.017)	0.06 (0.019)	27.6 (11.9)
Acanthophora spicifera	Tobacco Reef, SWCMR, Belize	2	1.07 (0.27)	0.05 (0.007)	52.9 (6.7)
Dictyota menstrualis	Tobacco Reef, SWCMR, Belize	2	1.30 (0.006)	0.08 (0.002)	36.1 (1.3)
Lobophora variagata	Tobacco Reef, SWCMR, Belize	2	0.81 (0.001)	0.03 (0.001)	71.2 (1.4)
Mean (SE)			1.00 (0.08)	0.048 (0.005)	51.4 (4.24)

Belize City transect are summarized by species collected at each site in Table 1. Sites are grouped as nearshore (0–0.7 km) located within the dry season "brown" plume of the Belize River and Haulover Creek (sites 1, 2, 3, 4, 5, 10) and offshore sites (2.8–22.0 km) in relatively "clear" water (sites 6, 7, 8, 9) (Figs. 1c, 2). Mean (+/− SE) tissue values for %N, %P and N:P ratios collected at the 3 sites (Curlew, Tobacco, Twin) in SWCMR during 2017 are presented in Table 2 and provide an additional set of information for an indication of the current broader impact of nutrient enrichment on the BBR.

3.2. Elemental composition and stoichiometry

The %C, %N and %P contents of macroalgae were generally higher at the nearshore sites for the Belize City transect compared to the offshore sites and consistent with closer proximity to land-based nutrient sources

Table 1
Stable isotopes (δ^{13}C, δ^{15}N), elemental contents (%C, %N, %P) and molar ratios (C:N, C:P, N:P) of macroalgae collected at 10 sites (see Fig. 1). Nearshore sites are within Haulover Creek or the Belize River plume (Fig. 1). All values represent means ($n = 2$).

Species	Site	Site description	d13C	d15N	%C	%N	%P	C:N	C:P	N:P
Gelidiopsis sp.	1	City dock/seawall	−19.6	5.8	28.8	3.3	0.19	10.1	392.6	39.0
Acanthophora spicifera	1	City dock/seawall	−16.4	5.6	30.6	4.1	0.06	8.8	1394.4	158.8
Hypnea musciformis	1	City dock/seawall	−16.7	7.4	28.2	3.3	0.13	10.1	577.6	57.7
Bostrychia moritziana	2	Moho caye	−25.4	1.9	27.6	3.3	0.09	9.8	828.5	84.4
Acetabularia crenulata	2	Moho caye	−13.2	3.1	22.3	0.7	0.06	54.0	1115.9	23.2
Bostrychia moritziana	3	Estuary/stump	−29.8	2.8	21.9	2.4	0.11	10.5	523.7	50.1
Bostrychia moritziana	4	Belize river bridge	−33.9	8.0	26.9	2.7	0.12	11.8	556.6	47.3
Bostrychia moritziana	5	mangrove/river mouth	−32.0	7.1	24.4	2.0	0.11	14.3	563.8	39.5
Laurencia filiformis	6	Riders caye	−15.4	6.4	23.7	1.1	0.03	26.4	1879.0	71.3
Hypnea spinella	6	Riders caye	−18.5	2.8	23.0	1.2	0.03	23.0	2273.8	99.0
Acanthophora spicifera	7	St. George's Caye	−15.0	4.9	23.2	1.6	0.05	16.6	1242.1	74.9
Laurencia intricata	7	St. George's Caye	−13.8	4.2	22.2	1.1	0.04	23.5	1287.1	54.8
Gracilaria ferox	7	St. George's Caye	−15.7	4.9	25.7	2.0	0.08	15.2	818.6	53.8
Dictyota cervicornis	8	Shallow forereef	−12.7	2.1	25.3	0.9	0.04	33.4	1815.0	54.3
Turbinaria turbinata	8	Shallow forereef	−14.3	2.3	24.6	0.7	0.03	39.3	1943.2	49.6
Sargassum polyceratium	8	Shallow forereef	−11.6	1.7	26.7	1.0	0.04	32.5	1577.6	48.8
Hypnea spinella	9	Swallow caye	−21.1	4.5	24.4	2.5	0.05	11.3	1210.1	107.4
Laurencia filiformis	9	Swallow caye	−15.5	4.3	20.2	1.5	0.05	16.3	1069.9	65.9
Gracilaria tikvahiae	10	Haulover seawall	−20.3	−4.4	27.9	2.4	0.05	13.8	1578.7	114.5
Ulva chaetomorphoides	10	Haulover seawall	−18.7	−7.0	24.6	1.7	0.06	16.8	1068.7	63.8
Acanthophora spicifera	10	Haulover seawall	−18.0	−2.2	26.6	3.0	0.07	10.4	1036.8	99.8
Mean			−18.9	3.2	25.2	2.0	0.07	19.4	1178.7	69.4
Standard error			1.38	0.82	0.57	0.22	0.01	2.58	114.8	6.9

(Table 1). The %C ranged from a maximum mean value of 30.6 in the red macroalga *Acanthophora spicifera* at the Belize City fishing dock (site 1) to a minimum value of 20.2 in *Laurencia filiformis* at SCWS (site 9). For % N, the maximum value of 4.1 was in the red macroalga *A. spicifera* at the Belize City fishing dock, with the minimum value of 0.70 in the green alga *Acetabularia crenulata* at Moho Caye (site 2) (Fig. 3a). A regression analysis of %N values across the shelf indicated a significant decline (R^2 = 0.375, P = 0.003) out to the shallow fore reef 22 km offshore (Fig. 4a). For %P, the maximum mean value of 0.19 was in the red macroalga *Gelidiopsis* sp. at the Belize City fishing dock (site 1) with the minimum value of 0.03 in the brown macroalga *Turbinaria turbinata* at the offshore shallow fore reef (site 8) (Fig. 3b). A regression analysis of %P values across the shelf indicated a significant decline (R^2 = 0.226, P = 0.03) in levels of phosphorus out to the shallow fore reef 22 km offshore (Fig. 4b).

