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## Isotopic composition of aquatic and semiaquatic plants from the Mexican Caribbean: A baseline for regional ecological studies

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### ABSTRACT

Primary producers in the aquatic community structure are fundamental elements because they are the first link in the trophic network as occur in most ecosystems. They produce oxygen and biomass, act as a shelter for several species, and provide food for a wide variety of megaherbivore species like manatees and sea turtles. Physico-chemical processes taken place in primary producers can be determined through stable isotope analysis (SIA) as natural tracers from elements like carbon and nitrogen, applied in ecological, and physiological studies. In the Mexican Caribbean ecosystems, SIA has been little applied in aquatic plants, where *Thalassia testudinum* is the main seagrass species studied. Here, we present the isotopic composition ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) from 95 aquatic and semiaquatic plant species of four vegetation types, from three different environments, hydroclimatic seasons, and geographical zones in the Mexican Caribbean. Main statistical differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were found according to vegetation type and environment. Besides, for  $\delta^{13}\text{C}$  were also found statistical differences among seasons, while for  $\delta^{15}\text{N}$  differences were found among zones (H-Test,  $p < 0.05$ ). This study provides an isotopic baseline for further ecological studies in the region. This information can contribute to understanding the structure of aquatic food webs and infer the diet and feedings habits of aquatic species, as well as to detect possible changes related to anthropogenic activities that can affect the survival of these plant species, and the fauna depending on them.

### 1. Introduction

Aquatic environments along the coastline of the Mexican Caribbean harbor a great diversity of aquatic and semiaquatic plant species. There are more than 540 types of macroalgae (Aguilar Rosas et al., 1998; González-Solis et al., 2018; Vilchis et al., 2018), seven varieties of seagrasses (Rioja-Nieto et al., 2019), and four mangrove species (Agraz-Hernández et al., 2006), among others. Macroalgae, seagrasses and

mangroves provide important benefits to ecosystems because they are photosynthetic organisms, and therefore, key elements in the ocean community structure. They are often the first link in the trophic chain of aquatic ecosystems as they produce oxygen and biomass, provide shelter for several animal species, and represent a food source for many others (León Álvarez et al., 2012). Macroalgae are considered good aquatic bioindicators since they fully depend on water and substratum to complete their physiological processes. Thus, any disturbance of physical and

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chemical characteristics of the environment leads to changes in their composition, abundance, and diversity (Valdez-Cruz et al., 2015). Seagrasses and mangroves play a significant ecological role in coastal marine zones, because they are an important source of carbon in the detritus cycle (Duarte et al., 2007). Furthermore, they help to mitigate impacts from natural phenomena like hurricanes by providing a natural protective barrier for the coastline, reducing the effects of erosion and catching and storing nutrients (Hemminga and Duarte, 2000; Torres and Rivera, 1989). These ecosystems act as habitat and breeding sites for fishes and invertebrates (Conabio, 2009; Green and Short, 2003) and provide food for a wide variety of megaherbivores (Castelblanco-Martínez et al., 2009; Green and Short, 2003).

Stable isotope analysis (SIA) applied to aquatic plants can provide knowledge about ecological changes in primary producers and modification of natural sources in a food web which could have significant impacts on herbivorous animals (Ehleringer and Cerling, 2002), like manatees (Alves-Stanley et al., 2010) and sea urchins (Cabanillas-Terán et al., 2019). Isotopes are natural tracers of physicochemical processes and application of SIA has mainly focused on ecological, physiological and paleontological studies (Clementz et al., 2007; Guerrero and Berlanga, 2000; Newsome et al., 2010). For the present study, we analyzed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in four aquatic and semiaquatic vegetation types (seagrasses, macroalgae, mangroves and "other vascular plants").  $\delta^{13}\text{C}$  of primary producers provide information about the way plants adjust their metabolism during the gaseous exchange, identify resource strategies (Farquhar et al., 1989), and infer the efficiency of water use during photosynthesis (Farquhar et al., 1982). Differences among primary producers are predicted by the photosynthetic pathway. For

instance,  $\delta^{13}\text{C}$  values of  $\text{C}_4$  plants are less impoverished in  $^{13}\text{C}$  (Peterson and Fry, 1987), range from  $-20\text{‰}$  to  $-10\text{‰}$ , whereas  $\delta^{13}\text{C}$  in  $\text{C}_3$  plants, usually fluctuates between  $-33\text{‰}$  and  $-22\text{‰}$  (Bender, 1971). Moreover,  $\delta^{15}\text{N}$  is useful to identify biogeochemical processes and nitrogen sources of the trophic net (Kohl et al., 1973), which can be affected by natural events (e.g. phytoplankton bloom, denitrification and nitrification processes, etc), or anthropogenic factors (e.g. domestic or industrial wastewater discharges) (Peterson, 1999). The  $\delta^{15}\text{N}$  values on plant tissues vary between  $-5\text{‰}$  and  $+10\text{‰}$  (Mariotti, 1983). Nitrogen isotopic fractionation in plants occurs as a result of nitrate ( $\text{NO}_3^-$ ) or ammonium ( $\text{NH}_4^+$ ) assimilation, translocation toward the leaves, and nitrogen metabolism in the cytoplasm (Mariotti et al., 1980).

The most common aquatic plant species studied using SIA are seagrasses and macroalgae (Ciotti, 2012; Fry, 1984; Loneragan et al., 1997; Reich and Worthy, 2006). *Thalassia testudinum* is the most studied species in the Mexican Caribbean (Camacho-Cruz et al., 2019; Carruthers et al., 2005; Mutchler et al., 2007, 2010; Rodríguez Juárez, 2011; Sánchez et al., 2013). The aim of this research was to analyze  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of aquatic and semiaquatic plants collected in the Mexican Caribbean, in order to identify possible isotopic differences among vegetation type (seagrasses, macroalgae, mangroves, and "other vascular plants"), environment (marine, estuarine and freshwater), geographic distribution (north, center, and south zone), and hydroclimatic season (rainy, dry, and cold-fronts). Results will provide isotopic baseline information for 95 aquatic and semiaquatic plant species along the Mexican Caribbean coast, which can be used for further ecological research in the region.

