An analysis of the macroalgal δ¹³C variability in the Gulf of California

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Abstract

The C isotopic composition in macroalgae (δ¹³C) is highly variable, and its prediction is very complex relative to terrestrial plants. To contribute to the knowledge on the variations and determinants of δ¹³C-macroalgal, we analyzed a large stock of specimens varying in taxa and morphology and inhabiting shallow marine habitats from the Gulf of California (GC) featured by distinctive environmental conditions. A large δ¹³C variability (-34.61‰ to -2.19‰) was observed, mostly explained on the life form (taxonomy, morphology, and structural organization), and modulated by the interaction between habitat features and environmental conditions. The intertidal zone specimens had less negative δ¹³C values than in the subtidal zone. Except for pH, environmental conditions of the seawater do not contribute to the δ¹³C variability. Specimens of the same taxa showed δ¹³C similar patterns, to increase or decrease, with latitude (21°-30°N). δ¹³C-macroalgal provides information on the inorganic carbon source used for photosynthesis (CO₂ diffusive entry vs HCO₃⁻ active uptake). Most species showed a δ¹³C belong into a range that indicates a mix of CO₂ and HCO₃⁻ uptake; the HCO₃⁻ uptake by active transport is widespread among GC macroalgae. About 20-34% of species showed the presence of carbon concentrating mechanism (CCM). Ochrophyta presented a high number of species with δ¹³C>-10‰, suggesting widespread HCO₃⁻ use by non-diffusive mechanisms. Few species belonging to Rhodophyta relied on CO₂ diffusive entry (δ¹³C<-30‰) exclusively. δ¹³C provides useful information about the physiological and environmental status of macroalgae.

**Keywords:** δ¹³C-macroalgal, carbon-concentrating mechanisms, CO₂ diffusive proxy
1. Introduction

Macroalgae shows a wide diversity of morphologies, structural organization (e.g., surface area/volume ratio), and various pigments. Based on these features, macroalgae can be classified into only three phyla, in agreement to the pigment contents in the thallus, or in dozens of groups considering morphologies and pigments (Littler and Littler, 1980; Littler & Arnold, 1982; Balata et al., 2011). For example, mixing of chlorophyll (a, b) and carotenoids are usually observed in Chlorophyta; chlorophyll (a, c) is dominant in Ocrophyta. Rhodophyta contains chlorophyll (a, d), carotenoid, and a mix of phycobilin (e.g., phycocyanin, phycoerythrina, allophycocyanin) (Bold and Wynne, 1978; Masojidek et al., 2004; Gateau et al., 2017). Both traits work as an excellent approximation to explain the fundamentals of metabolism, growth, zonation, and colonization (Littler and Littler, 1980; Littler and Arnold, 1982; Nielsen and Sand-Jensen, 1990; Vásquez-Elizondo and Enríquez, 2017).

Thallus thickness as the propriety of the morphology influences the diffusion boundary layer at the macroalgal surface, where the uptake of essential ions and dissolved gases by macroalgae occur (Hurd, 2000; San-Ford and Crawford, 2000). In marine environments, where pH~8.1±1, HCO$_3^-$ accounting 98% of total DIC due to the low diffusion rate of CO$_2$ in seawater that results in a high HCO$_3^-$ : CO$_2$ ratio (150:1) (Sand-Jensen and Gordon, 1984). The limitations for growth imposed by low seawater CO$_2$ concentrations are compensated by carbon concentrating mechanisms (CCMs) in most macroalgae that increase internal carbon inorganic concentration (near the site of RuBisCo activity (Giordano et al., 2005). For hence, HCO$_3^-$ uptake by most macroalgae is the principal inorganic carbon source for photosynthesis, but a few species depend exclusively on to use of dissolved CO$_2$ that enter by diffusion to the cells (Maberly et al., 1992; Beardall and Giordano, 2002; Raven et al.,
2002a, b; Giordano et al., 2005). So, macroalgal species with productivity limited by lacking CCM’s (have low plasticity for carbon inorganic forms uptake) seems to be restricted to submareal habitats and composed mainly by red macroalgae (but without a morphological patron apparent) (Cornwall et al., 2015, Kübler and Dungeon, 2015). The rest of the macroalgae with CCM occupies from the intertidal to the deep submareal.

Nevertheless, marine ecosystems have many environmental factors, including habitat features and environmental conditions in seawater that modify the main macroalgae photosynthesis drivers (light, DIC, and inorganic nutrients). These factors could generate negative consequences for their productivity, principally when they cause resources limitation. Each factor varies from habitat to habitat (e.g., local scale: from intertidal to subtidal and global scale: from temperate to tropical regions), and as in response to these environmental changes, macroalgae can modulate their photosynthetic mechanism (Lapointe and Duke, 1984; Dudgeon et al., 1990; Kübler and Davison 1993, Young et al., 2005). The modulation, to increase their photosynthetic activity (up-and-down-regulation processes), implies a physiological acclimation enhancing the transport of DIC (CO$_2$, HCO$_3^-$) into the cell and its fixation rates (Madsen and Maberly, 2003; Klenell et al., 2004; Zou et al., 2004; Giordano et al., 2005; Enríquez and Rodríguez-Román, 2006; Rautemberger et al., 2015).

The $\delta^{13}$C on the thallus of marine macrophytes is a proxy used to identify CO$_2$ or HCO$_3^-$ source in photosynthesis and to infer the presence or absence of CCM’s (Maberly et al., 1992; Raven et al., 2002a). Also, the $\delta^{13}$C signal in the algal thallus can be used as an indicator of the physiological state of photosynthetic metabolism (Kim et al., 2014; Kübler and Dungeon, 2015). Consequently, $\delta^{13}$C variability depends, in part, on the life form (taxonomy, morphology, and structural organization), but also is modulated by the interaction to
environmental conditions (light, DIC, and nutrients). Thus, the prediction of the δ\textsuperscript{13}C variability in marine macrophytes is very complex relative to terrestrial plants. In this study, our objective was to investigate the contributions of life form, the changes in the habitat features, and environmental conditions to the δ\textsuperscript{13}C macroalgal variability in communities in the Gulf of California (GC). A second objective was to describe the proportion of species that lacks CCM inferred by the δ\textsuperscript{13}C signal along and between the GCE bioregions. A third objective was to explore any geographical pattern in the δ\textsuperscript{13}C macroalgal. Macroalgae as biomonitor constitute an efficient tool in monitoring programs in large geographical regions (Balata et al., 2011) and for environmental impact assessments (Ochoa-Izaguirre and Soto-Jiménez, 2014).

To reach our objectives, we collected a large stock of macroalgae specimens of a diversity of species characterized by a variety of morphological and physiological properties. Besides high diversity, in terms of life forms, we selected various shallow marine habitats along a latitudinal gradient in the GCE for the sample collection, characterized by unique and changing environmental factors. The GCE features abundant and diverse macroalgal populations, which are acclimated and adapted to diverse habitats with environmental conditions, determining the light, DIC, and nutrients availability.

2. Materials and Methods

2.1. Gulf of California description

The Gulf of California is a subtropical, semi-enclosed sea of the Pacific coast of Mexico, with exceptionally high productivity being the most important fishing regions for Mexico and one of the most biologically diverse worldwide marine areas (Zeitzschel, 1969; Espinosa-Carreón and Valdez-Holguin 2007; Lluch-Cota et al., 2007; Páez-Osuna et al., 2017). GC represents only
0.008% of the area covered by the seas of the planet (265,894 km$^2$, 150 km wide, and 1000 km long covering >9 degrees latitude) but has a high physiographic diversity and is biologically mega-diverse with many species endemic (Wilkinson et al., 2009; Espinosa-Carreón and Escobedo-Urías, 2017).

Regionalization criteria of the GC include phytoplankton distribution (Gilbert and Allen, 1943), topography (Rusnak et al., 1964) and depth (Álvarez-Borrego, 1983), oceanographic characteristics (Roden and Emilson, 1979; Álvarez-Borrego, 1983; Marinone, 2003), biogeography (Santamaría-del-Ángel et al., 1994a), and bio-optical characteristics (Bastidas-Salamanca et al., 2014). The topography is variable along GC, includes submarine canyons, basins, and variable continental platform. Besides, GC presents complex hydrodynamic processes, including internal waves, fronts, upwelling, vortices, mixing of tides. The gulf’s coastline is divided into three shores: extensive rocky shores, long sandy beaches, numerous scattered estuaries, coastal lagoons, and open muddy bays tidal flats and coastal wetlands (Lluch-Cota et al., 2007).

The Gulf of California is different in the north and the south, related to a wide range of physicochemical factors. The surface currents seasonally change direction and flow to the Southeast with maximum intensity during the winter and to the Northwest in summer (Roden, 1958). The northern part is very shallow (<200 m deep averaged), divided into Upper Gulf, Northern Gulf, and Grandes Islas. The surrounding deserts largely influence this region (Norris, 2010) shows marked seasonal changes in coastal seawater temperatures (Martínez-Díaz de León et al., 2006; Marinone, 2007). Tidal currents induce a significant cyclonic circulation through June to September and anticyclonic from November to April (Carrillo et al., 2002; Bray, 1988a; Velasco-Fuentes and Marinone, 1999; Martínez-Díaz-de-León, 2001). The southern part consists of a series of basins whose depths increase towards the South (Fig. 1). The intertidal macroalgae in the
southern region are subject to desiccation, mostly during summer. The water column's physicochemical characteristics are highly influenced by the contrasting climatic seasons in the GC, the dry season (nominally from November to May), and the rainy season (from June to October). Annual precipitation (1,080 mm y\(^{-1}\)) and evaporation (56 mm y\(^{-1}\)) rates registered during the past 40 years were 881±365 mm y\(^{-1}\) and 53±7 mm y\(^{-1}\), respectively (CNA, 2012).