The resulting molar C:N:P ratios of macroalgae along the Belize City transect were elevated above the Redfield Ratio (106:16:1), indicating high levels of nutrient limitation, especially P. The overall mean C:N ratio was 19.4 with the minimum mean value of 8.8 found in the red macroalga *Acanthophora spicifera* at the Belize City fishing dock (site 1) and the maximum value of 54.0 in the green calcified macroalga *Acetabularia crenulata* at Moho Caye (site 2; Table 1). A Mann-Whitney Rank Sum test (U Statistic = 56.000, T = 227.000, P < 0.001), normality test (Shapiro-Wilk) failure, revealed a significantly lower C:N ratio (mean +/− SE) at nearshore sites (15.5 +/− 2.8) versus offshore sites (23.8 +/− 2.0). The overall mean for the C:P ratio was 1179 with the minimum mean value of 393 in *Gelidiopsis* sp. at the Belize City fishing dock (site 1), with the maximum value of 2274 in the red macroalga *Hypnea spinella* at Riders Caye (site 6) (Table 1). A *t*-test (t = −3.914, 40 df, Two-tailed P < 0.001), both normality and equal variance tests passed, revealed a significantly lower C:P ratio at nearshore sites (876 +/− 83) versus offshore sites (1511 +/− 102).

In contrast to the C:N and C:P ratios, a Mann-Whitney test (U Statistic = 205.000, T = 376.000, P = 0.790), run due to failure of normality, revealed no significant differences in N:P ratios (mean +/− SE) between nearshore (70.7 +/− 8.6) and offshore (68.0 +/− 4.6) sites for the Belize City data (Fig. 3c). The overall mean N:P ratio across the transect was 69.4 (+/− 7.0) with a minimum value of 23.2 in the green macroalga *Acetabularia crenulata* at Moho Caye (site 2) and the maximum value of 158.8 in the red macroalga *Acanthophora spicifera* at the Belize City fishing dock (site 1) (Table 1). Macroalgae at site 9, within the SCWS, had very high N:P values ranging from 61.1 to 109.2 (Table 1). A regression analysis of N:P values across the shelf from Belize City indicated a non-significant trend (R^2 = 0.061, P = 0.696) and describes elevated N:P ratios compared to the Redfield Ratio of 16:1 and historical data from SWCMR (29.3:1), and hence N enrichment relative to P, out to the shallow fore reef 22 km offshore (Fig. 5). Almost all N:P values along the Belize City transect (Fig. 5) exceed the mean N:P ratio found at the SWCMR in the 1980s (29.2 ±9.8, Table 2). The mean N:P ratio along the Belize City transect (69.4 ±6.9) is 138% higher than the mean N:P ratio at SWCMR in the 1980s (Table 1, Fig. 6). Mann-Whitney U Statistics reveal no significant differences in %N (P = 0.075) or %P (P = 0.336) at SWCMR between the 1980s and 2017, however, the N:P ratio has increased significantly (P < 0.001) (Fig. 6). With the perspective of the unique historical baseline in the area of SWCMR from the 1980s (Lapointe et al., 1987, 1992a), the %N and %P at SWCMR in 2017 have increased by 40% and 32%, respectively, while the N:P ratio has increased by 76% over approximately 30 years (Table 2, Fig. 6).

3.3. Stable isotopes

The δ^{13}C data for the Belize City transect ranged from depleted values of −33.9‰ in the red macroalga *Bostrichia moritziana* at the Belize River bridge (site 4, group 1) to the most enriched value of −11.6‰ in the brown macroalga *Sargassum polyceratium* on the offshore shallow fore reef (site 8, group 2) (Fig. 7a, Table 1). The general relationship of δ^{13}C across the lagoon to the shallow fore reef (22 km) indicated significantly increasing enriched (i.e. less negative) and decreasing terrestrial origins of C with the most depleted (i.e. terrestrial) signatures occurring at the outlet of the Belize River (Fig. 7a). The δ^{13}C values at site 10 (Haulover Creek, group 3) were separated given the considerably different δ^{15}N data for that site and a desire for consistency in the presentation of overall stable isotope results in Fig. 8. When data were grouped by distance from the mainland, significant differences in mean δ^{13}C values were only detected between nearshore sites, groups 1 and 3 (#1–5, 10) and offshore sites, group 2 (#6–9) (Fig. 8a). Failure of normality test (Shapiro-Wilk) required the use of the Kruskal-Wallis One-Way Analysis of Variance on Ranks (H = 16.201 with 2 degrees of freedom, P < 0.001) followed by pairwise multiple comparison procedures (Dunn's Method) for δ^{13}C: 1 vs. 2 (P < 0.001), 2 vs. 3 (P = 0.083), 1 vs. 3 (P = 1). (Fig. 8a).

