An analysis of the variability in $\delta^{13}$C in macroalgae from the Gulf of California: indicative of carbon concentration mechanisms and isotope discrimination during carbon assimilation

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Abstract. The isotopic composition of carbon in macroalgae ($\delta^{13}$C) is highly variable, and its prediction is complex concerning terrestrial plants. The determinants of $\delta^{13}$C macroalgal variations were analyzed in a large stock of specimens that vary in taxa and morphology and were collected in shallow marine habitats in the Gulf of California (GC) with distinctive environmental conditions. A large $\delta^{13}$C variability ($-34.6\text{‰}$ to $-2.2\text{‰}$) was observed. Life-forms (taxonomy 57%, morphology and structural organization 34%) explain the variability related to carbon use physiology. Environmental conditions influenced the $\delta^{13}$C macroalgal values but did not change the physiology, which is most likely inherently species-specific. Values of $\delta^{13}$C were used as indicators of the presence or absence of carbon concentrating mechanisms (CCMs) and as integrative values of the isotope discrimination during carbon assimilation in the life cycle macroalgae. Based on $\delta^{13}$C signals, macroalgae were classified in three strategies relative to the capacity of CCM: (1) HCO$_3^-$ uptake ($\delta^{13}$C $> -10\text{‰}$), (2) using a mix of CO$_2$ and HCO$_3^-$ uptake ($-10 < \delta^{13}$C $< -30\text{‰}$), and (3) CO$_2$ diffusive entry ($\delta^{13}$C $< -30\text{‰}$). Most species showed a $\delta^{13}$C that indicates a CCM using a mix of CO$_2$ and HCO$_3^-$ uptake. HCO$_3^-$ uptake is also widespread among GC macroalgae, with many Ochrophyta species. Few species belonging to Rhodophyta relied on CO$_2$ diffusive entry exclusively, while calcifying macroalgae species using HCO$_3^-$ included only Amphiroa and Jania. The isotopic signature evidenced the activity of CCM, but it was inconclusive about the preferential uptake of HCO$_3^-$ and CO$_2$ in photosynthesis and the CCM type expressed in macroalgae. In the study of carbon use strategies, diverse, species-specific, and complementary techniques to the isotopic tools are required.

1 Introduction

Macroalgae show a wide diversity of thallusmorphologies (e.g., filamentous, articulated, flattened), structural organization (e.g., surface area : volume ratio), and various photosynthetic pigments (e.g., Chlorophyll a, b, phycocyanin) (Lobban and Harrison, 1994). According to the predominant pigment contents in the thallus, macroalgae are classified into three phyla. The interaction of morphologies and photosynthetic pigments is classified into dozens of groups (Balata et al., 2011; Littler and Littler, 1980; Littler and Arnold, 1982). For example, the mixture of chlorophyll (a, b) and carotenoids is dominant in Chlorophyta, and chlorophyll (a, c) and fucoxanthin carotenoid are dominant in Ochrophyta, while Rhodophyta contains chlorophyll (a, d), carotenoid, and a mixture of phycobilin (e.g., phycocyanin, phycoerythrin, allophycocyanin) (Bold and Wynne, 1978; Gateau et al., 2017; Masojidek et al., 2004). Both traits work as an excellent approximation to explain the fundamentals of metabolism, growth, zonation, and colonization (Littler and

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Littler, 1980; Littler and Arnold, 1982; Nielsen and Sand-Jensen, 1990; Vázquez-Elizondo and Enríquez, 2017).

In marine environments, where pH = 8.1 ± 1, the diffusion rate of CO$_2$ in seawater is low. Thus, HCO$_3^-$ accounts for 98% of the total dissolved inorganic carbon (DIC), resulting in a high HCO$_3^-$ : CO$_2$ ratio (150:1) (Sand-Jensen and Gordon, 1984). Low CO$_2$ concentrations in seawater, which limit macroalgal growth, are compensated for by carbon concentrating mechanisms (CCMs) that increase the internal inorganic carbon concentration near the site of RuBisCo activity (Giordano et al., 2005). Therefore, the absorption of HCO$_3^-$ by most macroalgae is the primary source of inorganic carbon for photosynthesis, but some species depend exclusively on the use of dissolved CO$_2$ that enters cells by diffusion (Beardall and Giordano, 2002; Giordano et al., 2005; Maberly et al., 1992; Raven et al., 2002a, b). Hence, macroagal species with productivity limited by lacking CCMs (having low plasticity for inorganic carbon uptake) seems to be restricted to subtidal habitats and composed mainly of red macroalgae (but without a morphological patron apparent) (Cornwall et al., 2015; Kübler and Dudgeon, 2015). The rest of the macroalgae with CCM occupies the intertidal to the deep subtidal zone.