Previous macroalgae floristic studies of the CG, report around 580 species, including 116 endemic species (Norris, 1975; Espinoza-Avalos, 1993). Based on oceanographic characteristics (Roden and Groves, 1959) and in the endemic species distribution (Aguilar Rosas and Aguilar Rosas, 1993), the CGE can be classified into three phycofloristic zones: 1) First zone located from the imaginary line connecting San Francisquito Bay, B.C. to Guaymas, Sonora, with 51 endemic species. 2) the Second zone with an imaginary line from La Paz bay (B.C.S.) to Topolobampo (Sinaloa) with 41 endemic species. 3) the Third zone is located with an imaginary line from Cabo San Lucas (B.C.S.) to Cabo Corrientes (Jalisco) with 10 endemic species. Besides, 14 endemic species are distributed throughout the GCE (Espinoza-Ávalos, 1993). The macroalgal communities are subject to the changing environmental conditions in the diverse habitats in the GCE that delimits their zonation, which tolerates a series of anatomical and physiological adaptations to water movement, temperature, sun exposure, and light intensities, low pCO2, desiccation (Espinoza-Avalos 1993).

### 2.1 Macroalgae sampling

In this study, the GC coastline (21°-30°N latitude) was divided into six coastal sectors based on the three phycofloristic zones previously described (Fig. 1a). In each selected ecosystem, representative habitats were sampled based on macroalgae communities' presence and habitat characterization. Habitats were classified by substrate type (e.g., sandy-rock, rocky shore), hydrodynamic (slow to
faster water flows), protection level (exposed or protected sites), and immersion level (intertidal or subtidal) (Fig. 1b).

Based on the local environmental factors, macroalgae specimens (4-5) of the most representative species were gathered by hand (free diving) during low tide. A total of 809 composite samples were collected from marine habitats along both G.C. coastlines. The percentages of specimens collected for the substrate type were sandy-rock 28% and rocky shores 72% based on the habitat features. Related to the hydrodynamic, 30% of the specimens were collected in habitats with slow to moderate and 70% with moderate to fast water movement. Regarding the protection level, 57% were exposed specimens, and 43% were protected. Finally, 56% were intertidal and 44% subtidal macroalgae organisms concerning the emersion level. About half of the protected specimens were collected in isolated rock pools, which was noted.

In 4-5 sites of each habitat, we measured in situ the salinity, temperature, and pH by using a calibrated multiparameter sonde (Y.S.I. 6600V) and the habitat characteristics mentioned above noted. Besides, composite water samples were collected for nutrient and alkalinity in the laboratory. Briefly, the representative habitats were classified by pH levels in >9.0 “alkalinized”, 7.9-8.2 ‘typical’ and <7.9 “acidified”. Based on the temperature in colder <20°C, typical 20-25°C, and warmer >25°C. 72% of the specimens were collected at typical pH values, 22% in alkalinized and 6% in acidified seawater. Regarding the temperature, about 55% of the specimens were collected at typical, 31% at warmer, and 14% at colder seawaters. Regarding salinity, most of the ecosystems showed typical values for seawater (35.4±0.91 ups, from 34.5 to 36.1 ups). In this study, the collection surveys were conducted during spring (March-April) and dry season (nominally from November to May) from 2009 to 2014. Only in few selected ecosystems located at C1 and C2 sectors, one sampling survey was conducted at the end of the rainy season (nominally from June to October.
in 2014). Thus, these ecosystems were possible to include habitat with a salinity range varying from estuarine (23.5±3.0 ups) to hypersaline (42.7±7.0 ups) values. These habitats were mainly isolated rockpools, and only a few were sites near tidal channels receiving freshwater discharges. About 95% of the specimens were collected at typical seawater salinity and only 1.5 and 3.5% in estuarine and hypersaline environments. Detailed information on the selected shallow marine ecosystems, habitat characterization, and environmental conditions is summarized in the inserted table in Fig. 1.

2.2 Macroalgae processing and analysis of the isotopic composition of carbon

The collected material was washed in situ with surface seawater to remove the visible epiphytic organisms, sediments, sand, and debris and then thoroughly rinsed with MilliQ water. The composite samples were double-packed in a plastic bag, labeled with the locality's name and collection date, placed in an ice-cooler box to be kept to 4°C, and immediately transported to the laboratory UAS-Facimar in Mazatlán. In the field, sample aliquots were also preserved in 4% v/v formaldehyde solution for taxonomic identification to the genus or species level (when possible). The following GC macroalgal flora identification manuals were consulted: Dawson, 1944; 1954; 1956; 1961; 1962; 1963; Setchell and Gardner, 1920; 1924; Abbott and Hollenberg, 1976; Ochoa-Izaguirre et al., 2007; Norris, 2010).

In the laboratory, macroalgae samples were immediately frozen at -30°C until analysis. Then, samples freeze-dried at -38°C and 40 mm Hg for 3 days, upon which they were ground to a fine powder and exposed to HCl vapor for 4 h (acid-fuming) to remove carbonates and dried at 60°C for 6 h (Harris et al. 2001). Five milligrams aliquots were encapsulated in tin cups (5x9 mm) and stored in sample trays until analysis. Macroalgae samples were sent to the Stable Isotope Facility (SIF) at the University of California at Davis, CA, USA. Natural $^{13}$C relative abundance relative to
12C in samples was determined with mass spectrometry, using a Carlo Erba elemental analyzer attached to a Finnigan Delta S mass spectrometer equipped with a Europa Scientific stable isotope analyzer (ANCA-NT 20-20) and a liquid/solid preparation unit (PDZ, Europa, Crewz, UK).

Isotope ratios of the samples were calculated using the equation \( \delta (\%) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \), where \( R = ^{13}\text{C}/^{12}\text{C} \). The \( R_{\text{standard}} \) is relative to the international V-PDB (Vienna PeeDee Belemnite) standard. During the isotopic analysis, the SIF lab used different certified reference materials (e.g., IAEA-600, USGS-40, USGS-41, USGS-42, USGS-43, USGS-61, USGS-64, an USGS-65) for the analytical control quality. The analytical uncertainties reported for the SIF lab were 0.2‰ for \( \delta^{13}\text{C} \) (https://stableisotopefacility.ucdavis.edu/13cand15n.html). We also included triplicate aliquots of several specimens of the same species and condition, collected from one patch or attached to the same substrate, to assess the method error by sampling and processing procedural. The methodological uncertainties were <0.4‰.

2.3. Analysis of \( \delta^{13}\text{C} \)-macroalgal variability

The variability of \( \delta^{13}\text{C} \) values in macroalgae was analyzed in function of the taxonomy (phylum, genus, and species) and morpho-functional groups (e.g., thallus structure, growth form, branching pattern, and taxonomic affinities; Balata et al. 2011; Ochoa-Izaguirre and Soto-Jiménez, 2015).

Sampled specimens belong to three phyla, 63 genera, and 167 species. The phyla were identified as Rhodophyta (53%), Ochrophyta (22%) and Chlorophyta (25%). The most representative genus (and their species) were Ulva (\( U. \) lactua, \( U. \) lobata, \( U. \) flexuosa, and \( U. \) intestinalis), Codium (\( C. \) amplivesiculatum and \( C. \) simulans), Chaetomorpha (\( C. \) antenina), Padina (\( P. \) durvillaei), Dictyota (\( D. \) dichotoma), Colpomenia (\( C. \) tuberculata and \( C. \) sinuosa), Sargassum (\( S. \) sinicola and \( S. \) horridum), Amphiroa (\( A. \) spp.), Spyridia spp., Polysiphonia spp., Gymnogongrus spp.,
Gracilaria (*G. vermiculophylla*, *G. pacifica* and *G. crispate*), Hypnea (*H. pannosa* and *H. johnstonii*) Grateloupia (*G. filicina* and *G. versicolor*), and Laurencia (*L. papillosa* and *L. pacifica*). An analysis of the biogeographical diversity among sectors evidenced that P3 (43 genera of 63, 68%) and C3 (63%) at north recorded the highest number of genus, followed by C1 (38%) and P1 (29%) at the south, and P2 (27%) and C2 (22%). The same pattern was observed in the species richness, zones P3 (94 of 167 species, 56%) and C3 (52%) at the north, C1 (34%) and P1 (25%) at south, and C2 and P2 (19-20%) at the center.

In order to find a geographic pattern associated with the $\delta^{13}C$ signal of macroalgae in this study, macroalgae were grouped according to their characteristics morpho-functional proposed initially by Littler and Littler (1980) and modified by Balata et al. (2011). Not all morphofunctional groups and taxon were present in every site during each sampling survey, and the sample size in each group varied for taxa, location, and time. The morphofunctional groups identified were 21, of which the most common were C-tubular (6 spp., n=69; C-Blade-like (6 spp, n=55); C-Filamentous uniseriate (17 spp, n=49); C-Erect thallus (5 spp, n=33); O-Compressed with branched or divided thallus (19 spp., n=92); O-Thick leathery macrophytes (12 spp., n=104); O-Hollow with spherical or subspherical shape (4spp, n=87); R-Large-sized corticated (57 spp., n=225); R-Filamentous uniseriate and pluriseriate with erect thallus (9 spp., n=48); and R-Large-sized articulated corallines (6 spp, n=17). The diversity, in terms of presence/absence of the morphofunctional groups, varied among coastline sectors, higher in C3 (16 of 21, 76%) and P3 (71%) at the north, followed by C1 (57%) and P1 (48%) at the south, and C2 and P2 and (42-48%) at the center of both GC coastlines.

Detailed information on macroalgae specimens collected (ecosystem, habitat, number of composite samples, morphological group, and taxon) is given as Supplementary Information (Table SI-1).

A basic statistical analysis of $\delta^{13}C$ values in different macroalgae groups was applied to distribute...
and calculate the arithmetic mean, standard deviation, minimum and maximum. Because not all macroalgal species were present in sufficient numbers at different collection habitats, several macroalgal groups were not considered for statistical analysis. Regarding the life form, we compared among morphofunctional groups, taxon collected in the same habitat (within-subjects factor) by multivariate analysis of variance. When differences were noted, a Tukey-Kramer HSD (Honestly Significant Difference) test was performed. Besides, variations of $\delta^{13}$C macroalgal in specimens of the same morpho-functional and taxon collected in different habitats were also investigated with a Kruskal-Wallis test.