The spatial pattern of δ^{15}N indicated generally enriched (>+2‰) values with no significant trend across the shelf with a mean value of 3.2 +/− 0.82‰ (Fig. 7b). However, exceptionally depleted values were detected in the most urban setting at the outlet of Haulover Creek (site 10, mean (SE), −4.5 +/− 0.86‰), which included *Ulva chaetomorphoides* at −7.0‰ (Figs. 7b, 8b Table 1). With the exception of values at the outlet of Haulover Creek (site 10) in Belize City, an opposite terrestrial/oceanic trend compared to δ^{13}C values was revealed in the macroalgae δ^{15}N data. Enriched values, ranging up to +8.0‰ in the red macroalga *Bostrichia moritziana*, were found at the Belize River bridge (i. e. mouth/estuary) with lower values at the shallow fore reef (+2.0 +/− 0.16) 22 km offshore. Significant differences occurred between the nearshore site group (#1–5), offshore site group (#6–9), and the site at Haulover Creek (#10) using a One-Way Analysis of Variance (F = 83.1 with 2 degrees of freedom, P < 0.001), data passing tests of normality (Shapiro-Wilk) and equal variance (Brown-Forsythe), followed by pairwise multiple comparison procedures (Holm-Sidak method) δ^{15}N: 1
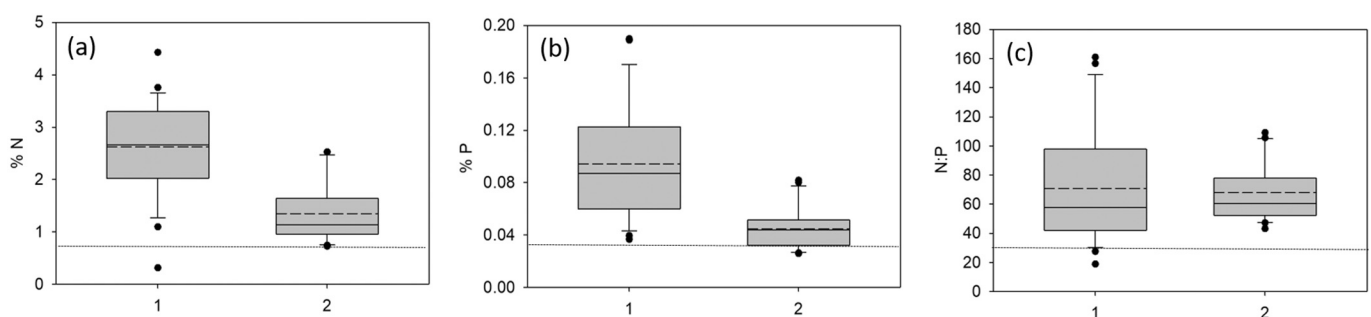

Fig. 3. (a) Contents of nitrogen (%N), (b) phosphorus (%P) and (c) molar ratio (N:P) for macroalgae at Belize City and adjacent shelf and shallow fore reef for nearshore (1) and offshore (2) site groups. Fine dashed horizontal lines indicate mean values for the 1980s at SWCMR (Fig. 6). (a) %N: t = 5.377 with 40 degrees of freedom, Two-tailed P-value < 0.001; (b) % P: Mann-Whitney U Statistic = 41.000, T = 251.000 n (small) = 20 n (big) = 22 ($P \leq$ 0.001); (c) N:P ratio: Mann-Whitney U Statistic = 189.000, T = 461.000 n (small) = 20 n (big) = 22 (P = 0.442).
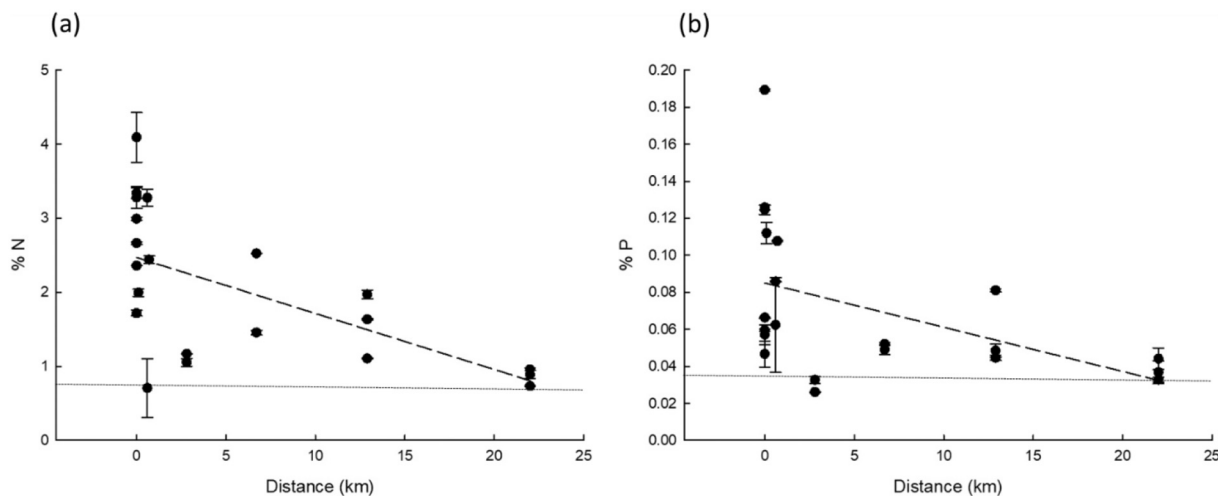
Fig. 4. Regression of mean (+/− SE) (a) percent nitrogen (%N = 2.471 - (0.0757 * Dist)) and (b) phosphorus (%P = 0.0850 - (0.00239 * Dist)) for macroalgae species tissue ($n = 2$) at 10 sites (Table 1) across the shelf (22 km) from Belize City to the fore reef. %N: $R^2 = 0.375$, P = 0.003, %P: $R^2 = 0.226$, $P = 0.03$. Fine dashed horizontal lines indicate mean values for %N (0.71) and %P (0.037) detected at SWCMR in the 1980s (Fig. 6).
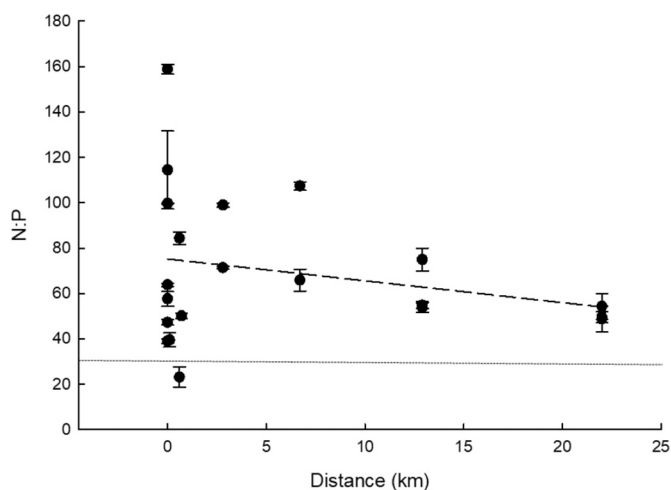