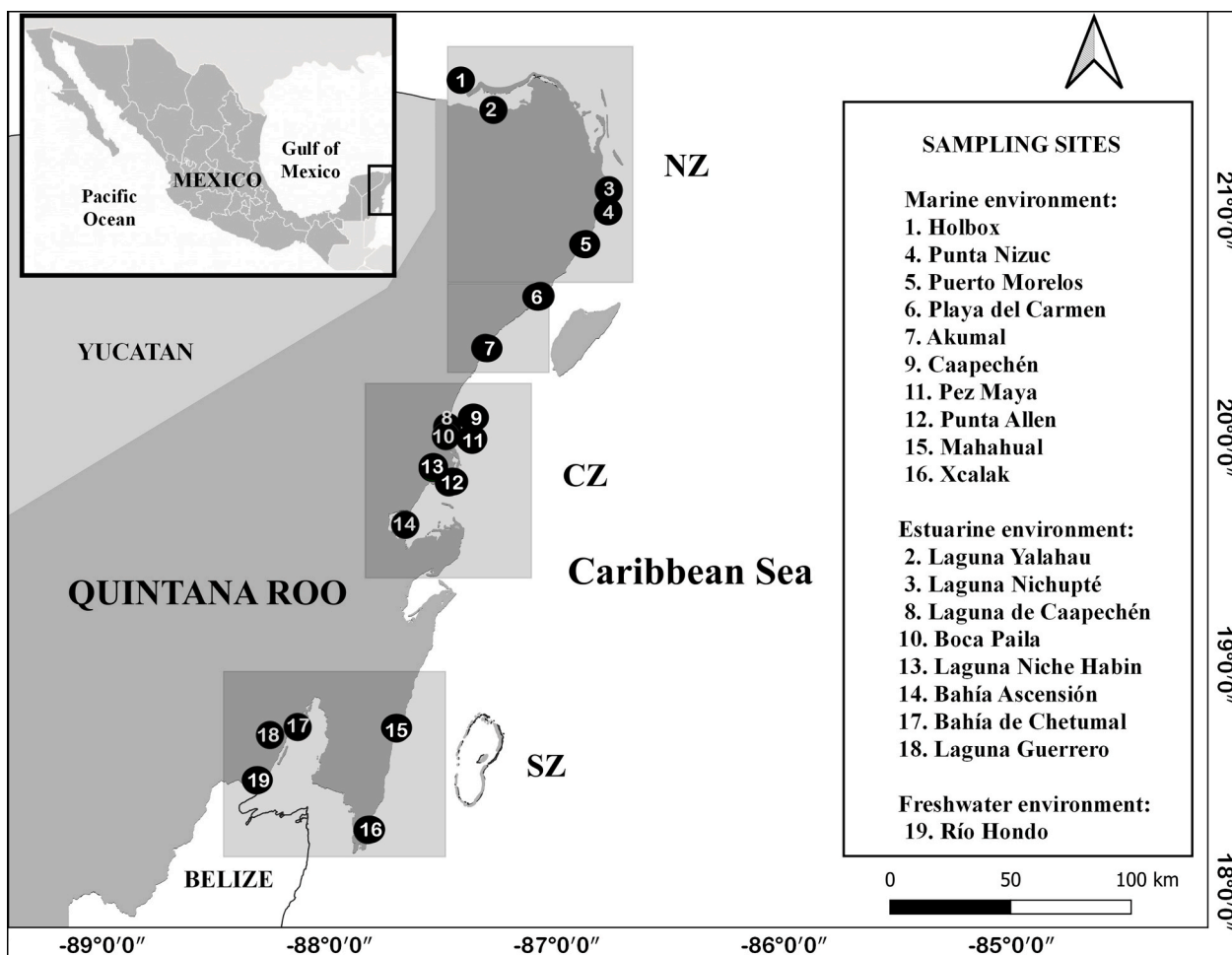


Fig. 1. Sampling sites along the Mexican Caribbean where aquatic and semiaquatic plants were collected. NZ = north zone; CZ = center zone; SZ = south zone.

## 2. Materials & methods

### 2.1. Study area

The Mexican Caribbean is located in the state of Quintana Roo, which is delimited to the north by Holbox Island (21° 31' N; 87° 23' W), and to the south by Chetumal Bay (17°52'-18°50'N, 87°50'-88°25'W) (Fig. 1). This region has a coastal system of karstic origin, including a great heterogeneity, high infiltration rate, and fast flux, making it a vulnerable ecosystem (Aranda-Cirerol et al., 2011; Bakalowicz, 2005). In addition, it belongs to the second most important coral reef barrier worldwide, the megadiverse Mesoamerican Barrier Reef System (Aguilar et al., 2008b). One of the most important vegetation communities in the coastal zone are mangroves, which typically border lagoons, estuaries and bays (De la Lanza Espino, 2004). The predominant climate in this region is warm and subhumid, with an annual mean temperature of 26 °C (De La Lanza Espino et al., 2013). The Mexican Caribbean has three different seasons (rainy, wet, and cold-fronts), and has frequent tropical storms and hurricanes (Carrillo et al., 2009). Three important bays exist in Quintana Roo: Ascensión, Espíritu Santo, and Chetumal (De La Lanza Espino et al., 2013). The Hondo river is the only superficial river in the State, which flows into Chetumal Bay (Herrera-Sansores and Heredia-Escobedo, 2011).

### 2.2. Aquatic plants sampling

From July 2017 to May 2018, samples of aquatic and semiaquatic plants were manually collected using freediving equipment in shallow waters (depth < 3 m) (Caricomp, 2001). The sampling was conducted along to the Mexican Caribbean coast, from the northernmost point at Holbox, to Chetumal Bay south of Quintana Roo. Three environments were covered (marine, estuarine and freshwater), during three hydroclimatic seasons (rainy, dry, and cold-fronts). The region was divided into three zones (north, center and south) according to the distribution of several aquatic and semiaquatic plants reported as food items for manatees in the study area (Castelblanco-Martínez et al., 2009; Espinoza-Avalos, 1996; Flores-Cascante et al., 2013), as well as based on previous studies related to manatee habitat use (Morales-Vela and Olivera-Gómez, 1997; Morales-Vela and Padilla-Saldívar, 2009). Moreover, 19 randomly selected sampling sites were defined (Fig. 1), according to SIA studies applied in aquatic plants from the Mexican Caribbean (Carruthers et al., 2005; Mutchler et al., 2007, 2010; Sánchez et al., 2013), and principal manatee habitats (coastal lagoons, estuaries, and rivers) (Morales-Vela and Padilla-Saldívar, 2001). In most cases, three specimens of each morphotype were collected and stored in labeled paper bags to keep them dry for further morphological identification and SIA. At the laboratory of Ecology and Molecular Biology of Quintana Roo University (UQROO), vegetation specimens were identified when able to species level using common keys (Agraz-Hernández et al., 2006; Guterres et al., 2008; Littler and Littler, 2000; Van Tussenbroek et al., 2010), stereoscopic and compound Olympus microscopes. The collected samples were grouped according to vegetation type in four groups: seagrasses, macroalgae, mangroves, and “other vascular plants”.

### 2.3. Sample preparation for SIA

Aquatic and semiaquatic plant samples were rinsed with distilled water. A piece of leaf was cut and submerged into hydrochloric acid (10%) to eliminate associated carbonates, followed by a second distilled water rinsing process. Each sample was then oven-dried at 60 °C for 24 h. Samples were grained in an agate mortar until a fine powder was obtained (Sánchez et al., 2013), and subsequently stored into 2 ml vials. Approximately 2 mg of powder per sample were encapsulated into micro tin cups. Later, nitrogen and carbon isotopic composition of samples was analyzed in a Carlo Erba NC1500 elemental analyzer coupled with a Delta Plus XP (ThermoQuest, Bremen, Germany) mass spectrometer

(EA-IRMS). Isotope measurements were carried out at the Stable Isotope Laboratory of the Instituto Andaluz de Ciencias de la Tierra (CSIC-UGR, Granada, España).