In this study, the relationships between $\delta^{13}$C with each independent variable related to the inherent macroalgae properties (morphology and taxon), biogeographical collection zone (GC coastline and coastal sector), habitat features (substrate, hydrodynamic, protection, and emersion level) and environmental conditions (temperature, pH, and salinity) were examined through simple and multiple linear regression analyses. Excepting temperature, pH, and salinity, most of the independent variables are categorical independent variables. However, these continue variables were also categorized, such as previously was described. Analyses of simple linear regression were performed to establish the relationships between $\delta^{13}$C-macroalgal with each environmental parameter analyzed as possible driving factors (e.g., temperature, salinity, pH). Multiple linear regression analyses were conducted to evaluate the combined effects of those independent variables (macroalgae properties, biogeographical collection zone, habitat features, and environmental conditions) on the $\delta^{13}$C-macroalgal. In the multivariable regression model, the dependent variable, $\delta^{13}$C-macroalgal, is described as a linear function of the independent variables $X_i$, as follows: $\delta^{13}$C-macroalgal = $a + b_1(X_1) + b_2(X_2) + \ldots + b_n(X_n)$ (1). Where $a$ is regression constant (it is the value of intercept and its value is zero); $b_1$, $b_2$, and $b_n$ are regression coefficients for each independent variable $X_i$. From each
one of the fitted regression models, we extracted the estimated regression coefficients for each of the predictor variables (e.g., Bayesian Information Criterion (BIC), Akaike Information Criterion (AIC), root-mean-square error (RMSE), Mallow’s Cp criterion, F Ratio test, p-value for the test (Prob > F), coefficients of determination ($R^2$) and the adjusted $R^2$ statistics) (SAS Institute Inc., 2018). All regression coefficients were used as indicators of the quality of the regression (Draper and Smith, 1998; Burnham and Anderson, 2002). Kolmogorov-Smirnov normality test was applied for all variables and all were normally distributed. Most of the $\delta^{13}$C values in each group showed a normal distribution. For all statistical tests, a probability $P<0.05$ was used to determine statistical significance. The statistical analysis of the results was done using JMP 14.0 software (SAS Institute Inc.).

3. Results

3.1. $\delta^{13}$C-macroalgal variability in function of taxonomy and morpho-functional groups

The variability of $\delta^{13}$C values in macroalgae was analyzed by taxon in the phylum, genus, species, and morphofunctional groups. Ochrophyta displayed the values from $-21.5$ to $-2.20\%$o ($-12.55\pm3.77\%$o), significantly higher to Chlorophyta ($-25.92$ to $-5.57\%$o, $-14.55\pm3.04\%$o) and Rhodophyta ($-34.61$ to $-4.55\%$o, $-14.84\pm3.96\%$o) (Fig. 2a-c). The $\delta^{13}$C-macroalgal values (average$\pm$SD) for the genus of Chlorophyta, Ochrophyta, and Rhodophyta (Fig. 2d-f) varied from $-33.79\pm1.17\%$o for Schizymenia to $-7.86\pm0.73\%$o for Amphiros. Multiple comparisons among the genera more representative of each taxon showed the following order Schizymenia $<$ Polysiphonia $<$ Ulva, Gracilaria and Spyridia ($-16.17\pm0.67\%$o to $-15.11\pm0.26\%$o) $<$ Gymnogongrus, Laurencia, Hypnea, Cladophora, Dictyota, Sargasumm, Chaetomorpha, and Grateloupia (from $-15.40\pm0.71\%$o...
to -13.86±0.78‰) < Codium and Padina (-12.52±2.46‰ to -12.45±2.54‰) < Colpomenia and
Amphiroa (-9.26±0.32 to -7.86±0.73‰). Aggregation of δ¹³C values based on morpho-functional
features on macroalgae id displayed in Fig. 3. The most representative groups in the phylum
Chlorophyta varied from -15.83±0.37‰ for C-Tubular to -12.45±0.54‰ for C-thallus erect. The
phylum Ochrophyta includes O-Thick leathery with the lowest mean (-14.79±0.30‰) and O-Hollow
with a spherical or subspherical shape with the highest values (-9.26±0.33‰). The lowest and highest
δ¹³C values for Rhodophyta were observed for R-flattened macrophytes (-24.0±9.63‰) and for R-
Larger-sized articulated coralline (-7.89±0.75‰), respectively. Significant differences were
observed among groups, which were ordered as follows: R-flattened macrophytes < R-blade like < C-
Tubular < O-Tick leathery and R-Large size corticated < C-Blade like and C-Filamentous uniseriate
< C-Erect thallus and O-Compressed with branch < O-Hollow with spherical < R-Larger-sized
articulated coralline.

By multiple comparison analysis of the same genus at different coastal sectors (Fig. 4), non-
significant differences were observed among coastal sectors for most of the genus, except for
Amphiroa, Codium, Padina, and Spyridia with δ¹³C values systematically more negatives in
continental than peninsular coastline (C1-C3 > P1-P3). Also, lower δ¹³C values were observed in the
C2 sector for most of the genus and higher at P1 and P3. Due to the strong influence of genera
composition on the morphofunctional group, similar results were found, and the graph is no showed.

For the most representative species, a detailed comparative analysis of macroalgal δ¹³C values was
also conducted and displayed on Table 1-3 for phyla Chlorophyta, Ochrophyta, and Rhodophyta,
respectively. For Codium, C. brandegeei (11.82±1.24‰) and C. simulans (-11.43±2.20‰) showed
higher δ¹³C values than C. amplivesculatum (-14.44±2.74‰). The three Colpomenia species had
higher δ¹³C values than the other genera. C. tuberculata (-8.75±3.2‰) showed values significantly
higher than *Colpomenia* sp. (-10.97±3.65‰) and *C. sinuosa* (-10.18±2.95‰). The four-representative species of *Gracilaria* showed comparable δ\(^{13}\)C values, averaging from -16.48±1.64‰ for *G. pacifica* to -15.48±2.43‰ for *Gracilaria* sp. Three representative species of *Hypnea* showed non-significant δ\(^{13}\)C differences, varied from -16.4±1.75‰ for *H. spinella* to -14.95±2.36‰ for *Hypnea* sp. Two species represented *Laurencia*, *Laurencia* sp. (-12.90±1.22‰) higher than *L. pacifica* (-14.9±2.20‰). Two species represented *Padina*, being *Padina* sp. (-11.10±1.53‰) higher than *P. durvillaei* (-13.20±2.59‰). *Sargassum* was one of the most diverse genera studied with six-representative species. Based on the δ\(^{13}\)C values the species were ordered as follow: *S. horridum* = *S. sinicola* = *S. johnstonii* (-15.52±2.89 to -15.10±2.41‰) < *S. lapazeanum* (-14.49±1.59‰) = *Sargassum* sp. (-14.25±2.36‰) < *S. herphorizum* (-13.65±1.63‰). *Spyridia* was represented by *Spyridia* sp. (-17.06±1.20‰) and *S. filamentosa* (-15.86±3.83‰) without significant differences. The six representative species of *Ulva* were divided in two morphological groups, filamentous and laminates. Filamentous species that averaged -16.35±2.01‰ for *U. clathrata*, -16.03±3.64‰ for *U. flexuosa*, -15.78±1.72‰ for *U. acanthophora* and -15.29±2.54‰ for *U. intestinalis* and Ulva laminates that included *U. linza* (-15.56±2.44‰) and *U. lactuca* (-14.10±3.13‰). Non-significant differences were observed between morphological groups and among species. A high intra-specific variability, 11-28%, explains average overlapping.

### 3.2. Taxonomy versus habitat features

Variability of δ\(^{13}\)C values for the most representative genera was evaluated by multiple comparative analyses in the habitat features' function, including the substrate, hydrodynamic, and emersion level. Large δ\(^{13}\)C variability observed between specimens of the same genus collected in the different habits does not show any significant pattern, and non-significant differences were observed. An exception was observed with the emersion level (showed in Fig. 5), where intertidal specimens
recorded less negative values than subtidal in most macroalgae genus. For example, for Hydroclathrus (intertidal -5.74±0.89‰; subtidal -11.46±5.93‰), Amphiroa (Intertidal -6.93±1.52%), Subtidal -9.91±6.14), Hypnea (intertidal -13.56±2.56‰; submareal -18.60±1.88‰), and Laurencia (intertidal -13.49±1.36‰; subtidal -17.11±1.80‰). Exceptions were observed for Polysiphonia (intertidal -19.74±2.27‰, subtidal -14.94±6.69‰), Spyridia (intertidal -16.97±3.33‰, subtidal -13.21±0.73‰) and Colpomenia (Intertidal -9.41±3.41‰, subtidal -7.76±1.34‰).

3.3. Taxonomy versus environmental conditions

Non-significant differences were observed for the same genera at different temperatures ranges, except for Grateloupia (cold, -19.28±4.70‰, typical -14.45±2.23‰, warm -14.57±2.25‰) and Polysiphonia (cold, -21.05±0.46‰, typical -18.12±5.54‰, warm -17.96±2.38‰) with more negative values in colder than warmer waters. Significant differences were observed in δ¹³C values in macroalgae specimens from the different genus in the same temperature range. For example, Colpomenia (cold -8.34±2.43‰, typical -9.47±3.77‰, warm -9.22±2.64‰), Codium (cold -11.98±1.91‰, typical -12.54±3.01‰, warm -13.61±0.62‰), and Padina (cold -11.34±2.55‰, typical -11.88±1.76‰, warm -13.42±2.77‰) (Fig. 6a), was less negative than the other genus.

Overall, more negative δ¹³C values in macroalgae specimens’ values of the same genus were observed at continental (C2) compared to peninsular CG coastline (P1-P3) and more negative southward than northward.