Fig. 5. Regression of the molar nitrogen to phosphorous ratio (N:P = 75.188 - (0.964 * Dist)) for macroalgae tissue (n = 2) at 10 sites (Table 1) across the shelf (22 km) from Belize City to the fore reef. Fine dashed horizontal line indicates mean N:P ratio (30:1) observed at South Water Caye Marine Reserve in the 1980s (Fig. 6). $R^2 = 0.061$ (heavy dashed line), $P = 0.696$. Error = SE.

vs. 2 ($P < 0.001$), 2 vs. 3 ($P \leq 0.001$), 1 vs. 3 ($P < 0.001$) (Fig. 8b).

3.4. Salinity

All distances for the Belize City transect sample sites of salinity and macroalgae were taken from the closest point of mainland and where a depth of 2 m was encountered so the salinity probe did not touch bottom. A distance of zero kilometers was used for sites 4 and 10, which are at the two main freshwater discharge points (Belize River, Haulover Creek), and site 1 located at a public dock along a seawall (Fig. 1). Low salinity (1.36 to 6.84 ppt) surface waters (0.5–1.0 m) measured in May and November 2019 and May 2020 at the lower reaches of the Belize River (sites 3, 4, 5) (Fig. 1c) indicated significant discharges of freshwater coming from the broader watershed into the estuary and lagoon (Fig. 9). These freshwater discharges also appeared to carry sediments that were visible as a brown plume around the peninsula of Belize City (Fig. 1c). All other sites indicated salinities between 30.7 and 40.2 ppt consistent with coastal marine waters out to the fore reef (Fig. 9), even as some sites (1, 2, 10) were found within the nearshore sediment plume (Fig. 1c) and grouped with nearshore sites. Values measured in November 2019 ("wet season") were very consistent with the patterns observed in May 2019 and 2020 ("dry season") with potential differences in salinity only evident immediately following heavy rain events associated with severe weather or stalled meteorological systems.
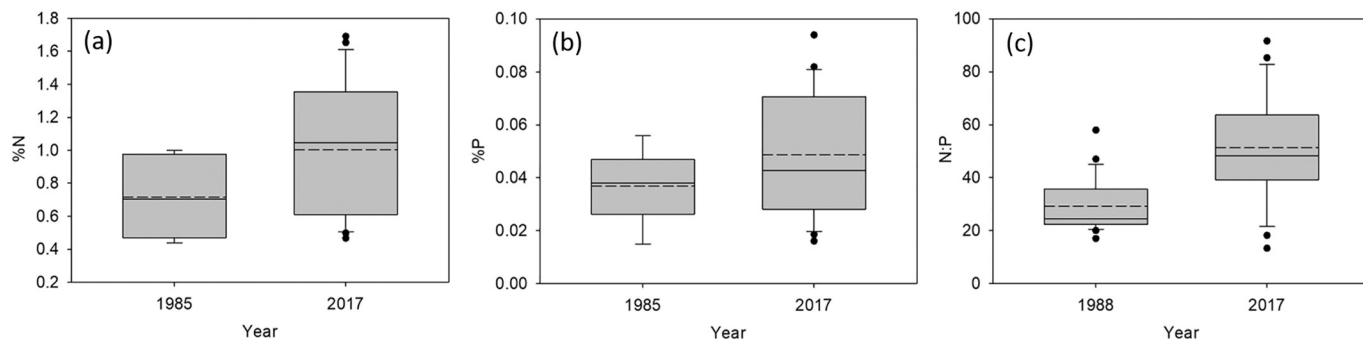