The habitat features and environmental conditions in marine ecosystems modify the main macroalgae photosynthesis drivers, such as light (Anthony et al., 2004; Johansson and Snoeijis, 2002), DIC (Brodeur et al., 2019; Zeebe and Wolf-Gladrow, 2001), and inorganic nutrients (Ochoa-Izaguirre and Soto-Jiménez, 2015; Teichberg et al., 2010). These factors could generate negative consequences for their productivity, principally when they cause resource limitation. Each factor varies from habitat to habitat (e.g., local scale: from intertidal to subtidal zone; and global scale: from temperate to tropical regions), and to these environmental changes, macroalgae can modulate their photosynthetic mechanism (Dudgeon et al., 1990; Kübler and Davison, 1993; Lapointe and Duke, 1984; Young and Beardall, 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 (Enriquez and Rodriguez-Román, 2006; Giordano et al., 2005; Klenell et al., 2004; Madsen and Maberly, 2003; Rautenberger et al., 2015; Zou et al., 2004).

The δ$^{13}$C macroalgal values indicate the carbon source used (CO$_2$ or HCO$_3^-$) in photosynthesis and allow the presence or absence of CCMs to be inferred (Giordano et al., 2005; Maberly et al., 1992; Raven et al., 2002a). However, the isotopic signature may be inconclusive for determining the carbon source’s preference (Rolda and Hurd, 2012). Also, the δ$^{13}$C signal in the algal thallus can be used to indicate the physiological state of photosynthetic metabolism (Kim et al., 2014; Kübler and Dungeon, 2015). For example, δ$^{13}$C variability depends, in part, on the life-forms’ taxonomy, morphology, and structural organization (Lovelock et al., 2020; Marconi et al., 2011; Mercado et al., 2009; Rolda and Hurd, 2012). δ$^{13}$C is also modulated by the interaction with environmental conditions (e.g., light, DIC, and nutrients) (Carvalho et al., 2010a, b; Cornelissen et al., 2007; Dudley et al., 2010; Mackey et al., 2015; Rautenberger et al., 2015; Rolda and Hurd, 2012). In this study, our objective was to investigate the contributions of life-forms, the changes in the habitat features, and environmental conditions to the δ$^{13}$C macroalgal variability in communities in the Gulf of California (GC). We collected a large stock of macroalgal specimens of a diversity of species characterized by various morphological and physiological properties to reach our objective. Besides high diversity, in terms of life-forms, we selected various shallow marine habitats along a latitudinal gradient in the GC or the sample collection, characterized by unique and changing environmental factors. The GC features abundant and diverse macroalgal populations, acclimated and adapted to diverse habitats with environmental conditions determining the light, DIC, and nutrient availability. The δ$^{13}$C signal from the thallus of macro algae was used as indicative of the presence or absence of CCMs and as integrative values of the isotope discrimination during carbon assimilation and respiration along the life cycle of macroalgae in macroalgal communities in the GC as a function of taxa and environmental factors (Díaz-Pulido et al., 2016; Hepburn et al., 2011; Maberly et al., 1992; Raven et al., 2002a). Because the GC is a subtropical zone with high irradiance and specimens were collected in the intertidal and shallow subtidal zone, we expect to find a high proportion of species with active uptake HCO$_3^-$ (δ$^{13}$C > −10‰). A third objective was to explore any geographical pattern in the δ$^{13}$C macroalgal along and between the GC bioregions. Previous studies have indicated changes in the δ$^{13}$C signal with latitude, mainly related to the light and temperature (Hofmann and Heesch, 2018; Lovelock et al., 2020; Marconi et al., 2011; Mercado et al., 2009; Stepien, 2015). 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, 2015).

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 making it the most important fishing region for Mexico and one of the most biologically diverse worldwide marine areas (Espinoza-Careón and Valdez-Holgún, 2007; Lluch-Cota et al., 2007; Páez-Osuna et al., 2017; Zeitzschel, 1969). The Gulf of California 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° latitude). However, the GC
has a high physiographic diversity and is biologically mega-
diverse with many endemic species, including ~766 macro-
fauna species and/or subspecies in which the major num-
ber belong to Arthropoda (118 species) and Mollusca (460
species) taxa (Brusca et al., 2005; Espinoza-Carreón and
Escobedo-Urías, 2017; Wilkinson et al., 2009) and 116 to
an macroalga species (Espinoza-Avalos, 1993; Norris, 1975,
1985).