### 2.4. Data analysis

Stable isotope ratios were expressed as  $\delta$  (delta) in parts per thousand (‰), using the equation:

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000 \quad (1)$$

where X is  $^{13}\text{C}$  or  $^{15}\text{N}$ ,  $R_{\text{sample}}$  and  $R_{\text{standard}}$  represent the  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  ratio of the sample and standard, respectively. The precision of the measurement was calculated after correction of the mass spectrometer daily drift. The analytical error for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  determinations was <0.1‰. The reference standard for reporting  $\delta^{13}\text{C}$  carbon measurements was Vienna Pee Dee Belemnite (V-PDB) and for  $\delta^{15}\text{N}$  the atmospheric nitrogen (AIR). For carbon, 22 internal standards (organic and inorganic material) ranging between -49.44 and +28.59‰ (V-PDB), contrasted with the IAEA international references NBS-28, NBS-29, NBS-20 (carbonates) and NBS-22, IAEA-CH-7, IAEA-CH-6 (organic material), are used in relation to the isotopic range of samples to be analyzed. This study comprised two internal standards of -30.63‰ and -11.65‰ (V-PDB). For nitrogen, nine internal standards (organic and inorganic material) ranging between -1.94 and +16.01‰ (AIR) served as contrasts to the IAEA international references IAEA-N-1, IAEA-N-2, IAEA-NO-3, USGS32, USGS34 and USGS35. This study also resorted to the two internal standards of -1.02‰ and +16.01‰ (AIR).

### 2.5. Statistical analysis

Data were tested for normality using the Shapiro-Wilk test, as well as Levene's test of homogeneity of variances, in order to find the proper test to determine differences in populations' distribution (Zar, 2010). Since the variables  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  did not present a normal distribution ( $W p = 0.00$  and  $p = 0.01$ , respectively) the non-parametric Kruskal-Wallis test using the H statistic (Zar, 2010) was performed for each factor: vegetation type, environment, zone, and season. A post-hoc Dunn test with Bonferroni correction was used to determine statistical differences among levels into each group. Regarding zones, “other vascular plants” and freshwater samples were excluded from the statistical analysis, because these samples were only collected in the south zone (SZ). A descriptive analysis from the samples was performed with violin graphics in order to represent the distribution of the sample data. We used the mean as a measure of central tendency in order to describe and compare the data with other studies. All statistical analyses were performed using basic functions from R language (R Core Team (2019), and a  $p < 0.05$  was considered statistically significant in all cases.

## 3. Results

A total of 560 samples of aquatic and semiaquatic plants were collected, including 73 algal taxa, five seagrass species, three mangrove species, and 14 taxa of “other vascular plants”, along the Mexican Caribbean (Appendix 1). Stable isotope analysis revealed that seagrasses contained the lesser negative values of  $\delta^{13}\text{C}$ , while “other vascular plants” contained the most negative values. On the other hand, “other vascular plants” had the highest values of  $\delta^{15}\text{N}$ , while seagrasses had the lowest values. When comparing environments, the marine environment had the lesser negative  $\delta^{13}\text{C}$  values while freshwater environments had the highest  $\delta^{15}\text{N}$  values. Stable isotope ratios also differ according to season and geographic distribution. Rainy season had less -negative  $\delta^{13}\text{C}$  values than dry and cold-fronts, and displayed the lowest  $\delta^{15}\text{N}$  values, when compared to the center and north zone (Table 1).

**Table 1**  
 $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values according to vegetation type, environment, hydroclimatic season and geographical distribution.

Vegetation type	$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)		
	Mean $\pm$ SE	Min	Max	Mean $\pm$ SE	Min	Max
Macroalgae (n = 351)	-16.67 $\pm$ 0.20	-29.64	-6.92	3.85 $\pm$ 0.18	2.46	10.33
Seagrass (n = 130)	-10.65 $\pm$ 0.33	-21.79	-2.31	1.85 $\pm$ 0.28	-7.89	10.63
Mangrove (n = 37)	-28.63 $\pm$ 0.24	-31.29	-25.75	3.35 $\pm$ 0.59	-2.55	9.06
Other vascular plants (n = 42)	-29.90 $\pm$ 0.48	-40.17	-21.72	4.05 $\pm$ 0.48	-3.44	9.06
<b>Environment</b>						
Marine (n = 318)	-14.36 $\pm$ 0.26	-29.91	-2.31	3.01 $\pm$ 0.15	-4.21	12.46
Estuarine (n = 184)	-18.28 $\pm$ 0.44	-30.70	-6.11	3.27 $\pm$ 0.32	-7.89	14.24
Freshwater (n = 58)	-27.99 $\pm$ 0.70	-40.17	-14.16	5.53 $\pm$ 0.45	-3.44	13.37
<b>Hydroclimatic season</b>						
Rainy (n = 354)	-16.05 $\pm$ 3.14	-31.61	-2.97	3.30 $\pm$ 1.87	-6.80	14.24
Dry (n = 134)	-18.18 $\pm$ 0.61	-34.60	-2.31	3.61 $\pm$ 0.32	4.03	7.26
Cold-fronts (n = 72)	-20.02 $\pm$ 1.00	-40.17	-5.41	3.24 $\pm$ 0.37	-2.95	10.63
<b>Geographical distribution</b>						
North zone (n = 243)	-15.95 $\pm$ 0.33	-29.91	-2.97	3.24 $\pm$ 0.23	-7.89	14.24
Center zone (n = 105)	-15.31 $\pm$ 0.55	-30.70	-6.11	3.64 $\pm$ 0.31	-4.55	13.98
South zone (n = 149)	-19.23 $\pm$ 0.54	-40.17	-2.31	2.61 $\pm$ 0.26	-5.71	12.46

### 3.1. Changes in $\delta^{13}\text{C}$ y $\delta^{15}\text{N}$ from the aquatic and semiaquatic plants

Bulk sample  $\delta^{13}\text{C}$  values ranged from  $-40.17\text{‰}$  to  $-2.31\text{‰}$  with an average of  $-17.07\text{‰}$  and a median of  $-16.01\text{‰}$ . Statistical differences were found among vegetation type ( $H = 319.22$ ,  $df = 3$ ,  $p < 0.00$ ), environment ( $H = 161.19$ ,  $df = 2$ ,  $p < 0.00$ ), and season ( $H = 16.84$ ,  $df = 2$ ,  $p = 0.00$ ) (Fig. 2). On the other hand, the  $\delta^{15}\text{N}$  values fluctuated between  $-7.89\text{‰}$  and  $14.24\text{‰}$ , with an average of  $3.37\text{‰}$ , and a median of  $3.14\text{‰}$ . Statistical differences were found among vegetation type ( $H = 32.70$ ,  $df = 3$ ,  $p = 0.00$ ), environment ( $H = 27.03$ ,  $df = 2$ ,  $p = 0.00$ ), and zone ( $H = 13.08$ ,  $df = 2$ ,  $p = 0.00$ ) (Fig. 3).

According to vegetation type (seagrasses, macroalgae, mangroves and "other vascular plants"),  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values are differentiated by three principal groups (Fig. 4). Significant statistical differences in  $\delta^{13}\text{C}$  were between macroalgae ( $-16.67 \pm 0.2\text{‰}$ ), and other vegetation types (seagrasses =  $-10.65 \pm 0.33\text{‰}$ , mangroves =  $-28.63 \pm 0.24\text{‰}$ , and "other vascular plants" =  $-29.9 \pm 0.48\text{‰}$ ) ( $p = 0.00$ ), and seagrasses vs. mangroves and "other vascular plants" ( $p = 0.00$ ). For  $\delta^{15}\text{N}$  values, statistical differences were obtained between seagrasses ( $1.85 \pm 0.28\text{‰}$ ), and macroalgae ( $3.85 \pm 0.18\text{‰}$ ), and "other vascular plants" ( $4.05 \pm 0.48\text{‰}$ ) ( $p = 0.00$ ) (Fig. 4).