Fig. 6. (a) Contents of nitrogen (%N), (b) phosphorus (%P) and (c) molar ratio (N:P) for macroalgae at 3 offshore sites in South Water Caye Marine Reserve (SWCMR) assessed in the 1980s (Lapointe et al., 1987, 1992a) and 2017 (Lapointe and Tewfik, 2018) (Table 2). Dashed line in box plot is the mean value, solid line is median, box represents 25% and 75% percentile, caps are 10% and 90% percentile, points indicate all outliers. (a) %N: Mann-Whitney U Statistic = 32.000, $T =$ 53.000 n(small) = 6 n (big) = 21 ($P = 0.075$); (b) %P: Mann-Whitney U Statistic = 46.000, $T = 67.000$ n(small) = 6 n (big) = 21 ($P = 0.336$); (c) N:P ratio: Mann-Whitney U Statistic = 77.000, $T = 658.000$ n (small) = 21 n (big) = 24 ($P < 0.001$).
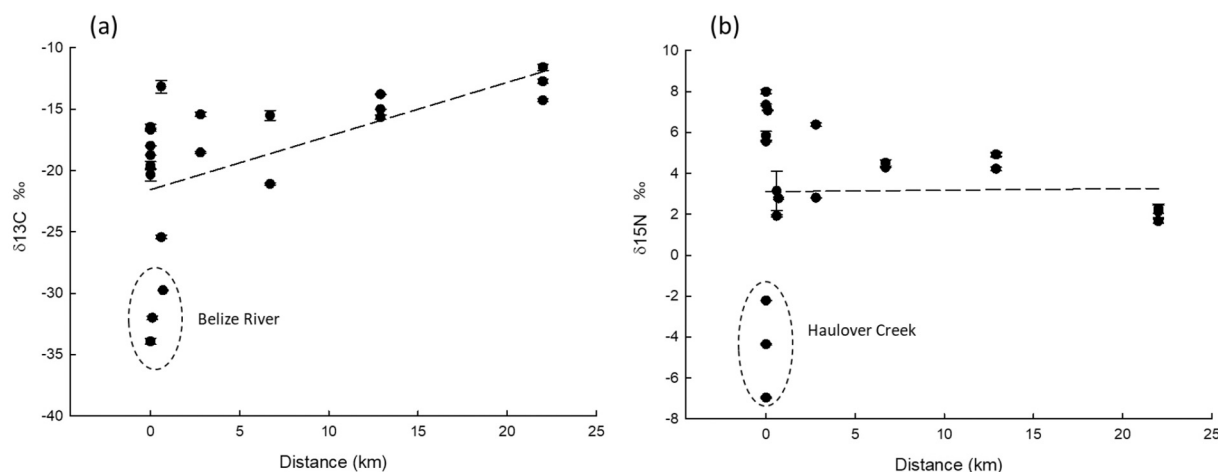
Fig. 7. Regression of mean (+/− SE) (a) $\delta^{13}C$ ($\delta^{13}C = -21.551 + (0.437 * Dist)$) and (b) $\delta^{15}N$ ($\delta^{15}N = 3.114 + (0.00696 * Dist)$) for macroalgae tissue (N = 2) at ten sites (Table 1) across the shelf (22 km) from Belize City to the fore reef. $\delta^{13}C$: $R^2 = 0.312$, $P = 0.008$, $\delta^{15}N$: $R^2 = 0.0002$, $P = 0.949$. Ellipses highlight groups of data at the outlet of the (a) Belize River (site 3, 4, 5) and (b) Haulover Creek (site 10) which indicate noteworthy terrestrial carbon and bacterial fractionation signatures respectively.
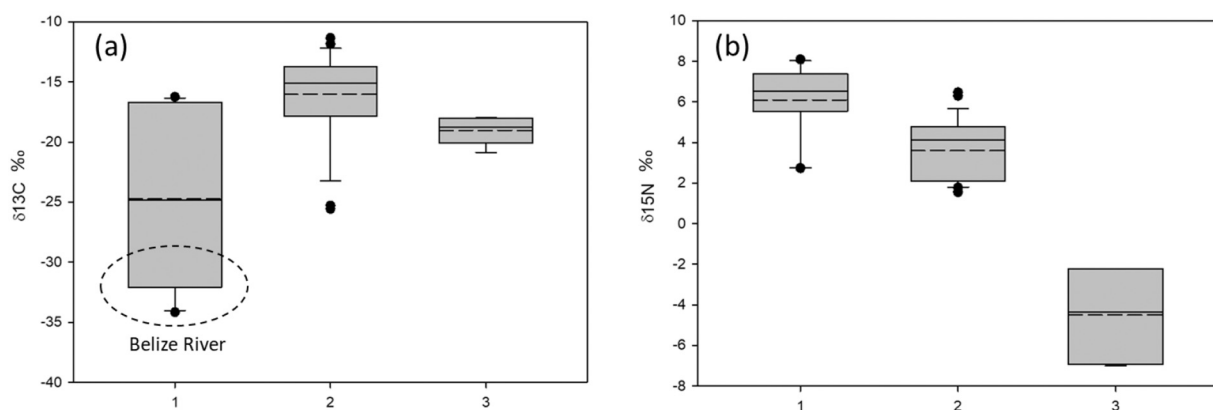

Fig. 8. Stable isotopes of (a) $\delta^{13}C$ and (b) $\delta^{15}N$ for macroalgae in nearshore (1), offshore (2) and Haulover Creek (3) sites. Ellipse (a) indicates range of signatures at the outlet of the Belize river. Dashed line in box plot is the mean value, solid line is median, box represents 25% and 75% percentile, caps are 10% and 90% percentile, points indicate all outliers. $\delta^{13}C$ Kruskal-Wallis One-Way Analysis of Variance on Ranks, H = 16.201 with 2 degrees of freedom (P < 0.001). Pairwise Multiple Comparison Procedures (Dunn's Method) for $\delta^{13}C$: 1 vs. 2 (P < 0.001), 2 vs. 3 (P = 0.083), 1 vs. 3 (P = 1). $\delta^{15}N$ One-Way Analysis of Variance, F = 83.1 with 2 degrees of freedom, (P < 0.001). Pairwise Multiple Comparison Procedures (Holm-Sidak method) $\delta^{15}N$: 1 vs. 2 (P < 0.001), 2 vs. 3 (P ≤ 0.001), 1 vs. 3 (P < 0.001).