Regionalization criteria of the GC include phytoplankton
distribution (Gilbert and Allen, 1943), topography (Rusnak
et al., 1964) and depth (Álvarez-Borrego, 1983), oceanog-
graphic characteristics (Álvarez-Borrego, 1983; Marinone
and Lavín, 2003; Roden and Emilion, 1979), biogeography
(Santamaría-del-Ángel et al., 1994), and bio-optical charac-
teristics (Bastidas-Salamanca et al., 2014). The topography
is variable along the GC and includes submarine canyons,
basins, and variable continental platforms. Besides, the GC
presents complex hydrodynamic processes, including inter-
nal waves, fronts, upwelling, vortices, and mixing of tides.
The gulf’s coastline is divided into three shores: extensive
rocky shores, long sandy beaches, numerous scattered estu-
aries, coastal lagoons, 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 on average), divided
into the upper gulf, northern gulf, and Midriff Islands re-
gions (Roden, 1958; Roden and Groves, 1959). The sur-
rounding deserts largely influence this region (Norris, 2010),
which shows marked seasonal changes in coastal surface
seawater temperatures (Marinone, 2007; Martínez-Díaz-de-
León et al., 2006). Tidal currents induce a significant cy-
clonic circulation through June to September and anticy-
clonic from November to April (Bray, 1988; Carrillo and
Palacios-Hernández, 2002; Martínez-Díaz-de-León, 2001;
Velasco-Fuentes and Marinone, 1999). The southern part
consists of a series of basins whose depths increase south-
wards (Fig. 1). The intertidal macroalgae in the southern re-
gion are subject to desiccation, mostly during summer. The
water column’s physiochemical 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
(1080 mm yr\(^{-1}\)) and evaporation (56 mm yr\(^{-1}\)) rates reg-
istered during the past 40 years were 881 ± 365 mm yr\(^{-1}\) and
53 ± 7 mm yr\(^{-1}\), respectively (CNA, 2012).

In the GC around 669 macroalga species exist, includ-
ing 116 endemic species (Espinoza-Avalos, 1993; Norris,
1975; Pedroche and Sentíes, 2003). Many endemic species
currently have a wide distribution along the Pacific Ocean
coast but with GC origin (Aguilar-Rosas et al., 2014; Dreck-
man, 2002). Based on oceanographic characteristics (Ro-
den and Groves, 1959) and in the endemic species distribu-
tion (Aguilar-Rosas and Aguilar-Rosas, 1993; Avalos, 1993),
the GC can be classified into three phycofloristic zones:
(1) the first zone located from the imaginary line connecting
San Francisquito Bay, B.C. (Baja California), to Guaymas,
Sonora, with 51 endemic species; (2) the second zone with
an imaginary line from La Paz Bay (B.C.S.; Baja Califor-
nia Sur) 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 en-
demic species. Besides, 14 endemic species are distributed
throughout the GC (Espinoza-Avalos, 1993). The macroal-
gal communities are subject to the changing environmental
conditions in the diverse habitats in the GC that delimit
their zonation, which tolerates a series of anatomical and
physiological adaptations to water movement, temperature,
sun exposure, light intensities, low \( p\text{CO}_2 \), and desiccation
(Espinoza-Avalos, 1993).

2.2 Macroalgae sampling

In this study, the GC coastline (21–30° N latitude) was di-
vided into six coastal sectors based on the three phycoflor-
istic zones along peninsular and continental GC coastlines
(Fig. 1a). In each coastal sector, selected ecosystems and
representative habitats were sampled based on macroalgae
communities’ presence and habitat characterization. Habi-
tats were classified by substrate type (e.g., sandy-rock, rocky
shore), hydrodynamic (slow to faster water flows), protec-
tion level (exposed or protected sites), and immersion level
(intertidal or subtidal) (Fig. 1b).

Based on the local environmental factors, four to five
macroalgae specimens of the most representative species
were gathered by hand (free diving) during low tide. A total
of 809 composite samples were collected from marine habi-
tats along both GC coastlines. The percentages of specimens
collected for the substrate type were 28 % sandy-rock and
72 % rocky shores based on the habitat features. In the hy-
drodynamic, 30 % of the specimens were collected in habi-
tats 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 speci-
mens were collected in isolated rock pools, which was noted.