Significant differences were observed among genus related to the pH level at seawater (Fig. 6b). Typical pH seawater, Amphiroa (-8.80±5.44) and Colpomenia (-10.29±3.66‰) were 1-2‰ more negatives than in alkaline waters, while Ulva (-15.08±2.47‰) and Spyridia (-15.34±2.12‰) were 3-5‰ less negative than in acidic waters. Amphiroa and Colpomenia were not collected in acidic water,
and neither Spyridia in alkaline waters to compare. Another genus also showed extremes values between alkaline (Tacanoosca -7.60±1.01‰) and acidic waters (Schizymentia, -32.96±2.01‰). The following order was observed in the genus collect at the three pH ranges: alkaline > typical > acidic. Significant differences were observed for genus Ahnfeltiopsis, Caulerpa, Gymnogongrus, Padina, and Ulva, with higher values at alkaline than in acidic waters. Values of δ¹³C for specimens of the same genus collected at typical pH waters are mostly overlapped between those for alkaline and acidic seawaters. Non-significant differences in δ¹³C values were observed for Grateloupia, Hypnea, and Polysiphonia concerning pH-type waters.

Regarding the δ¹³C variability for all data set in response to temperature and salinity, a non-significant trend was observed between δ¹³C-macroalgal in both parameters' function. A poor bivariate correlation, but significant, was observed between δ¹³C with pH (R² = 0.04) (Fig. 7).

3.4. Variation latitudinal of δ¹³C-macroalgal

The δ¹³C-macroalgal variation in the GC biogeography was evaluated by regression linear analysis between δ¹³C values along the nine degrees latitude in both GC coastlines. A non-significant latitudinal trend was observed for datasets, but for the three taxa's most representative genera, δ¹³C values correlated with latitude (Fig. 8a-f). In Chlorophyta, with the higher genera number, δ¹³C values increased with latitude (Fig. 8a) with a weak but significant correlation. Contrarily, in Ochrophyta (Fig. 8b) and Rhodophyta (Fig. 8c) specimens, the δ¹³C values decreased with latitude.

Significant correlations (p<0.001) were observed for δ¹³C-macroalgal versus latitude in the most representative morphofunctional groups. Representative morphofunctional groups of Chlorophyta (e.g., C-Tubular, C-Filamentous uniseriate, Fig. 8d) showed a positive correlation, while those belonging to Ochrophyta (e.g., O-thick leathery; Fig. 8e) and Rhodophyta (e.g., R-large sized...
corticated.; Fig. 8f) showed a negative trend with latitude.

3.5. Analyses of $\delta^{13}$C macroalgal variability

An analysis of the effects, independent and combined, on the $\delta^{13}$C-macroalgal variability related to life form and environmental factors was conducted. Firstly, simple linear regression analyses were performed to evaluate the dependent variable's prediction power ($\delta^{13}$C-macroalgal) in the function of several independent variables controlling the main macroalgae photosynthesis drivers (light, DIC, and inorganic nutrients). Regression coefficients were estimated for each fitted regression model, which are used as indicators of the quality of the regression (Draper and Smith, 1998; Burnham and Anderson, 2002) as was described in Methods; however, our results description focused on the coefficients of determination ($R^2$ and adjusted $R^2$). The coefficient $R^2$ describes the overall relationship between the independent variables $X_i$ with the dependent variable $Y$ ($\delta^{13}$C-macroalgal), and it is interpreted as the % of contribution to the $\delta^{13}$C variability. While the adjusted $R^2$ statistics compensate for possible confounding effects between variables.

Results of the analysis of the relationships between $\delta^{13}$C with each independent variable are summarized in Table 4. Regarding the inherent macroalgae properties, Phyla explain only 7% variability, the morphofunctional properties 35%, and taxon by genus 46%, and by species 57%. The biogeographical collection zone, in terms of coastline (continental vs. peninsular) and coastal sectors (C1-C3 and P1-P3), explained a maximum 5% variability. Related to the habitat features, only emersion level (6%) contributed to the $\delta^{13}$C variability. The contribution of the seawater's environmental conditions was marginal for pH (4%) and negligible for temperature and salinity. A marginal reduction in the percentage of
contribution was observed for Phyla (1%) and morphofunctional properties (1%), but significant for genus (5%) and species (10%).

Multiple regression analyses were also performed to interpret the complex relationships among δ\(^{13}\)C-macroalgal, considering the life form (morphofunctional and taxon by genus) and their responses to environmental parameters. Results for the fitted regression models performed for morphofunctional groups (Table 5) and genus (Table 6) evidenced that the effect of the coastal sector and pH ranges on the δ\(^{13}\)C-macroalgal increased the contribution by 9-10% each one. The emersion level increased by 5-6%, the contribution respect to individual effect of morphofunctional group and genus, the temperature and pH in 1 and 3%, respectively, while salinity decreased by 1-2%. Adding the effect of the biogeographical collection zone, represented by the coastline sector, to those for morphofunctional group (Table 5) and genus (Table 7), a notable increase of 11-12% was observed.

The full model considering the combined effect of the coastline sector + Habitats features for Morphofunctional group or Genus (Table 7), showed R\(^2\) of 0.60 and 0.71. In contrast, Coastline sector + Environmental conditions + Morphofunctional group or Genus the R\(^2\) increased to 0.62 and 0.72, respectively. The interactive explanations of environmental factors increased the explanation percentage of δ\(^{13}\)C variability; however, these contributions were significantly lower than the explained by life forms, such as the morphofunctional properties and taxa by genus and species.

The combined effect of environmental condition on the δ\(^{13}\)C variability was tested for the best-represented morphological groups and genus. Results evidenced that 9 of 21 morphological groups showed significant effects on the δ\(^{13}\)C variability (Table 8), five increasing and four decreasing the model constant of δ\(^{13}\)C=-14.21‰. For example, for the O-Hollow with spherical or subspherical shape (+4.96‰) and R-Larger-sized articulated corallines (+6.32‰) the predicted values are -
7.89±0.80‰ and -9.25±0.47‰. For R-Filamentous uniseriate and pluriseriate with erect thallus (-2.15‰) and C-Tubular (-1.62‰) the predicted values are -16.36±0.55‰ and -15.83±0.50‰, respectively. Regarding taxon, a significant effect was observed only in 13 genera, including Colpomenia (+5.45‰), Amphiroa (+6.84‰), and Padina (+2.19‰) increasing the signal, and Polysiphonia (-3.75‰), Gracilaria (-0.89‰), and Spyridia (-1.46‰) decreasing the signal of the model constant (Table 9). In 33 species was observed a significant effect on the δ¹³C variability, including C. tuberculata +5.87‰, C. sinuosa +4.42‰, H. pannosa +4.42‰, H. johnstonii +4.42‰, and Amphiroa spp. (+4.42 to 8.20‰) increasing the model constant δ¹³C= -14.59‰, and Spyridia sp. (-2.46‰), G. filicina (-2.37‰), P. mollis (-5.22‰) and S. pacifica (-19.19‰) (Table 9).

4. Discussions

4.1. Relationship among taxonomy and habitat with δ¹³C signal

Our analyses showed high variability in the δ¹³C signal in the large inventory of macroalgae collected along GC coastline for five years. Most authors studying the isotopic composition of C in these organisms have reported the high isotopic variability, which has been attributable to the taxon-specific photosynthetic Ci acquisition properties (Raven et al., 2002, Mercado et al., 2009, Marconi et al., 2011, Stepien, 2015). Following the mechanistic explanation of δ¹³C signal for algal thallus, values of δ¹³C more negative than -30‰ indicate that photosynthesis is exclusively dependent on CO₂ diffusion (absence of CCM), whereas values above -10‰ indicate non-diffusive Ci transport mechanism (HCO₃⁻ users by the presence of CCM; Maberly et al., 1992; Raven et al., 2002). To explain our results, no considerate of the CO₂ leak out inside the cell could occur and change the cutoffs for CO₂ or HCO₃⁻ users (Sharkey and Berry, 1985; Raven et al., 2005).
In our study, 84% of the analyzed specimens belong into the intermediate range between -30‰ and -10‰, averaging -14.05±3.98‰, which is slightly higher than the global mean for intertidal macroalgal -17.35±0.43‰ based on the meta-analysis of macroalgal δ¹³C compiled by Stepień (2015). The apparent differences in the δ¹³C averages can be related to the organism origin, mostly from temperate and polar marine ecosystems (142 sampling sites temperate, eight sites from tropics, and six from polar zones) in the Stepień (2015) compilation concerning the subtropical marine ecosystems in our study. Our global mean includes the specimens collected at submareal and intertidal habitats because non-significant differences were observed in most macroalgae groups.

These results suggest that macroalgal communities from subtropical marine ecosystems record higher values than communities from temperate. Seawater from temperate zones has more CO₂ dissolved availability, which results in more negative carbon isotopic values in macroalgae when the Ci is incorporated into the tissue (Raven et al., 2002ab). δ¹³C values evidence that most of the sampled macroalgae in our study have an active CCM to fix involves the direct use of HCO₃⁻ with little CO₂ diffusive uptake (Giordano et al., 2005; Hopkinson et al., 2011; Hopkinson, 2014; Raven and Beardall, 2016). However, based only on the δ¹³C values, it is not possible to discern that CCM type is expressing in the organisms (e.g., direct HCO₃⁻ uptake by the anion-exchange protein AE; Drechsler and Beer 1991; Drechsler et al. 1993). However, it is possible to assume that at least one basal carbon concentrating mechanism (bCCM) is active. The most primitive mechanism is the CO₂ diffusion (Cerling et al., 1993) that could be composed of two types of mitochondrial carbonic anhydrase (e.g., internal and external) that enhance the fixation of Ci by recycling mitochondrial CO₂ (Zabaleta et al., 2012). The role of carbonic anhydrase (CA) in algal photosynthesis was described since the end-1960s (Bowes, 1969) and more recently detailed by Jensen et al. (2020), who described the CA types and their functions. Also, the co-existence of different CCM’s have...
been described for the same species (Axelsson et al., 1999, Xu et al., 2012), even that different CCM’s can operate simultaneously, generating different Ci contribution to RuBisCo internal pool (Rautemberger et al., 2015). The variety of CCMs and their combinations contribute to the high δ13C variability for the same species.