4. Discussion

Several lines of evidence from this study support the hypothesis that increasing nutrients, especially N, from the Belize River and Haulover Creek, sustain macroalgal blooms from nearshore to offshore waters of the BBR. First, significant offshore gradients of $\delta^{13}C$, %N, and %P in macroalgae all indicated land-based sources of these key nutrients. Second, C:N and C:P ratios of macroalgae were lower in nearshore waters with lower salinity values, pointing to freshwater runoff as the source of nutrient enrichment. Third, $\delta^{15}N$ values of macroalgae were overall enriched well above values for N_2 fixation, especially in nearshore waters, where values matched those reported for sewage pollution. Last, the N:P ratios of macroalgae were elevated across the entire nearshore-to-offshore gradient to values of ~70:1, some four-fold higher than the Redfield Ratio (16:1) for oceanic waters and more than two-fold higher than values for macroalgae on the BBR in the mid-1980s (~30:1) (Lapointe et al., 1992a). This indicates widespread N-enrichment, increasing N:P ratios and a strengthening of P-limitation, which is consistent with pelagic *Sargassum* across large areas of the Atlantic basin and Caribbean Sea (Lapointe et al., 2021).

The wide range in macroalgae $\delta^{13}C$ values in the study represents a gradient of terrestrial to oceanic influence, with more depleted values typical of terrestrial dissolved inorganic carbon (DIC) from watersheds in the lower salinity water (<5 ppt). The more enriched values across the transect to the offshore fore reef are typical of higher salinity (>34 ppt) and marine DIC (Olsen et al., 2010). The opposite trend of enriched macroalgal $\delta^{15}N$ values in nearshore urbanized areas to more depleted values in offshore waters has been widely reported for sewage-stressed coral reefs in the Caribbean region, including southeast Florida (Lapointe et al., 2005; Lapointe and Bedford, 2010), Florida Keys (Lapointe et al., 2004), Tobago (Lapointe et al., 2010), Jamaica (Lapointe et al., 2011) and the Bahamas (Barile and Lapointe, 2005). The more enriched $\delta^{15}N$ values > +3‰ in the nearshore macroalgae reflect the more processed N from sewage, manure effluent and animal processing waste whereas values < +3‰ reflect more depleted N sources such as N_2 fixation (0‰), atmospheric deposition (−3.0 to +1.0‰), and synthetic fertilizers (0 to +3‰) (Lapointe and Bedford, 2007). While we were unable to evaluate the details of the pattern of freshwater discharge and flow from the Belize River and Haulover Creek into the nearshore and broader BBR lagoon, a number of approaches (hydrodynamic modelling, remote sensing) have recently been used to assess freshwater flows and associated impacts of land-based pollution
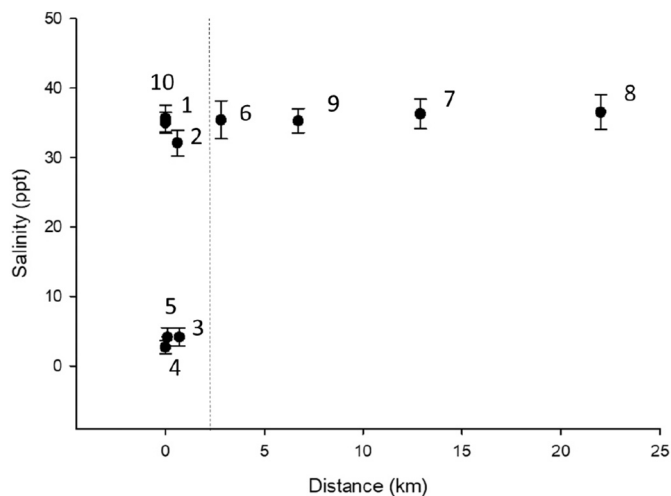
Fig. 9. Mean (+/− standard error) salinity values (ppt) in surface waters (0.5–1.0 m depth) at ten sampling sites (see Table 1) from around Belize City, the Belize River outfall and to the offshore reef 22 km east. Measurements were collected between May 2019, November 2019 and May 2020 in close proximity to macroalgae sample collections in March 2019. Dotted vertical line indicates division between sites grouped as nearshore (0–0.7 km) located within the dry season "brown" plume of the Belize River and Haulover Creek, sites 1, 2, 3, 4, 5, 10, and offshore (2.8–22.0 km) in "clear" water, sites 6, 7, 8, 9 (Fig. 1c).

on coral reef systems elsewhere (Alvarez-Romero et al., 2013; Wolff et al., 2018). These could be applied to our study sites and the broader BBR in future work.

The mouth of Haulover Creek was clearly the most polluted site examined in the study, as it drains directly through the commercial center of the Belize City watershed that is heavily impacted by untreated sewage (Fig. 1c). In addition, the impact of seafood processing effluents from the Northern and National Fishermen's Cooperatives, which are responsible for most of the country's hundreds of metric tonnes of queen conch and Caribbean spiny lobster exports annually (Huitric, 2005), cannot be ignored. The high organic pollutant loads associated with untreated discharge of decaying animal matter would result in ammonium-rich anoxic/hypoxic waters. The macroalgae collected at this site included the red macroalgae *Gracilaria tikvahiae* and *Acanthophora spicifera*, as well as the green macroalga *Ulva chaetomorphoides*, all of which are well-known nutrient pollution indicator species (Lapointe and Bedford, 2011; Whitehouse and Lapointe, 2015). In such nutrient polluted surface waters where these algae were collected (seawall, intertidal zone), high rates of bacterial nitrification convert ammonium to nitrite and nitrate under aerobic conditions. This microbial processing can result in isotopic fractionation and depletion of macroalgae $\delta^{15}N$ values by −6 to −9‰ (Ochoa-Izaguirre and Soto-Jimenez, 2015). More specifically, given the average $\delta^{15}N$ signature that we observed in all other sites (enriched, +4.4‰), depletion by −6 to −9‰ as described above could result in $\delta^{15}N$ values from −2.4 to −5.4‰, where the mean $\delta^{15}N$ value at site 10 (Haulover Creek) was −4.5‰ (+/−0.87). In addition, the average natural $\delta^{15}N$ signature of the majority of product processed at local seafood facilities on Haulover Creek (both <500 m from site 10), queen conch (*L. gigas*) and Caribbean spiny lobster (*P. argus*), is approximately +4.6‰ (Tilley et al., 2013) and could be depleted by microbial fractionation at this site to signatures we detected. Unfortunately, the processing of the two most important fisheries products in Belize (conch and lobster) (Tewfik et al., 2019; Tewfik et al., 2020) is negatively impacting the water quality where these species live and are harvested from. Depleted $\delta^{15}N$ source values from atmospheric deposition of N could also contribute to these low isotope values at this site.