On the other hand, significant statistical differences were found for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values when compared among environments (Fig. 5).  $\delta^{13}\text{C}$  values were different among environments (marine =  $-14.36\text{‰} \pm 0.26\text{‰}$ , estuarine =  $-18.28\text{‰} \pm 0.44\text{‰}$  and freshwater =  $-27.99\text{‰} \pm 0.7\text{‰}$ ) ( $p = 0.00$ ).  $\delta^{15}\text{N}$  values in freshwater ( $5.53\text{‰} \pm 0.45\text{‰}$ ) was significantly different from the other two environments: marine ( $3.01\text{‰} \pm 0.15\text{‰}$ ), and estuarine ( $3.27\text{‰} \pm 0.32\text{‰}$ ) ( $p = 0.00$ ) (Fig. 5).

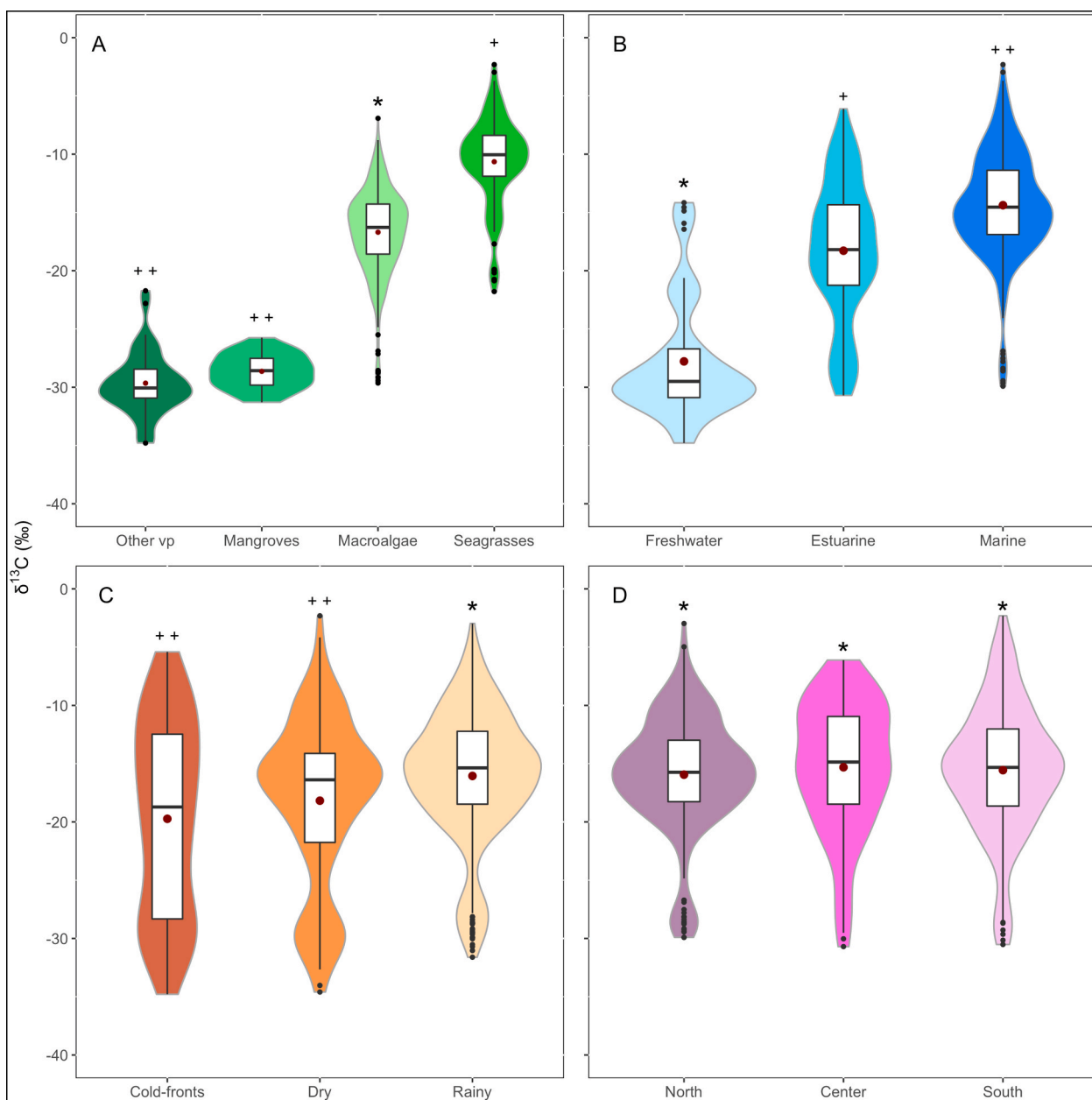
## 4. Discussion

### 4.1. Changes in $\delta^{13}\text{C}$ of aquatic and semiaquatic plants

The  $\delta^{13}\text{C}$  results found among vegetation type, coincide with the values reported by previous studies (Ciotti, 2012; Fry, 1984, 2006; Fry and Sherr, 1989; Mutchler et al., 2010; Ramírez Palomeque, 2013), where the lesser negative  $\delta^{13}\text{C}$  values belong to seagrasses ( $-10.65 \pm 0.33\text{‰}$ ). While all aquatic plants follow  $\text{C}_3$  photosynthesis pathways, our values seem to be closer to those of  $\text{C}_4$  plants (Hemminga and Mateo, 1996). This can be due to a limitation of dissolved  $\text{CO}_2$  in water, which results in a decrease of  $^{13}\text{C}$  discrimination in the plant coupled with the use of bicarbonate ( $\text{HCO}_3^-$ ) as a source of carbon (Anderson and Fourqurean, 2003; García-Sánchez et al., 2016). This characteristic distinguishes between marine and continental species (Berry, 1989). On the other hand, the most negative  $\delta^{13}\text{C}$  values obtained for mangroves ( $-28.63 \pm 0.24\text{‰}$ ), and "other vascular plants" ( $-29.90 \pm 0.48\text{‰}$ ) are typical values of atmospheric  $\text{CO}_2$  sources and photosynthesis type  $\text{C}_3$ , and similar to reports in previous studies (Ciotti, 2012; Del Río Salas, 2014; Loneragan et al., 1997; Rao et al., 1994; Rodelli et al., 1984). Likewise, macroalgae usually present intermediate  $\delta^{13}\text{C}$  values ( $-16.67 \pm 0.2\text{‰}$ ) between seagrasses and the group formed by mangroves and "other vascular plants" (Ciotti, 2012; France, 1995; Loneragan et al., 1997; Mutchler et al., 2010). Variations in  $\delta^{13}\text{C}$  are determined by the isotopic composition of the dissolved inorganic carbon (DIC). As DIC is assimilated ( $\text{CO}_2$  or  $\text{HCO}_3^-$ ), isotopic discrimination of the enzyme is responsible for the carbon fixation, and intracellular concentration of  $\text{CO}_2$  or  $\text{HCO}_3^-$  (Farquhar et al., 1982; France, 1995; Keeley and Sandquist, 1992). Some types of aquatic vegetation use  $\text{HCO}_3^-$  (which is less negative in  $\delta^{13}\text{C}$  values than  $\text{CO}_2$  in approximately  $8\text{‰}$  at  $25^\circ\text{C}$ ) in addition to dissolved  $\text{CO}_2$  (Mook et al., 1974), and as a result, have  $\delta^{13}\text{C}$  values between  $-11.03\text{‰}$  and  $-21.4\text{‰}$  (Maberly et al., 1992). This was observed in  $\delta^{13}\text{C}$  values of seagrasses and macroalgae obtained in our study. Also, more negative  $\delta^{13}\text{C}$  values are present in species for which their principal source of carbon is  $\text{CO}_2$ , varying between  $-30\text{‰}$  and  $-34.5\text{‰}$ , (Maberly et al., 1992), which are similar to those obtained for the group of mangroves and "other vascular plants" in the present study.