Because the carbon isotopic discrimination decreases when photosynthesis rates increase (Kübler and Dungeon, 2015), less negative values in GC macroalgae could evidence higher productivity in subtropical seaweed communities than those in temperate marine ecosystems. A high δ13C on macroalgal tissue require saturating light intensity and enough nutrients availability (Dudley et al., 2010), conditions occurring in the GC waters. Based on the plant communities’ pattern, G.C.’s macroalgal community productivity with intermediates values (so-called hump-back) belonging to intermediate productivity (Grime, 1970; Pärtel et al., 2007; Pärtel and Zobel, 2007).

On the other hand, species that high efficiently HCO₃⁻ uptake, according to their δ₁³C signal, were to 35 (20%, >-10‰). Alternatively, 58 species (34%) of 170 species, if -11.5‰ (Δ of 1.5‰ as respiratory effect) is the cutoff value for HCO₃⁻ users according to Carvalho and Eyre (2011). About 20-34% of species could have the biochemical machinery to fix directly HCO₃⁻, an efficient CCM that potencies the productivity when is growing under optimal conditions. Furthermore, the highest δ₁³C values have been associated with the intermediate C3-C4 or C4 pathway (Valiela et al., 2018), which suggests a more efficient C.C.M.’s than the typical C3 pathway. The C4 pathway reduces photorespiration, the antagonist process of RuBisCo that causes a reduction in Ci assimilation about 25-40% (Ehleringer et al., 1991; Bauwe et al., 2010; Zabaleta et al., 2012). C4 pathway plants’ photorespiration reduction could be explained by their resource allocation, where they have more investment in C.C.M. than in RuBisCo protein content than plants with C3 pathway (Young et al., 2016). Also, the reports of C4 or C4-like pathway in marine algae have increased in the last years.
High activity of key enzymes of C4 metabolism, such as pyruvate orthophosphate dikinase (PPDK), phosphoenolpyruvate carboxylase (PEPC), and phosphoenolpyruvate carboxykinase (PCK), has been described in macroalgae species. The establishment of a true C4 pathway in marine algae is not clear since the massive changes in gene expression patterns seem to be incomplete and it is suggested that many marine algae have high plasticity to use a combination of CCM to overcome C\textsubscript{i} limitations (Roberts et al., 2007; Doubnerová and Ryslavá, 2011; Xu et al., 2012, 2013). A stepwise model of the path from C3 to C4 photosynthesis is explained in Gowik and Westhoff (2011).

An elevated \(\delta^{13}C\) signal in macroalgae can also be associated to calcifying species. For instance, in our study, the genus *Amphiroa* and *Jania* both Rhodophyta with articulated-form, averaged -7.86\(\pm\)3.7\%\textsubscript{o} and -9.37\(\pm\)0.75\%\textsubscript{o}, respectively, which suggest the activity of a CCM using HCO\textsubscript{3}\textsuperscript{-} efficiently. Stepien (2015) reported a global mean of -14.83\(\pm\)1.0\%\textsubscript{o} for calcifying species compared to -20.11\(\pm\)0.31\%\textsubscript{o} for non-calcifying species. High \(\delta^{13}C\) values for calcifying species are related to the excess of H\textsuperscript{+} released as residuals products of the calcifying process, the acidified boundary layers benefit the HCO\textsubscript{3}\textsuperscript{-} uptake (McConnaughey & Whelan 1997, Courneau et al., 2012). The high \(\delta^{13}C\) values can also be related to the highly efficient light properties enhanced in the carbonate skeleton, resulting in an optimization of photosynthetic activity (Vasquez-Elizondo et al., 2016, 2017). Hofmann and Heesch (2018) reported high \(\delta^{13}C\) values in eight rhodoliths species (calcifying species) collected in deep habitats (25-40m) where light availability is low. High \(\delta^{13}C\) has been reported for other calcifying species (e.g., *Halimeda*, *Udotea*, *Penicillus* with \(\delta^{13}C\) usually >10\%\textsubscript{o}) inhabiting seagrass meadows, where the light availability is limited by the *Thalassia testudinum* canopy structure (Berger, 1981; Aharon, 1990; Oehlert et al., 2012; Enríquez et al., 2019). Another case is *Padina* (frondose), a genus with lesser capacity to precipitate CaCO\textsubscript{3}, but that show relatively
According to our fitted regression model to explain the variability of δ\textsubscript{13}C by genera can be classified from high (e.g. *Schizymenia* = -19.09‰), moderate (e.g., *Hydroclathrus* = 7.33‰; *Amphiroa* = 6.84‰) and low variability (e.g. *Gracilaria* = -0.89; *Spyridia* = -1.46‰). Most species belong into the moderate category, and these range of δ\textsubscript{13}C values found is similar to those reported for algae growing up between saturating (less negative values) or sub-saturating light intensity (more negative values) (Hu et al., 2012; Rautemberger et al., 2015; Kübler and Dungeon, 2015). For instance, experimental evidence by Rautemberger et al. (2015) showed *Ulva prolifera* growing under saturated light at different pCO\textsubscript{2} levels showed the highest growth rates and activity of internal carbonic anhydrase reached δ\textsubscript{13}C signal >-10‰, higher than signal under low light regimen at same pCO\textsubscript{2} level. The authors concluded that CCM activity is energy and light dependent. Also, Kübler and Dudgeon (2015) reported that pCO\textsubscript{2} and temperature depend on light intensities. Under sub-saturating light intensities, pCO\textsubscript{2} has a stronger effect on photosynthetic rates, and the temperature effect increases at saturating light intensities. Light limitation effect on δ\textsubscript{13}C signal has been observed in deep subtidal habitats (Mercado et al., 2009; Hepburn et al., 2011; Marconi et al., 2011; Stepien 2015). Nevertheless, our study’s shallow water samples’ depth was insufficient to find significant differences in δ\textsubscript{13}C between submaraeal and intertidal habitats. Even so, according to multivariate linear regression analyses, the emersion level could explain a high percentage of the variability be genus and morpho-functional groups.

Belonging to submaraeal habitats, we found three non-calcifying species (*Schizymenia pacifica, Halymenia* sp., *Gigartina* sp.) of Rhodophyta with negatives values lesser than -30‰, which suggest that are diffusive CO\textsubscript{2} users and for hence lack CCM. Their δ\textsubscript{13}C signal are consistent with the results of Murru and Sandgreen (2004) whose described *S. pacifica* and two species of *Halymenia* (e.g., *H.*
schizymenioides and H. gardner) as a restricted CO$_2$ user based on measurements of pH drift. Red macroalgae that lack CCM, tend to inhabit in low-light habitats like subtidal or low intertidal and be abundant in cold waters (Kübler et al., 1999, Raven et al., 2002a, Cornwall et al., 2015). According to these authors, approximately 35% of the total red algae tested on a global scale are strictly CO$_2$ dependents. Our study evaluated 91 species of 453 red algae species reported in the Gulf of California (Pedroche and Sentíes, 2003), which <3% of red macroalgae specimens could be Ci limited. The low percentage of red macroalgae in the GC lack of CCM, which can be partially explained by the low solubility of CO$_2$ due to relatively high temperatures in subtropical waters (Zeebe & Wolf-Gladrow, 2007). The percentage of macroalgae species representative of Arctic and Antarctic ecosystems is 42-60% (Raven et al., 2002b; Iñiguez et al., 2019), 50% for temperate waters of New Zealand (Hepburn et al., 2011) and until 90% found for a single site of Tazmania Australia (Cornwall et al., 2015). The GC represents close 97%.

4.2. Environment factors and $\delta^{13}$C values

We expected differences in $\delta^{13}$C values between eco-regions (e.g., north vs. south, peninsular vs. continental), but non-geographical patterns were observed; neither differences associated with the temperature for the same species or genus was observed. A slightly low $\delta^{13}$C signal in communities from C2 eco-region was observed, influenced by the Sonora desert.

Based on pH, differences in $\delta^{13}$C were found only for a few genera (e.g., Amphiroa, Colpomenia, Ulva, Spyridia), with a trend to increase in the $\delta^{13}$C values with pH (Maberly et al., 1992, Raven et al. 2002b). Similar results were reported for Cornwall et al. (2017) with the differential response of the $\delta^{13}$C signals to pH among 19 species, in which only four species were sensitive to pH changes. Our in-situ pH measurements do not represent the pH compensation point; the physiology
measurement indicates the presence or absence of CCM in photosynthetic organisms. Based on the complete dataset, a weak but significant positive linear regression was observed between δ\textsubscript{13}C and pH, similar to the reported by Iñiguez et al. (2009) in three taxa of polar macroalgae. According to Stepien (2015), the result of meta-analyses between pH values and δ\textsubscript{13}C was positive only for Rhodophyta (R\textsuperscript{2}=0.41, p<0.001) and Ochrophyte (R\textsuperscript{2}=0.19, p<0.001), but not for Chlorophyta (R\textsuperscript{2}=0.002, p<0.10). About 86% of the Stepien metadata met the theoretical CCM assignment based on both parameters, exceptions for species with δ\textsubscript{13}C<-30‰ that has been capable of raising pH>9.

Our linear regression analyzes for latitudes showed a weak but significant correlation for the dataset classified by morphofunctional groups and genus, negative in the cases of Rhodophyta and Ochrophyta groups (R\textsuperscript{2}=0.2 and 0.5, p<0.001), and a positive for Chlorophyta. The negative correlation between latitude and δ\textsubscript{13}C-algal was described by Stepien (2015), concluding that δ\textsubscript{13}C signal increased by 0.09‰ for each latitude degree from the Equator. Hofmann and Heesch (2018) recently show a strong decrease in latitudinal effect (R\textsuperscript{2}= 0.43 \textsubscript{δ\textsuperscript{13}C\textsubscript{total}} and 0.13, for \textsubscript{δ\textsuperscript{13}C\textsubscript{organic-tissue}}, p=0.001) for rhodolite of the northern hemisphere and macroalgae from coral reefs in Australia. In both cases, the latitude range is higher than we tested (30° to 80° and from 10° to 45°, respectively). These differences on a big scale tend to be associated with a temperature effect (Stepien, 2015) and their effect on CO\textsubscript{2} solubility in S.W. (Zeebe & Wolf-Gladrow, 2007). Even so, our multivariate linear regression analyses showed that the environmental factors were significant (p=0.001), explaining up to 50% of the δ\textsubscript{13}C variability.