The macroalgal C:N ratios revealed spatial patterns of land-based N enrichment from the combined runoff of Haulover Creek and the Belize

River. The low C:N ratios (8.8–10.1) in macroalgae at the Belize City dock (site 1, Table 1) reflect the high concentrations and availability of dissolved inorganic nitrogen (DIN = ammonium + nitrate + nitrite) typical of sewage polluted urban environments (Lapointe et al., 2010; Lapointe et al., 2011). The lower salinity values (<5 ppt) observed at the discharge of the Belize River sites are indicative of freshwater runoff into the coastal zone, which can carry high DIN concentrations that support macroalgal blooms (Lapointe et al., 1987; Whitehouse and Lapointe, 2015). Several abundant red and green macroalgae species collected at these inshore sites, including *Hypnea musciformis*, *Acanthophora spicifera*, *Gracilaria tikvahiae*, and *Ulva chaetomorphoides*, are well known nutrient indicator species that form widespread blooms in sewage polluted, DIN enriched waters of Florida's Indian River Lagoon (Lapointe et al., 2015), Buccoo Reef Complex, Tobago (Lapointe et al., 2010), and Hawaii (Dailer et al., 2010; Lapointe and Bedford, 2011). Experimental lab studies show how the growth rate of bloom-forming red algae *Gracillaria foliifera* becomes saturated at low DIN concentrations of <1 μM and at higher concentrations initiates rapid uptake kinetics to sequester DIN to support future growth demands (Deboer et al., 1978; Delia and Deboer, 1978). Higher C:N values (32.5–39.3) in the brown macroalgae *Sargassum polyceratium*, *Dictyota cervicornis*, and *Turbinaria turbinata* at the offshore fore reef site reflected lower DIN concentrations and less eutrophic conditions.

It is not widely understood that the human alteration of the global N and P cycles (Steffen et al., 2015) has resulted in global patterns of N enrichment and widespread increases in the N:P ratio well above the Redfield Ratio of 16:1 (Lapointe et al., 2021). However, the very high N: P ratios in macroalgae (mean ~70:1) across the entire Belize City shelf transect (Figs. 3c, 5) provides evidence that cumulative land-based runoff has impacted the N:P stoichiometry well above the historical levels (mean ~30:1) from relatively clean waters, indicating strengthening P-limitation out to the fore reef 22 km offshore (Fig. 5). The high N:P values detected within the SCWS, declared primarily for the protection of the endangered West Indian manatee, are similar to those detected in the N-enriched northern Indian River Lagoon, Florida in 2012 (N:P = 56:1), where harmful algal blooms and catastrophic seagrass die-off led to an Unusual Mortality Event (UME) of the endangered Florida manatee *Trichechus manatus latirostris* in 2013 (Capper et al., 2013; Lapointe et al., 2015) and more recently in the winter of 2021 (Chesnes, 2021). In comparison, mean N:P ratios in a broad sampling of macroalgae from across the SWCMR, Belize (Fig. 1) from 1987 to 1990 were all <36:1, with macroalgae on patch coral reefs at Curlew Caye and Tobacco Reef averaging 22.1 and 27.1, respectively (Lapointe et al., 1992a). This suggests that N:P ratios have tripled over the past three decades within the main BBR lagoon, remarkably similar to the three-fold increases in N:P of macroalgae at Looe Key reef in the lower Florida Keys since the 1980s (Lapointe et al., 2019). This dramatic increase in the N:P at Looe Key followed increased flows of freshwater from the Everglades watershed (N:P = 260:1), which begins at the urban center of Orlando, Florida, discharging into Florida Bay and flowing south through the Florida Keys region (Lapointe et al., 2019). While symbiotic scleractinians are adapted to N-limitation and low N:P ratios (Muscatine and Porter, 1977), the currently high N:P ratios and strong P-limitation at Looe Key and the BBR are well known to cause metabolic stress in scleractinian corals through P starvation that exacerbates coral diseases, bleaching, and die-off (D'Angelo and Wiedenmann, 2014; Lapointe et al., 2019; Rosset et al., 2017; Wiedenmann et al., 2013; Wooldridge, 2009). This nutritional imbalance was apparent in Negril, Jamaica, a major tourist destination, when N:P ratios of macroalgae on shallow fringing reefs averaged ~65:1 in 1998 during a period of catastrophic scleractinian coral decline from diseases, bleaching, and overgrowth by macroalgae (Lapointe et al., 2011). However, others concluded that such phase-shifts from coral to macroalgae was simply due to overfishing of herbivores and reduced grazing (Hughes, 1994). Unfortunately, nutrient data were not collected and it was assumed that Negril and other fringing reef monitoring sites in Jamaica, including the nearby urban

center of Montego Bay with a population of almost half a million, were not affected by land-based nutrient pollution (Hughes, 1994).