Similar to other studies comparing  $\delta^{13}\text{C}$  values plants of marine, estuarine and freshwater environments, freshwater samples showed the most negative  $\delta^{13}\text{C}$  values (Alves-Stanley et al., 2010; Reich and Worthy, 2006). These  $\delta^{13}\text{C}$  differences are principally attributed to intrinsic physicochemical and biological conditions of each environment (Mateo et al., 2004). In freshwater and estuarine environments, the pool of inorganic carbon is influenced by the input of carbon depleted in  $^{13}\text{C}$  derived from organic matter decomposition (respiration) from land (Hemminga and Mateo, 1996). In general, seawater has higher  $\delta^{13}\text{C}$  values of DIC, and as consequence, marine primary production is less negative than continental aquatic production (Fry, 2002). The current study found similar results, since marine vegetation had the less negative  $\delta^{13}\text{C}$  values.

Significant differences found among hydroclimatic seasons (rainy, dry and cold-fronts) could be attributed to the increased freshwater inputs proceeding from runoff and groundwater during rainy season. Thus, less negative  $\delta^{13}\text{C}$  values in primary production are expected in dry season compared to other two seasons since contributions of freshwater during the dry season from runoff or groundwater are scarce due to decrease in precipitation rates. Therefore, decrease in precipitation rates would contribute to higher isotopic values in the tissues of the plant grown in this season. In contrast,  $\delta^{13}\text{C}$  values should be most negative during the rainy season as freshwater contribution leads to most negative DIC (more heterotrophic systems with abundant  $\text{C}_3$  biomass respiration), an important nutrient and land organic matter input into water bodies. This can also be due to the increased influence of DIC depleted in  $^{13}\text{C}$  which in turn originates more negative  $\delta^{13}\text{C}$  values in aquatic and semiaquatic vegetation (Chanton and Lewis, 2002). Despite Quintana Roo is one of the Mexican states with highest



**Fig. 2.** Violin plots showing the variation of  $\delta^{13}\text{C}$  values in aquatic and semiaquatic plants from the Mexican Caribbean according to several factors. The boxplot inside represent the three quartiles, with a median represented by a central horizontal line, and the mean is the red point. Significant differences among categories within each factor are shown with different symbol. **A.** Vegetation type; **B.** Environment; **C.** Hydroclimatic season; **D.** Zone. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

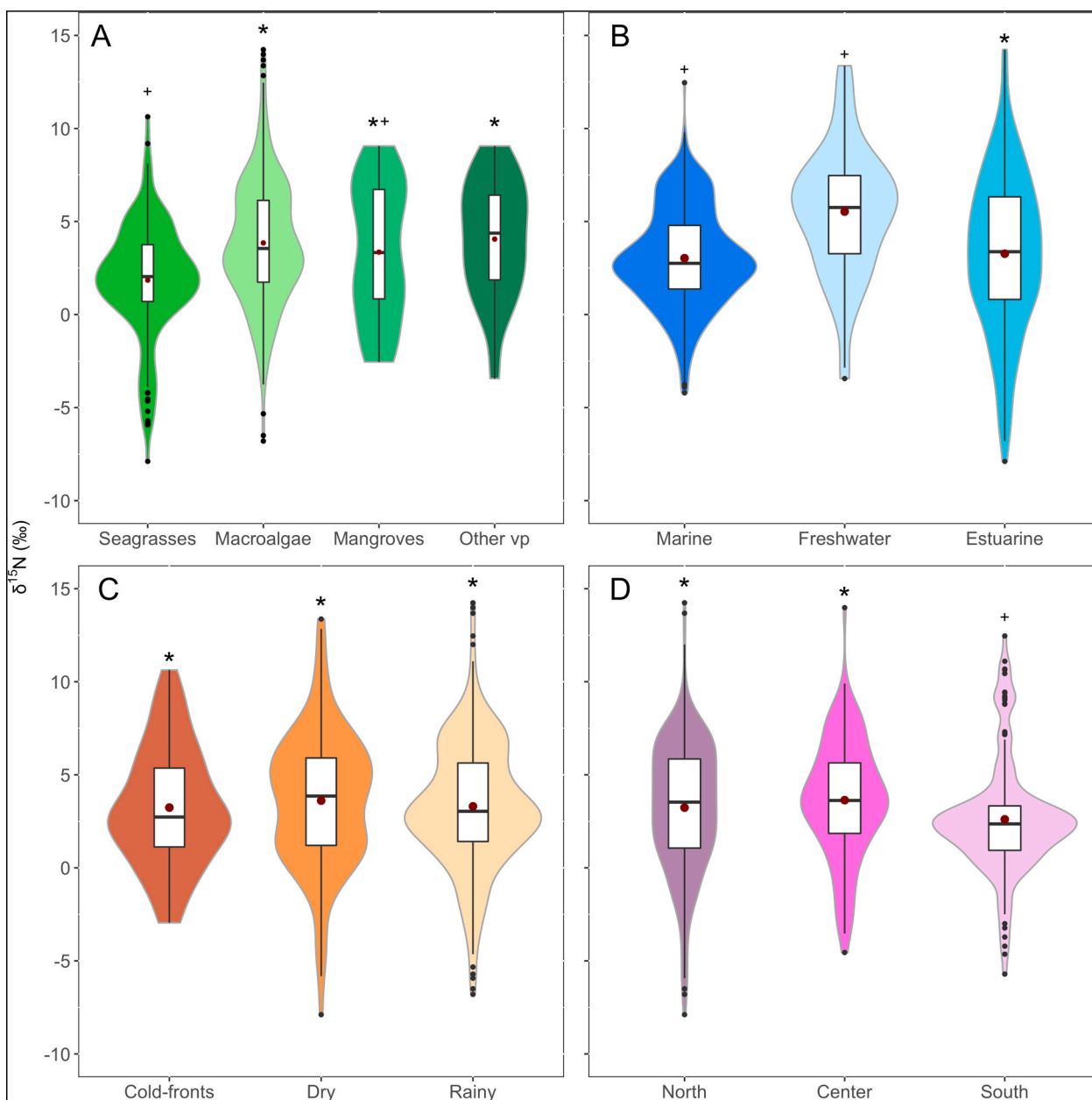
precipitation rates ( $>1000 \text{ mm year}^{-1}$ ), there are no large rivers or streams, except for the Hondo river (Herrera-Sansores and Heredia-Escobedo, 2011). However, it is important to consider the karstic nature of the region which is characterized by underground drainage systems and very permeable soils (Aranda-Cirerol et al., 2011), as a result, the coastal areas receive important inputs of underground, dissolved inorganic and organic carbon.