**4.3. Morphofunctional groups and δ\textsubscript{13}C**

The variability recorded on morphofunctional groups was high, mostly influenced by the genus. The highest δ\textsubscript{13}C values were found in R-larger size articulated y R-smaller-side articulated composed
by Amphiroa and Jania spp, respectively, also O-hollow with spherical composed Colpomenia spp.  

Based on the literature, Stepien (2015) made an analysis about morphofunctional groups and $\delta^{13}\text{C}$ by following the group proposed by Littler & Littler (1980) and modified by Balata et al., (2012) and they agreed that morphofunctional groups that are composed calcifying species (e.g., crust calcifiers) have highest $\delta^{13}\text{C}$ signal. Our regression models showed that morphofunctional groups have a $R^2$ adjusted =0.34, and increase to genus ($R^2$ adjusted =0.41) and to species ($R^2$ adjusted =0.46). This result is consistent with reported by Lovelock et al., (2020), which found that 66% of $\delta^{13}\text{C}$ variability was explained by taxonomy. Although morphofunctional groups could explain less than genus or species, it is a great tool to increase the possibility of analyzes on a big spatial scale, especially when the species distribution could be limited.

5. Conclusions

Our work confirms that taxonomy is the main cause of $\delta^{13}\text{C}$ variability among seaweed communities analyzed and explained until 46%. Most species showed a $\delta^{13}\text{C}$ belong into a range that indicates a mix of CO$_2$ and HCO$_3^-$ uptake. About 20-34% species depending on cutoff limits for CCM presence showed at least one specimen with $\delta^{13}\text{C}$$> -10\%\text{o}$, suggesting that potentially could have highly efficient CCM. On the other extreme, some Rhodophyta species relied exclusively on diffusive CO$_2$ entry, as inferred from their $\delta^{13}\text{C}$ values (i.e. $\delta^{13}\text{C}$ lower than -30‰; Schizymenia pacifica, Halymenia sp., and Gigartina sp.). Even so, $\delta^{13}\text{C}$ variability associated with species can be classified in high (-19‰), moderate (7%), low (0.89‰). This variability range is similar to $\delta^{13}\text{C}$ values between growing under saturating light (high values) and no saturating (low values). Specimens collected from the subtidal habitat showed more negative $\delta^{13}\text{C}$ values (higher discrimination) than the intertidal habitat but without significant difference. The percent of Rhodophyta species (3.26%) that could be Ci limited (without evident CCM activity) is relatively low in comparison that reported
for temperate regions (40-90%). The data presented indicate that \text{HCO}_3^-\text{uptake by active transport is widespread among GC algae. In this sense, } \delta^{13}\text{C provides information about the physiological and environmental status of macroalgae.}

6. Data Availability Statement

Data set are each permanently deposited Soto-Jimenez, MARTIN F; Velázquez-Ochoa, Roberto; Ochoa Izaguirre, Maria Julia. Earth and Space Science Open Archive ESSOAr; Washington, Nov 25, 2020. DOI: 10.1002/essoar.10504972.1

https://search.proquest.com/openview/2060de58b217ca47495469b53ae2f347/1?pq-origsite=gscholar&cbl=4882998

7. Author contribution

Velázquez-Ochoa R. participate in the collection, processing and analysis of the samples as a part of his master’s degree thesis. Ochoa-Izaguirre J. also participate in sample collections and identified macroalgae specimens. Soto-Jiménez M.F. coordinated the research, he was the thesis director and prepared the manuscript with contributions from all co-authors.

8. Competing interests

The authors declare that they have no conflict of interest.

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Figure captions

Fig. 1. Sites collection along the continental (C1-C3) and peninsula (P1-P3) Gulf of California coastlines (A), range of environmental factors supporting or limiting the life processes for the macroalgal communities within a habitat (B), and inserted Table with the features and environmental conditions in the diverse habitats in the GC ecoregion that delimits the macroalgal community’s zonation.

Fig. 2. Variability δ¹³C values for specimens of different macroalgae genera collected along GC coastlines classified by taxon, Chlorophyta and Ochrophyta (a) and Rhodophyta (b). Different letters indicate significant differences (P<0.05): a>b>c>d>e.

Fig. 3. Variability δ¹³C values for the most representative genus collected along continental (C1 to C3) and peninsula (P1 to P3) coastline of the Gulf of California.

Fig. 4. Variability of δ¹³C values in macroalgae specimens for the most representative genera in function of habitat features (emersion level).
Fig. 5. Variability of δ\textsuperscript{13}C values in macroalgae specimens for the most representative genus in function of temperature (a) and pH (b) ranges in samples collected along continental (C1-C3) and peninsula (P1-P3) Gulf of California coastline.

Fig. 6. Trends in the δ\textsuperscript{13}C-macroalgal from GC along latitude gradients for genus classified by phyla Chlorophyta (a), Ochrophyta (b), and Rhodophyta (c). Solid lines indicate the significant relationships with P<0.05.

Fig. 7. Trends in the δ\textsuperscript{13}C-macroalgal in specimens collected along continental (C1-C3) and peninsula (P1-P3) Gulf of California coastline in function of pH in seawater.
Fig. 1 Habitat features and environmental conditions in sampling sites

| Environmental Factors | Continental GC coastline | Peninsula GC coastline |
|------------------------|--------------------------|------------------------|
| Substrate at shore     | Rocky and sandy-rocky    | Rocky and sandy-rocky  |
| Hydrodynamics          | Slow to fast             | Slow to fast           |
| Protection level       | Exposed and protected    | Exposed and protected  |
| Sediment level         | Intertidal and subtidal  | Intertidal and subtidal|
| pH range               | Acid, typical and alkaline| Acid, typical and alkaline|
| Temperature range      | Typical and warmer       | Colder, typical and warmer|
| Salinity range         | Marine                    | Marine                 |
Fig 2
Fig 3
Fig 4
Fig 5
Fig 6

13C values (‰) vs Latitude

- Fig 6a: \( \delta^{13}C = -23.53 - 0.34 \times \text{Latitude} \)
  \( R^2 = 0.09, n=206, p<0.0001 \)

- Fig 6b: \( \delta^{13}C = -3.84 - 0.31 \times \text{Latitude} \)
  \( R^2 = 0.05, n=277, p<0.0003 \)

- Fig 6c: \( \delta^{13}C = -9.10 - 0.21 \times \text{Latitude} \)
  \( R^2 = 0.02, n=253, p<0.02 \)
Fig 7

\[ \delta^{13}C = -32.45 - 2.197 \times \text{pH} \]

\[ R^2 = 0.04, n = 809, p < 0.0001 \]
Table 1. Carbon isotopic composition (‰) in species of phyla Chlorophyta collected along Gulf of California coastlines.

| Species (n composite samples) | $\delta^{13}$C±SD (Min to Max, ‰) |
|------------------------------|----------------------------------|
| Chaetomorpha sp. (3)         | -13.7±0.83 (-14.56 to -12.9)     |
| C. antennina (10)            | -14.58±1.10 (-16.29 to -12.79)   |
| C. linum (5)                 | -16.84±1.65 (-18.45 to -14.6)    |
| Codium sp. (5)               | -11.6±3.01 (-14.07 to -6.65)     |
| C. amplivesiculatum (8)      | -14.44±2.74 (-20.42 to -11.25)   |
| C. brandegeei (7)            | -11.82±1.24 (-13.67 to -10.43)   |
| C. fragile (4)               | -13.0±2.66 (-14.78 to -9.04)     |
| C. simulans (9)              | -11.4±2.20 (-14.92 to -8.26)     |
| Ulva sp. (12)                | -13.98±3.85 (-19.16 to -7.11)    |
| U. acanthophora (25)         | -15.78±1.72 (-18.27 to -11.44)   |
| U. clathrata (8)             | -16.35±2.01 (-20.54 to -14.52)   |
| U. compressa (4)             | -17.84±2.39 (-20.58 to -15.42)   |
| U. flexuosa (13)             | -16.03±3.67 (-25.92 to -10.38)   |
| U. intestinalis (16)         | -15.29±2.54 (-20.29 to -8.95)    |
| U. lactuca (31)              | -14.1±3.14 (-19.56 to -7.67)     |
| U. linza (6)                 | -15.56±2.44 (-19.43 to -13.21)   |
| U. lobata (5)                | -13.18±1.87 (-15.33 to -11.11)   |
| U. prolifera (3)             | -14.24±1.76 (-15.49 to -12.22)   |
Table 2. Carbon isotopic composition (‰) in species of phyla Ochrophyta collected along Gulf of California coastlines.

| Species (n composite samples) | δ¹³C±SD (Min to Max, ‰) |
|-------------------------------|-------------------------|
| Colpomenia sp. (11) | -10.97±3.65 (-18.98 to -5.42) |
| C. ramosa (4) | -11.43±2.55 (-13.76 to -7.81) |
| C. sinuosa (7) | -10.18±2.95 (-16.27 to -7.18) |
| C. tuberculata (64) | -8.72±3.20 (-19.19 to -2.20) |
| Padina sp. (15) | -11.1±1.53 (-13.06 to -7.94) |
| P. crispata (3) | -11.27±1.71 (-12.47 to -10.06) |
| P. durvillaei (36) | -13.2±2.59 (-19.97 to -9.19) |
| Sargassum sp. (34) | -14.25±2.36 (-18.71 to -7.95) |
| S. herporhizum (7) | -13.65±1.63 (-16.59 to -11.51) |
| S. horridum (12) | -15.52±2.89 (-19.72 to -9.52) |
| S. johnstonii (10) | -15.41±1.98 (-17.71 to -11.8) |
| S. lapazeanum (7) | -14.49±1.59 (-17.19 to -12.81) |
| S. sinicola (31) | -15.11±2.41 (-21.1 to -12.13) |
Table 3. Carbon isotopic composition (‰) in species of phyla Rhodophyta collected along Gulf of California coastlines.