The current study did not include measurement of dissolved nutrient concentrations in seawater, which requires rigorous protocols for collection, handling, filtration, storage, and appropriate methods for analysis, specifically low Method Detection Limits (MDLs) (D'Elia et al., 1989). Because the measurement of dissolved nutrients on coral reefs is often poorly done using methods not designed for low MDLs (e.g. HACH kits) (Lapointe, 2004; McClanahan et al., 2002), its' usefulness in assessing levels of nutrient enrichment is limited. This is also due to the rapid uptake, short-lived nature, and high variability of these biologically reactive chemical constituents (DIN; soluble reactive phosphorus, SRP) in naturally oligotrophic waters around coral reefs, which may nevertheless have significant impacts on coral reefs even at low levels (Bell, 1992; Lapointe, 1997; Lapointe et al., 1992a). The problems associated with poor quantification of dissolved nutrients may have substantively contributed to the arguments that "bottom-up" effects of nutrients are unimportant compared to "top-down" effects of reduced grazing as a cause of macroalgal blooms and decline in scleractinian coral cover and functional loss (Hughes, 1994; McClanahan et al., 2002; McClanahan et al., 2003; Mumby et al., 2006). On DIN-enriched Caribbean coral reefs with macroalgal blooms and high tissue N:P ratios like the BBR, SRP is strongly growth-limiting and concentrations are very low, for example 0.03 to 0.07 μM in the Florida Keys (Lapointe et al., 2004) and 0.01 to 0.07 μM on the north coast of Jamaica (Lapointe et al., 2011). These low SRP concentrations can exacerbate coral diseases, bleaching and die-off in DIN-enriched systems due to high N:P ratios and associated P starvation in scleractinian corals (Lapointe et al., 2019; Rosset et al., 2017), so accurate measurement of both DIN and SRP are critical to understanding coral stress and decline, if they are to be used. However, common macroalgae species, functioning as bioindicators, are relatively easily collected, processed and analyzed for % N, %P, and N:P ratio as well as δ^{13}C and δ^{15}N. They present a much more practical and cost-effective approach to detecting sources of nutrient contamination and targets for long-term monitoring of changes in nutrient regimes across large spatial scales, especially where resources and analytical facilities are limited.

In summary, this study, using benthic macroalgae as cumulative bioindicators of nutrient enrichment, is the first to suggest that nutrient stress caused by N enrichment and elevated N:P ratios is a primary mechanism by which human activities within watersheds and coastal environments of Belize (Auil-Gomez, 2014; Boles and Requena, 2011; Cherrington et al., 2012) are negatively impacting the BBR. This is perhaps most acute for the health and biodiversity of scleractinian corals that are the critical ecosystem engineers of tropical reefs. Specific concerns over the impacts of agriculture, sewage, increasing numbers and capacity of cruise ships and tourism infrastructure, effluent from large-scale aquaculture production (e.g. shrimp), and large amounts of nutrient-rich ash from forest fires in the region have also been highlighted as contributing to examples of declining water quality and hyper-eutrophication in areas such as the Placencia Lagoon and the New River, Belize (Fig. 2d) (Auil-Gomez, 2014; Diedrich, 2010; Garcia et al., 2017; Ledwin, 2010). This coincides with concerns over the impacts of poor water quality across the Belize Barrier Reef Reserve System UNESCO World Heritage Site and a potentially premature removal of the site from the 'In Danger' list (Byron and Osipova, 2013; Murry, 2020). Such nutrient stress contributes significantly to the coral-algae phase shift and loss of biodiversity even in the presence of reasonably protected and healthy herbivore communities (Arias-González et al., 2017; Suchley et al., 2016; Tewfik et al., 2017). The science supporting the need for a proactive approach to nutrient management for the health and protection of coral reefs is sound (D'Angelo and Wiedenmann, 2014; El-Khaled et al., 2021; Lapointe et al., 2019; Rosset et al., 2017).

To manage the adverse effects of nutrient over-enrichment, nutrient thresholds (i.e. nutrient criteria, nutrient standards) have been adopted in Florida's oligotrophic Everglades (Florida Public Law 62-302.540) and coastal waters that include coral reefs in South Florida (Florida Public Law Chapter 62-302.532). The United States Environmental Protection Agency has also established a DIN maximum target value of 0.75 μM for coral reefs in the Florida Keys National Marine Sanctuary (Lapointe et al., 2019). Nutrient enrichment and eutrophication have long been an environmental concern on the Great Barrier Reef, with similar management efforts as in Florida having been instituted in Australia to protect the GBR from nutrient impacts, especially focused on reductions in DIN (Alluvium, 2019; Bell, 1992; Brodie et al., 2011). Although development of similar nutrient criteria may not be immediately practical for Belize, a number of actions could be taken that include the promotion of Best Management Practices (BMPs) and Basin Management Action Plans (BMAPs) to reduce nutrient loading from urban effluents and agricultural areas to improve water quality and restore the shallow coastal ecosystems of the BBR. The practicality of such actions are complicated by the ongoing dispute over territorial boundaries with Guatemala who share the area of several watersheds including that of the Belize River (Perez et al., 2009). Positive change will be facilitated by continuing efforts in standardizing appropriate MDLs and water quality monitoring protocols, compiling ecological assessments of impacts, adopting a focused ridge-to-reef conservation strategy and increasing awareness of nutrient contamination among the general public and political bodies (Boles, 2018; Boles and Requena, 2011; D'Elia et al., 1989; Winemiller et al., 2011). Coral reefs and associated seagrass ecosystems provide significant livelihoods (fishing, tourism) and other critical ecological services (coastal protection) to the growing population of Belize (Auil-Gomez, 2014). Furthermore, they represent a globally significant natural ecosystem complex and as such must be protected from ongoing impacts of unsustainable development in upland watersheds and the coastal environment.

CRediT authorship contribution statement

Brian E. Lapointe: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Alexander Tewfik:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Myles Phillips:** Investigation, Resources, Writing – review & editing, Visualization.

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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