As reported by other authors in other temperate places, they relate the changes in seasonal isotopic composition of seagrasses with temperature and light (Fourqurean et al., 2005). Also, with increasing temperatures, a decrease in solubility of  $\text{CO}_2$  can lead to a smaller  $\text{CO}_2$  pool and less isotopic discrimination with heavier  $\delta^{13}\text{C}$  values which happens in marine plants (Francois et al., 1993). Otherwise, during cold-fronts, the temperature is lower compared to rainy and dry seasons. Lower temperatures are associated with less rain and less surface runoff

or underground contributions, sources characterized by a contribution of DIC with more negative values (Aguirre, 2002; De La Lanza Espino et al., 2013; Herrera-Sansores, 2011). Consequently, DIC will be less negative in  $\delta^{13}\text{C}$  values as we found in the present study.

#### 4.2. Changes in $\delta^{15}\text{N}$ of aquatic and semiaquatic plants

The differences founded in  $\delta^{15}\text{N}$  values according to vegetation type provide evidence that the “other vascular plants” group showed the highest  $\delta^{15}\text{N}$  values with an average of  $4.05 \pm 0.48\text{‰}$ . Our values are similar to results reported in several studies for some vascular plants like *Thypha* sp. ( $4 \pm 3\text{‰}$ ), *Pontedeira crassipes* ( $4.1 \pm 0.3$  and  $5.9 \pm 0.1\text{‰}$ ), and *Vallisneria americana* ( $4.7 \pm 0.1\text{‰}$ ) (Reich and Worthy, 2006), *Cabomba* spp. ( $4.7\text{‰}$ ), *Salvinia* spp. ( $3.3\text{‰}$ ), *Coccoloba* spp. ( $3.3\text{‰}$ ), and from Cyperacea family ( $3.2\text{‰}$  and  $4.6\text{‰}$ ) (Carvalho Crema et al., 2019).



**Fig. 3.** Violin plots showing the variation of  $\delta^{15}\text{N}$  values in aquatic and semi-aquatic plants from the Mexican Caribbean according to several factors. The boxplot inside represent the three quartiles, with a median represented by a central horizontal line, and the mean is the red point. Significant differences among categories within each factor are shown with different symbol. **A.** Vegetation type; **B.** Environment; **C.** Hydroclimatic season; **D.** Zone. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Moreover, the “seagrasses” group had the lowest  $\delta^{15}\text{N}$  values with an average of  $1.86 \pm 0.27\%$  which agree with previously reported values for this group (Alves-Stanley et al., 2010; Anderson and Fourqurean, 2003; Duarte et al., 2018; Mutchler et al., 2010; Reich and Worthly, 2006). Variations in  $\delta^{15}\text{N}$  could be due to diverse factors, such as different metabolic pathways used for the nitrogen assimilation, changes in assimilated nitrogen compounds (nitrates, ammonium, etc.), and their concentration, changes in the relative activity from the nitrogen fixation, and bacterial denitrification (Handley and Raven, 1992). For instance, seagrasses incorporate nutrients from the water column and sediment, while macroalgae only depends on the surficial DIN (generally low in concentration and enriched in  $^{15}\text{N}$  due to uptake processes) from the water column to supply their nitrogen requirements and are more enriched in  $^{15}\text{N}$  in comparison to seagrasses (Dillon and Chanton, 2008; Kuramoto and Minagawa, 2001). A similar occurrence was reported in

the present study. Natural populations of macroalgae are usually enriched in  $^{15}\text{N}$  relative to the baseline, and often utilize their N source completely, reflecting in their isotopic composition (Wada and Hattori, 1978). Since isotopic fractionation in primary producers are induced by N uptake processes (Kuramoto and Minagawa, 2001), and seagrasses incorporate nutrients from water column and sediment, it is possible that they have different strategies and speed for nutrient uptake and assimilation, preferring to incorporate it from the sediments than the water column, with a higher fractionation resulting in a  $^{15}\text{N}$  depleted compared to macroalgae (Owens, 1988).

Our results show the highest  $\delta^{15}\text{N}$  values in plants collected from freshwater environments compared to those obtained from marine and estuarine environments are possibly due to the input increase of nutrients, which enriches  $^{15}\text{N}$  values (Fry, 2002; Olin et al., 2013). Overall, changes in  $\delta^{15}\text{N}$  are related to biogeochemical processes that have

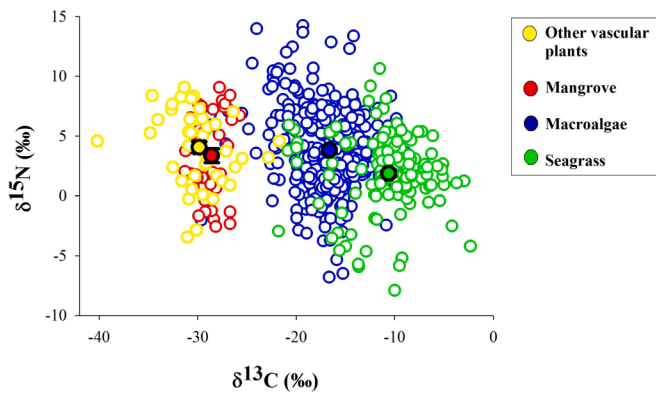


Fig. 4.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values according to vegetation type, integrated with mean values and standard error (SE).

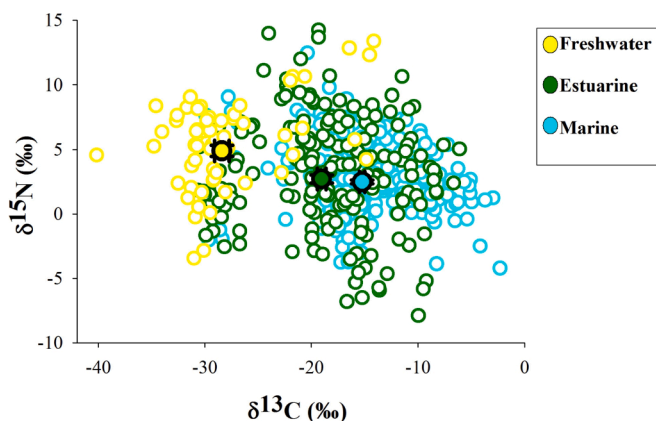


Fig. 5.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values according to environment, integrated with mean values and standard error (SE).

affected bioavailable nitrogen (fixed nitrogen): denitrification, nitrification, anammox. Additionally, the distribution of the DIN (changes in the proportion of nitrate and ammonium) are related to isotopic variability. The first process is the isotopic composition from dissolved inorganic nitrogen (DIN), which can change as proportions of nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) change. The second one is the fractionation happening during the absorption and transformation (McCusker et al., 1999). In rivers and brackish environments, the supply of nutrients from terrestrial materials generate high production and  $\delta^{15}\text{N}$  values are higher (Kuramoto and Minagawa, 2001). By contrast, marine plants usually have lower  $\delta^{15}\text{N}$  values than the nitrate source used by the plant (Anderson and Fourqurean, 2003), and this is related to the availability and use of nutrients (Fourqurean et al., 2005).