| Species (n composite samples)         | δ¹³C±SD (Min to Max, ‰) |
|--------------------------------------|-------------------------|
| *Gracilaria* sp. (18)                | -15.48±2.43 (-21.83 to -12.24) |
| *Gracilaria* sp2 (3)                 | -14.41±3.71 (-18.7 to -12.26) |
| *G. crispata* (7)                    | -15.07±2.96 (-19.13 to -10.14) |
| *G. pacifica* (6)                    | -16.48±1.64 (-18.57 to -13.61) |
| *G. spinigera* (3)                   | -14.94±3.84 (-17.66 to -12.23) |
| *G. subsecundata* (8)                | -15.93±2.82 (-20.31 to -12.78) |
| *G. tepocensis* (3)                  | -15.1±1.92 (-17.01 to -13.16)  |
| *G. textorii* (4)                    | -16.2±2.62 (-18.05 to -14.35)  |
| *G. turgida* (5)                     | -15.34±3.56 (-20.72 to -12.04) |
| *G. vermiculophylla* (16)            | -15.88±3.83 (-23.35 to -8.81)  |
| *Hypnea* sp. (14)                    | -14.95±2.56 (-20.85 to -11.41) |
| *H. johnstonii* (5)                  | -11.18±3.52 (-13.76 to -6.54)  |
| *H. pannosa* (5)                     | -11.8±3.31 (-14.95 to -6.39)   |
| *H. spinella* (6)                    | -16.44±1.75 (-19.23 to -14.87) |
| *H. valentiae* (6)                   | -15.24±2.32 (-19.16 to -12.66) |
| *Laurencia* sp. (8)                  | -12.92±1.22 (-14.65 to -10.95) |
| *L. pacifica* (8)                    | -14.86±2.19 (-18.97 to -12.69) |
| *L. papillosa* (3)                   | -15.75±0.28 (-15.95 to -15.55) |
| *Spyrida* sp. (5)                    | -17.06±1.120 (-19.11 to -16.13) |
| *S. filamentososa* (14)              | -15.86±3.83 (-26.16 to -11.46) |
Table 4. Summary of the estimated regression coefficients for each simple linear regression analyses and on the constant of fitted regression models. Estimated regression coefficients includes degrees of freedom for the error (DFE), root-mean-square error (RMSE), coefficients of determination (R²) and the adjusted R² statistics, Mallow’s Cp criterion (Cp), Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC) minimum, F Ratio test, and p-value for the test (Prob > F). Models information includes value of the constant a (δ¹³C, ‰), standard error (SE), t ratio and Prob > |t| (values * are significant).

| Independent variables            | DFE  | RMSE | R²   | Adjust R² | Cp   | AICC | BIC   | F   | Prob > F | Δ¹³C (%) | SE  | t ratio | Prob > |t| |
|----------------------------------|------|------|------|-----------|------|------|-------|-----|----------|----------|-----|---------|--------|--------|
| Inherent macroalgae properties   |      |      |      |           |      |      |       |     |          |          |     |         |        |        |
| Phyllia                          | 806  | 3.66 | 0.08 | 0.07      | 3    | 4,401| 4,420 | 33.1| <0.0001**| -13.98   | 0.13| -107.4  | <0.001**|
| Morphofunctional                 | 788  | 3.10 | 0.35 | 0.34      | 21   | 4,149| 4,251 | 21.6| <0.0001**| -14.21   | 0.35| -40.80  | <0.001**|
| Genero                           | 746  | 2.92 | 0.46 | 0.41      | 63   | 4,104| 4,393 | 10.1| <0.0001**| -14.71   | 0.23| -62.64  | <0.001* |
| Species                          | 641  | 2.79 | 0.57 | 0.46      | 168  | 4,195| 4,898 | 5.2 | <0.0001**| -14.60   | 0.16| -93.22  | <0.001**|
| Biogeographical collection zone  |      |      |      |           |      |      |       |     |          |          |     |         |        |        |
| GC coastline                     | 807  | 3.79 | 0.01 | 0.01      | 2    | 4,456| 4,470 | 7.4 | 0.0067*  | -13.97   | 0.13| -104.5  | <0.001**|
| Coastal sector                   | 803  | 3.73 | 0.05 | 0.04      | 6    | 4,333| 4,465 | 7.9 | <0.001**  | -14.12   | 0.16| -90.85  | <0.001**|
| Latitude                         | 807  | 3.80 | 0.00 | 0.00      | 2    | 4,462| 4,476 | 1.5 | 0.23      | -12.25   | 1.41| -8.71   | <0.001* |
| Longitude                        | 807  | 3.81 | 0.00 | 0.00      | 2    | 4,463| 4,477 | 0.1 | 0.80      | -15.44   | 5.83| -2.65   | 0.0082*|
| Substrate                        |      |      |      |           |      |      |       |     |          |          |     |         |        |        |
| Hydrodynamie                     | 807  | 3.80 | 0.00 | 0.00      | 2    | 4,460| 4,474 | 3.2 | 0.08      | -13.82   | 0.15| -92.06  | <0.001* |
| Emerision level                  | 807  | 3.89 | 0.06 | 0.06      | 2    | 4,412| 4,427 | 5.2 | <0.001**  | -14.05   | 0.13| -107.6  | <0.001**|
| Environmental conditions         |      |      |      |           |      |      |       |     |          |          |     |         |        |        |
| Temperature                      | 802  | 3.70 | 0.01 | 0.01      | 2    | 4,390| 4,404 | 5.4 | 0.0207*  | -16.11   | 0.96| -16.78  | <0.001* |
| pH                               | 807  | 3.73 | 0.04 | 0.04      | 2    | 4,430| 4,444 | 33.4| <0.001**  | -32.45   | 3.21| -101.3  | <0.001**|
| Salinity                         | 806  | 3.80 | 0.00 | 0.00      | 2    | 4,456| 4,470 | 0.9 | 0.34      | -15.77   | 1.91| -8.27   | <0.001**|

*p<0.05, **p<0.001
Table 5. Summary of the estimated regression coefficients for each multivariate linear regression analyses and on their constant of fitted regression models performed in individuals binned by genus. Estimated regression coefficients include degrees of freedom for the error (DFE), root-mean-square error (RMSE), coefficients of determination (R\(^2\)) and the adjusted R\(^2\) statistics, Mallow’s Cp criterion (Cp), Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC) minimum, F Ratio test, and p-value for the test (Prob > F). Model information includes value of the constant a (δ\(^{13}\)C, ‰), standard error (SE), t ratio and Prob > |t| (values * are significant).

| Independent variables | DFE | RMSE | R\(^2\) | R\(^2\) | Cp | AICc | BIC | F ratio | Prob > F | δ\(^{13}\)C (%) | SE | t ratio | Prob > |t| |
|------------------------|-----|------|--------|--------|----|------|-----|--------|---------|-------------|----|--------|---------|-----|
| Coastal sector         | 652 | 2.78 | 0.57   | 0.47   | 157| 4,169| 4,834| 20.0   | <.0001* | -17.52      | 0.64| -27.24 | <.0001* |
| Substrate              | 711 | 2.90 | 0.49   | 0.42   | 98 | 4,140| 4,577| 0.4    | 0.52    | -16.35      | 0.62| -26.20 | <.0001* |
| Hydrodynamic           | 714 | 2.87 | 0.50   | 0.43   | 95 | 4,120| 4,545| 0.1    | 0.78    | -16.53      | 0.64| -25.95 | <.0001* |
| Emersion level         | 713 | 2.77 | 0.53   | 0.47   | 96 | 4,060| 4,489| 153.0  | <.0001* | -16.65      | 0.60| -27.85 | <.0001* |
| Temperature            | 695 | 2.81 | 0.50   | 0.43   | 109| 4,083| 4,564| 98.4   | <.0001* | -14.60      | 0.92| -15.91 | <.0001* |
| Temperature ranges     | 686 | 2.87 | 0.49   | 0.40   | 118| 4,128| 4,645| 97.7   | <.0001* | -12.91      | 0.40| -31.97 | <.0001* |
| pH                     | 701 | 2.86 | 0.51   | 0.43   | 108| 4,134| 4,611| 156.6  | <.0001* | -28.57      | 2.69| -10.64 | <.0001* |
| pH ranges              | 697 | 2.67 | 0.57   | 0.51   | 112| 4,028| 4,522| 152.2  | <.0001* | -16.39      | 0.58| -28.05 | <.0001* |
| Salinity               | 697 | 2.89 | 0.50   | 0.42   | 111| 4,151| 4,640| 162.2  | <.0001* | -17.75      | 1.63| -10.88 | <.0001* |
| Salinity ranges        | 721 | 2.91 | 0.47   | 0.41   | 86 | 4,117| 4,504| 167.8  | <.0001* | -17.64      | 0.74| -23.68 | <.0001* |
Table 6. Summary of the estimated regression coefficients for each multivariate linear regression analyses and on their constant of fitted regression models performed in individuals binned by coastline sector and genus. Estimated regression coefficients include degrees of freedom for the error (DFE), root-mean-square error (RMSE), coefficients of determination ($R^2$) and the adjusted $R^2$ statistics, Mallow’s Cp criterion (Cp), Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC) minimum, F Ratio test, and p-value for the test (Prob > F). Model information includes value of the constant $a$ (δ$^{13}$C, ‰), standard error (SE), t ratio and Prob > |t| (values * are significant).