According to geographic distribution, we found that the SZ had the lowest  $\delta^{15}\text{N}$  mean values ( $2.61 \pm 0.26\text{‰}$ ; min:  $5.71\text{‰}$ , max:  $12.46\text{‰}$ ) in comparison to NZ ( $3.24 \pm 0.23\text{‰}$ ; min:  $7.89\text{‰}$ , max:  $14.24\text{‰}$ ), and CZ ( $3.64 \pm 0.31\text{‰}$ ; min:  $4.55\text{‰}$ , max:  $13.98\text{‰}$ ), suggesting that the N source in this zone is different than the other two zones. Quintana Roo is a region with great tourist demand, and tourist activities can modify habitat, increase pollution and have an intense use of marine resources (Aguilar et al., 2008a). Several studies applying nitrogen stable isotopes analysis in macroalgae and seagrasses in the Mexican Caribbean found that  $\delta^{15}\text{N}$  values are related to human activities and development (Camacho-Cruz et al., 2019; Mutchler et al., 2007, 2010; Sánchez et al., 2013, 2020; Umezawa et al., 2002), showing higher  $\delta^{15}\text{N}$  values in NZ compare to SZ. However, the study area has a variety of plant communities that could contribute with N input to the system (Pacheco Ávila and Cabrera Sansores, 2003) due to plants decomposition, increase in nitrates, and an enrichment in  $^{15}\text{N}$  from primary producers, which

would increase  $\delta^{15}\text{N}$  values (Medina-Gómez and Herrera-Silveira, 2003). Also, denitrification of an N source, and N limitation during periods of rapid growth could induce high  $\delta^{15}\text{N}$  values in marine plants (Fourqurean et al., 2005).

Our results, specifically for macroalgae and seagrasses, showed a high variability between  $-7.89\text{‰}$  and  $14.24\text{‰}$  influenced possibly by both natural process and anthropogenic activities. We found  $\delta^{15}\text{N}$  values  $> 10\text{‰}$  in different stations from our three zones, without a visible relation between the high  $\delta^{15}\text{N}$  values and the areas with more development and human activities, coinciding with other studies in the zone (Null et al., 2014). Nevertheless, Sánchez et al. (2013) suggest that high  $\delta^{15}\text{N}$  values are a result of wastewater discharges derived from anthropogenic activities. Our highest values (between  $12\text{‰}$  and  $14.24\text{‰}$ ) found in Nichupté lagoon (NZ), are consistent with the results of other authors, for this specific station (Camacho-Cruz et al., 2019; Carruthers et al., 2005; Sánchez et al., 2020), suggesting pollution by sewage discharges in groundwater, with high nitrate concentration ( $8.56 \mu\text{M}$ ) as found in this lagoon by other authors (Herrera-Silveira and Morales-Ojeda, 2010). However, in other places like Boca Paila at Sian Ka'an Biosphere Reserve (SKBR) (CZ) values  $> 10\text{‰}$  could be due to the decomposition process of organic matter from aquatic vegetation and mangroves contributing an increase in nitrate, producing an enrichment in  $^{15}\text{N}$  in primary producers (Medina-Gómez and Herrera-Silveira, 2003). It is also plausible that high  $^{15}\text{N}$  values in areas far from anthropic effects are due to natural processes, such as denitrification occurring in oxygen-poor areas/depths.

On the other hand,  $\delta^{15}\text{N}$  values between  $-4\text{‰}$  and  $4\text{‰}$  could be the result of fertilizer runoff into the wetlands (Heaton, 1986). Hence, the presence of crop areas within the SKBR (CZ) (Bello et al., 2009) would explain the obtained  $\delta^{15}\text{N}$  values in samples from some stations of this reserve. Nevertheless, those particular agricultural areas are located further than 50 km from the sampling points and have relatively low coverage area (Vázquez-Lule et al. (2009), therefore more studies are needed to investigate if this zone is polluted by fertilizers.

## 5. Conclusions

This study provides an isotopic baseline information from a wide variety of aquatic and semiaquatic plant species from the Mexican Caribbean. Our results in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values show a wide variation according to vegetation type (seagrasses, macroalgae, mangroves, and "other vascular plants", and aquatic environment (marine, estuarine, and freshwater). This information can contribute to future ecological studies aiming to understand the structure of aquatic food webs and possible changes related to anthropogenic activities affecting aquatic and semiaquatic vegetation. Additionally, it can be used to infer diet and feeding habits of endangered aquatic megaherbivores, like manatees and sea turtles, as well as to investigate spatial and temporal changes in feeding habits of these species, since isotopic composition of an animal is primarily determined by the isotopic composition of its food. All this information is critical to understand the impacts of habitat modification, pollution and intense use of marine resources on species and natural ecosystems, which is particularly relevant for Quintana Roo as this megadiverse state has shown a steady growth of tourism and other human activities.

## Credit author statement

Natalia Garcés-Cuartas: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. Carlos Alberto Niño-Torres: Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision. Delma Nataly Castelblanco-Martínez: Conceptualization, Methodology, Investigation, Visualization, Writing – review & editing, Supervision. Antonio Delgado-Huertas: Resources, Writing – review & editing. Neidy Pauline Cetz-Navarro: Investigation, Writing – review & editing. Raúl Ortiz-

Pulido: Formal analysis, Writing – review & editing. Jaime Cuevas: Formal analysis, Writing – review & editing.

### 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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## APPENDIX

### Appendix 1

Aquatic and semiaquatic plant species collected in the Mexican Caribbean, indicating year, hydroclimatic season, environment, and collection zone.

#	Species	Year	Season	Environment	Zone
<b>Seagrasses</b>					
1	<i>Halodule wrightii</i> (n: 49)	2017/2018	R, D, CF	M, E	N, C, S
2	<i>Ruppia maritima</i> (n: 7)	2017	R, CF	M, E	C, S
3	<i>Ruppia</i> sp. (n: 4)	2017/2018	D, CF	M, E, FW	C, S
4	<i>Syringodium filiforme</i> (n: 24)	2017/2018	R, D, CF	M	N, C, S
5	<i>Thalassia testudinum</i> (n: 46)	2017/2018	R, D, CF	M, E	N, C, S
<b>Macroalgae</b>					
6	<i>Acanthophora spicifera</i> (n: 1)	2017	D	M	N
7	<i>Acanthophora muscoides</i> (n: 1)	2018	D	M	S
8	<i>Acetabularia crenulata</i> (n: 1)	2017	R	M	S
9	<i>Acetabularia schenckii</i> (n: 9)	2017	R	E	N, C
10	<i>Acetabularia</i> sp. (n: 4)	2017	R, CF	M, E	N, C
11	<i>Avrainvillea nigricans</i> (n: 3)	2017	R	E	N
12	<i>Avrainvillea</i> sp. (n: 6)	2017/2018	R, D	M, E	N, C
13	<i>Batophora</i> sp. (n: 20)	2017/2018	R, D, CF	M, E, FW	C, S
14	<i>Bostrychia moritziana</i> (n: 1)	2017	D	M	N
15	<i>Canistrocarpus cervicornis</i> (n: 3)	2017/2018	R	M, E	C, S
16	<i>Caulerpa brachypus</i> (n: 2)	2017	R	E	N
17	<i>Caulerpa mexicana</i> (n: 2)	2017	R	M	S
18	<i>Caulerpa paspaloides</i> (n: 4)	2017/2018	R, D	M, E	N
19	<i>Caulerpa prolifera</i> (n: 3)	2017/2018	D	M, E	N
20	<i>Caulerpa sertularioides</i> (n: 1)	2018	D	M	S
21	<i>Caulerpa sertularioides f. longiseta</i> (n: 1)	2017	R	E	C
22	<i>Caulerpa</i> sp. (n: 16)	2017	R	M, E	N, S
23	<i>Centroceras clavulatum</i> (n: 2)	2017	R	M	C
24	<i>Ceramium nitens</i> (n: 6)	2017/2018	R, D	M, E	N, C
25	<i>Chaetomorpha cf. crassa</i> (n: 2)	2017	R	M	S
26	<i>Chaetomorpha clavata</i> (n: 2)	2018	D	M	N
27	<i>Chaetomorpha gracilis</i> (n: 4)	2017	R	M, E	C, S
28	<i>Chara</i> sp. (n: 10)	2017/2018	R, D, CF	E, FW	S
29	<i>Chondria capillaris</i> (n: 1)	2017	R	M	C
30	<i>Chondria cnicophylla</i> (n: 6)	2017	R	E	C
31	<i>Chondria collinsiana</i> (n: 1)	2017	D	M	N
32	<i>Chondria floridana</i> (n: 2)	2017	D	M	N
33	<i>Chondria</i> sp. (n: 1)	2018	D	E	N
34	<i>Cladophora laetevirens</i> (n: 2)	2018	D	E	N
35	<i>Cladophora prolifera</i> (n: 1)	2017	R	M	C
36	<i>Cladophora</i> sp. (n: 4)	2017/2018	D	M	N, S
37	<i>Cladophoropsis macromeres</i> (n: 1)	2017	R	M	S
38	<i>Cladophoropsis membranacea</i> (n: 1)	2017	D	M	N
39	<i>Cladophoropsis</i> sp. (n: 1)	2017	D	M	N
40	<i>Dasycladus vermicularis</i> (n: 3)	2017	R	E	S
41	<i>Dasycladus</i> sp. (n: 3)	2017	R	E	N
42	<i>Dictyota bartayresiana</i> (n: 1)	2018	D	M	S
43	<i>Dictyota menstrualis</i> (n: 1)	2017	D	M	N
44	<i>Dictyota pulchella</i> (n: 2)	2017	R	M	S
45	<i>Dictyota</i> sp. (n: 7)	2017	R, D, CF	M	N, S
46	<i>Digenea simplex</i> (n: 2)	2017	R	E	C
47	<i>Gelidiopsis</i> sp. (n: 2)	2018	D	E	N
48	<i>Gracilaria blodgettii</i> (n: 1)	2017	R	M	S
49	<i>Gracilaria bursa-pastoris</i> (n: 1)	2017	D	M	N
50	<i>Halimeda incrassata</i> (n: 8)	2017/2018	R, D	M, E	N, S
51	<i>Halimeda monile</i> (n: 5)	2017	R	M	N, S

(continued on next page)



## Appendix 1 (continued)

#	Species	Year	Season	Environment	Zone
52	<i>Halimeda opuntia</i> (n: 2)	2017	R	M	S
53	<i>Halimeda</i> sp. (n: 20)	2017/2018	R, D, CF	M	N, C, S
54	<i>Hypnea spinella</i> (n: 1)	2017	R	M	S
55	<i>Laurencia</i> sp. (n: 7)	2017	R	M, E	N
56	<i>Padina</i> sp. (n: 5)	2017/2018	R, D, CF	M, E	N, S
57	<i>Palisada perforata</i> (n: 1)	2017	R	M	C
58	<i>Penicillus capitatus</i> (n: 26)	2017/2018	R, D	M, E	N, C, S
59	<i>Penicillus dumetosus</i> (n: 8)	2017	R, D	M, E	N
60	<i>Penicillus</i> sp. (n: 18)	2017/2018	R, D, CF	M	N, S
61	<i>Rhypocephalus oblongus</i> (n: 3)	2017	R	M	S
62	<i>Rhypocephalus phoenix</i> (n: 3)	2017/2018	D	M	N
63	<i>Rhypocephalus</i> sp. (n: 9)	2017	R	M	N, S
64	<i>Sargassum fluitans</i> (n: 26)	2017/2018	R, D, CF	M, E	N, C, S
65	<i>Sargassum natans</i> (n: 9)	2017/2018	R, D	M	N, C, S
66	<i>Sargassum</i> sp. (n: 1)	2017	CF	M	C
67	<i>Spyridia filamentosa</i> (n: 2)	2017	R	M	S
68	<i>Turbinaria tricostrata</i> (n: 3)	2017	R	M	C
69	<i>Turbinaria turbinata</i> (n: 3)	2017/2018	D	M, E	N
70	<i>Turbinaria</i> sp. (n: 4)	2017/2018	D, CF	M	S
71	<i>Udotea fibrosa</i> (n: 2)	2017	R	M	S
72	<i>Udotea flabellum</i> (n: 8)	2017	R	M	N, C, S
73	<i>Udotea luna</i> (n: 3)	2017	R	E	N
74	<i>Udotea spinulosa</i> (n: 1)	2017	D	M	N
75	<i>Udotea wilsonii</i> (n: 1)	2017	D	M	N
76	<i>Udotea</i> sp. (n: 19)	2017/2018	R, D, CF	M, E	N, C, S
77	<i>Yuzurua poiteaui</i> (n: 4)	2017	R	M, E	C, N
78	<i>Yuzurua poiteaui</i> var. <i>gemmaifera</i> (n: 1)	2017	D	M	N
<b>Mangroves</b>					
79	<i>Conocarpus erectus</i> (n: 8)	2017/2018	R, D, CF	FW	S
80	<i>Laguncularia racemosa</i> (n: 16)	2017	R	M, E	N, C, S
81	<i>Rhizophora mangle</i> (n: 13)	2017/2018	R, D, CF	M, E, FW	N, S
<b>Other vascular plants</b>					
82	<i>Bucida buceras</i> (n: 4)	2018	D	FW	S
83	<i>Cabomba palaeformis</i> (n: 6)	2017/2018	D, CF	FW	S
84	<i>Chrysobalanus icaco</i> (n: 6)	2017/2018	R, D, CF	FW	S
85	<i>Cladium jamaicense</i> (n: 5)	2017/2018	R, D	E, FW	S
86	<i>Coccoloba uvifera</i> (n: 1)	2018	CF	E	S
87	<i>Crinum erubescens</i> (n: 1)	2018	CF	FW	S
88	<i>Cyperus</i> sp. (n: 1)	2018	CF	FW	S
89	<i>Pontedeira crassipes</i> (n: 4)	2018	D, CF	FW	S
90	<i>Manilkara zapota</i> (n: 2)	2018	CF	E	S
91	<i>Nymphaea ampla</i> (n: 3)	2018	D, CF	FW	S
92	<i>Salvinia</i> sp. (n: 2)	2017	CF	FW	S
93	<i>Thrinax radiata</i> (n: 4)	2017/2018	D, CF	FW	S
94	<i>Typha domingensis</i> (n: 1)	2018	CF	FW	S
95	<i>Vallisneria</i> sp. (n: 2)	2018	D	FW	S

Season (R: rainy; D: dry; CF: Cold-fronts). Environment (M: marine; E: estuarine; FW: freshwater). Zone (N: north zone; C: center zone; S: south zone).

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