| Independent variables | DFE | RMSE | Adj $R^2$ | $R^2$ | Cp | AICc | BIC | F ratio | Prob > F | $δ^{13}$C (‰) | SE | t ratio | Prob > |t| |
|-----------------------|-----|------|-----------|-------|----|------|------|---------|----------|-------------|-----|---------|----------|-----|
| Substrate             | 590 | 2.76 | 0.62      | 0.47  | 219| 4,287| 5,155| 15.8    | <.0001*  | -17.08      | 0.66| -25.72  | <.0001* |
| Hydrodynamic          | 592 | 2.73 | 0.62      | 0.49  | 217| 4,266| 5,128| 18.6    | <.0001*  | -17.18      | 0.67| -25.70  | <.0001* |
| Protection level      | 590 | 2.75 | 0.62      | 0.48  | 219| 4,285| 5,153| 20.0    | <.0001*  | -17.51      | 0.64| -27.22  | <.0001* |
| Emerson level         | 603 | 2.69 | 0.63      | 0.50  | 206| 4,217| 5,045| 18.6    | <.0001*  | -17.47      | 0.64| -27.49  | <.0001* |
| Temperature ranges    | 569 | 2.74 | 0.61      | 0.46  | 235| 4,293| 5,202| 28.0    | <.0001*  | -13.73      | 0.45| -30.32  | <.0001* |
| pH ranges             | 580 | 2.50 | 0.69      | 0.57  | 229| 4,155| 5,051| 9.7     | 0.0019*  | -16.88      | 0.62| -27.15  | <.0001* |
| Salinity ranges       | 631 | 2.76 | 0.58      | 0.47  | 176| 4,183| 4,913| 21.2    | <.0001*  | -18.30      | 0.79| -23.05  | <.0001* |
Table 7. Summary of the estimated regression coefficients for each multivariate linear regression analyses and on their constant of fitted regression models performed in individuals binned in coastline sector, habitats features, environmental conditions, and Physiological performed separately by morpho-functional groups and genus. Estimated regression coefficients include degrees of freedom for the error (DFE), root-mean-square error (RMSE), coefficients of determination (R$^2$) and the adjusted R$^2$ statistics, Mallow’s Cp criterion (Cp), Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC) minimum, F Ratio test, and p-value for the test (Prob > F). Model information includes value of the constant a ($\delta^{13}$C, ‰), standard error (SE), t ratio and Prob > |t| (values * are significant).

| Model Information | Estimated regression coefficients | Prob > F | $\delta^{13}$C (%) | SE | t ratio | Prob > |t|
|-------------------|----------------------------------|---------|------------------|----|---------|---------|
| Full model        | DFE | RMSE | Adjust $R^2$ | $R^2$ | Cp | AICc | BIC | F ratio | Prob > F | $\delta^{13}$C (%) | SE | t ratio | Prob > |t|
| Coastline sector + Habitats features + Morphofunctional group | 593 | 2.79 | 0.60 | 0.46 | 216 | 4,301 | 5,160 | 20.8 | <.0001* | -13.49 | 0.57 | -23.52 | <.0001* |
| I-Morpho-functional | 680 | 2.90 | 0.51 | 0.42 | 129 | 4,189 | 4,750 | 25.1 | <.0001* | -13.42 | 0.54 | -24.74 | <.0001* |
| II-Morpho-functional | 482 | 2.66 | 0.71 | 0.51 | 327 | 4,565 | 5,655 | 15.8 | <.0001* | -16.93 | 0.73 | -23.27 | <.0001* |
| Coastline sector + Habitat features + Genus | 494 | 2.49 | 0.72 | 0.55 | 310 | 4,374 | 5,438 | 14.8 | 0.0001* | -13.55 | 0.64 | -21.17 | <.0001* |
Table 8. Constant of fitted regression model explaining the $\delta^{13}C$ variability by morpho-functional groups. Model information includes value of the constant $a$ ($\delta^{13}C$, ‰), standard error (SE), t ratio and Prob > $|t|$. Only morpho-functional groups with significant effects are enlisted.

| Term                                         | Estimated | SE  | Razón t | Prob > $|t|$ |
|----------------------------------------------|-----------|-----|---------|-------------|
| Model constant                               | -14.21    | 0.35| -40.80  | <.0001**    |
| R-Smaller-sized articulated corallines       | 4.48      | 1.74| 2.58    | 0.0100*     |
| O-Compressed with branched or divided thallus| 1.24      | 0.46| 2.66    | 0.0079*     |
| C-Erect thallus                              | 1.76      | 0.62| 2.84    | 0.0046*     |
| R-Larger-sized articulated corallines        | 6.32      | 0.80| 7.95    | <.0001*     |
| O-Hollow with spherical or subspherical shape| 4.96      | 0.47| 10.51   | <.0001*     |
| R-Blade-like with one of few layers of cells | -5.89     | 2.97| -1.98   | 0.0476*     |
| C-Tubular                                    | -1.62     | 0.50| -3.26   | 0.0012**    |
| R-Filamentous uni&pluriseriate with erect thallus | -2.15     | 0.55| -3.92   | <.0001*     |
| R-Flattened macrophytes with cortication     | -8.89     | 1.25| -7.10   | <.0001*     |

*p<0.05, **p<0.0001
Table 9. Constant of fitted regression model explaining the $\delta^{13}\text{C}$ variability by genus. Model information includes value of the constant $a$ ($\delta^{13}\text{C}$, ‰), standard error (SE), $t$ ratio and Prob $>|t|$. Only genus with significant effects are enlisted.

| Term      | Estimated | SE  | Razón $t$ | Prob $>|t|$ |
|-----------|-----------|-----|-----------|-------------|
| Model constant      | -14.70    | 0.23 | -62.64    | <.0001**    |
| Corallina           |  6.40     | 2.88 |  2.22     |  0.0269*    |
| Tacanoosca          |  3.54     | 1.31 |  2.71     |  0.0070*    |
| Jania               |  4.98     | 1.68 |  2.97     |  0.0031*    |
| Struveopsis         |  4.12     | 1.31 |  3.15     |  0.0017*    |
| Codium              |  2.26     | 0.55 |  4.08     |  <.0001**   |
| Padina              |  2.19     | 0.46 |   4.8     |  <.0001**   |
| Hydroclathrus       |  7.33     | 1.11 |  6.59     |  <.0001**   |
| Amphiroa            |  6.84     | 0.76 |  9.05     |  <.0001**   |
| Colpomenia          |  5.45     | 0.39 | 14.02     |  <.0001*    |
| Spyridia            | -1.46     | 0.70 | -2.10     |  0.0361*    |
| Gracilaria          | -0.89     | 0.41 | -2.18     |  0.0294*    |
| Polysiphonia        | -3.75     | 0.78 | -4.82     |  <.0001**   |
| Schizymenia         | -19.08    | 2.05 | -9.33     |  <.0001**   |

*p<0.05, **p<0.001
Table 10. Constant of fitted regression model explaining the $\delta^{13}$C variability by species. Model information includes value of the constant $a$ ($\delta^{13}$C, ‰), standard error (SE), t ratio and Prob > $|t|$. Only genus with significant effects are enlisted.

| Term                       | $\delta^{13}$C, ‰ estimated | SE  | Razón t | Prob > $|t|$ |
|----------------------------|-------------------------------|-----|---------|-------------|
| Constante del modelo       | -14.59                        | 0.16| -93.22  | <.0001**    |
| *Hypnea pannosa*           | 2.79                          | 1.25| 2.24    | 0.0256*     |
| *Colpomenia ramosa*        | 3.16                          | 1.39| 2.27    | 0.0237*     |
| *Corallina vancouverensis* | 6.29                          | 2.78| 2.27    | 0.0238*     |
| *Caulerpa peltata*         | 3.86                          | 1.61| 2.4     | 0.0165*     |
| *Codium sp.*               | 3.00                          | 1.25| 2.4     | 0.0167*     |
| *Amphiroa misakiensis*     | 7.08                          | 2.78| 2.55    | 0.0110*     |
| *Jania* sp.*               | 5.04                          | 1.97| 2.56    | 0.0106*     |
| *Codium brandegeei*        | 2.78                          | 1.06| 2.63    | 0.0088**    |
| *Hypnea johnstonii*        | 3.42                          | 1.25| 2.74    | 0.0063**    |
| *Tacanoosca uncinata*      | 3.43                          | 1.25| 2.74    | 0.0062**    |
| *Struveopsis* sp.*         | 3.98                          | 1.39| 2.86    | 0.0044**    |
| *Padina durvillaei*        | 1.40                          | 0.49| 2.87    | 0.0043**    |
| *Amphiroa* sp.1            | 8.20                          | 2.78| 2.95    | 0.0033**    |
| *Codium simulans*          | 3.19                          | 0.94| 3.41    | 0.0007**    |
| *Amphiroa* sp.2            | 6.59                          | 1.61| 4.1     | <.0001**    |
| *Colpomenia sinuosa*       | 4.42                          | 1.06| 4.17    | <.0001**    |
| Species                        | Mean | SD  | T-value | P-value |
|-------------------------------|------|-----|---------|---------|
| *Colpomenia* sp.              | 3.63 | 0.85| 4.27    | <.0001**|
| *Padina* sp.                  | 3.50 | 0.73| 4.77    | <.0001**|
| *Hydroclathrus clathratus*    | 7.22 | 1.06| 6.82    | <.0001**|
| *Amphiroa* sp.                | 8.12 | 0.94| 8.67    | <.0001**|
| *Colpomenia tuberculata*      | 5.87 | 0.38| 15.45   | <.0001**|
| *Spyrida* sp.                 | -2.46| 1.25| -1.97   | 0.0496* |
| *Pyropia thuretii*            | -5.50| 2.78| -1.98   | 0.0480* |
| *Ulva acanthophora*           | -1.19| 0.58| -2.06   | 0.0399* |
| *Grateloupia filicina*        | -2.37| 1.14| -2.08   | 0.0382* |
| *Rhodymenia* sp.              | -4.08| 1.97| -2.08   | 0.0380* |
| *Ulva compressa*              | -3.24| 1.39| -2.33   | 0.0203* |
| *Rhizoclonium riparium*       | -5.06| 1.61| -3.15   | 0.0017**|
| *Polysiphonia* sp.            | -4.81| 1.39| -3.44   | 0.0006**|
| *Halymenia actinophysa*       | -9.91| 2.78| -3.57   | 0.0004**|
| *Cladophora microcladioides*  | -7.16| 1.97| -3.64   | 0.0003**|
| *Polysiphonia mollis*         | -5.22| 1.06| -4.93   | <.0001**|
| *Schizymenia pacifica*        | -19.19| 1.97| -9.76   | <.0001**|

*p<0.05, **p<0.001