In four to five sites of each habitat, we measured in situ
the salinity, temperature, and pH by using a calibrated multi-
parameter sonde (Y.S.I. 6600V) and the habitat characteristics
mentioned above noted. Besides, composite water samples
were collected for a complementary analysis of nutrients,
alkalinity (and their chemical components), and \( \delta^{13}\text{C} \) DIC
(data not included). Briefly, the representative habitats were
classified by pH levels of > 9.0 “alkalinized”, 7.9–8.2 “typi-
cal”, and < 7.9 “acidified”. Based on colder (< 20°C), typi-
cal (20–25°C), and warmer (> 25°C) temperatures, 72 % of
the specimens were collected at typical, 22% at alkalinized, and 6% at acidified pH values. 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 PSU, from 34.5 to 36.1 PSU). In this study, the collection surveys were conducted during spring (March–April) and dry season (nominally from November to May) from 2008 to 2014. Only in a few selected ecosystems located at C1, C2, and C3 sectors was one sampling survey 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 PSU) to hypersaline (42.7 ± 7.0 PSU) values. These habitats were mainly isolated rock pools, and only a few were sites near tidal channels receiving freshwater discharges. About 95% of the specimens were collected at typical seawater salinity (34–36 PSU) and only 1.5% and 3.5% in estuarine (<30 PSU) and hypersaline (>37 PSU) environments, respectively. Detailed information on the selected shallow marine ecosystems, habitat characterization, and environmental conditions is summarized in the inserted table in Fig. 1.

2.3 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 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 (Abbot and Hollenberg, 1976; Dawson, 1944, 1954, 1956, 1961, 1962, 1963; Norris, 2010; Ochoa-Izaguirre et al., 2007; Setchell and Gardner, 1920, 1924).

In the laboratory, macroalgae samples were immediately frozen at −30°C until analysis. Then, samples were freeze-dried at −38°C and 40 mm Hg for 3 d, 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). Aliquots of ~5 mg were encapsulated in tin cups (5 × 9 mm) and stored in sample trays until analysis. Macroalgae samples were sent to the Sta-
ble Isotope Facility (SIF) at the University of California at Davis, CA, USA. Natural $^{13}$C relative abundance relative to $^{12}$C 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-NJT 20-20) and a liquid-solid preparation unit (PDZ, Europa, Crewz, UK). Isotope ratios of the samples were calculated using the equation $\delta^{13}C = [(R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000]$, where $R = ^{13}C/^{12}C$. 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, and USGS-65) for the analytical control quality. The analytical uncertainties reported for the SIF lab were $0.2\%$ for $\delta^{13}C$ (https://stableisotopefacility.ucdavis.edu/carbon-and-nitrogen-solids, last access: 18 January 2021). 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 procedures. The methodological uncertainties were $<0.4\%$.

### 2.4 Analysis of $\delta^{13}$C macroalgal variability

The variability in $\delta^{13}$C values in macroalgae was analyzed as a function of the taxonomy (phylum, genus, and species) and morphofunctional groups (e.g., thallus structure, growth form, branching pattern, and taxonomic affinities; Balata et al., 2011; Ochoa-Izaguirre and Soto-Jiménez, 2015). The carbon fixation strategies in the macroalgae communities of the GC were identified by $\delta^{13}C$ (Díaz-Pulido et al., 2016; Hepburn et al., 2011) in agreement with the Maberly et al. (1992) and Raven et al. (2002a) thresholds. So, macroalgae were classified into three strategies for DIC uptake: (1) CCM only by active uptake $\text{HCO}_3^-$, (2) CCM active uptake $\text{HCO}_3^-$ and diffusive uptake $\text{CO}_2$ ($\delta^{13}C < -11$ to $-30\%$), and (3) non-CCM $\text{CO}_2$ by diffusion only ($\delta^{13}C < -30\%$). The measured $\delta^{13}$C macroalgal signals are integrative of the discrimination by photosynthesis ($\Delta^{13}C_{\text{P}}$) on the carbon source ($\delta^{13}C$ DIC in seawater), respiration ($\Delta^{13}C_{\text{R}}$), and probable $\text{CO}_2$ leak out inside the cell during the CCM process (Carvalho et al., 2009a, b; Raven et al., 2005; Sharkey and Berry, 1985).

Macroalgae were grouped according to their morphofunctional characteristics proposed initially by Littler and Littler (1980) and modified by Balata et al. (2011). Most of the macroalgae species showed a limited distribution along the GC coastlines. Few cosmopolites’ species included Colponemia tuberculata, Sargassum sinicola, Padina durvillei, and Ulva lactuca. Also, not all morphofunctional groups and taxa were present in every site during each sampling survey, and the sample size in each group varied for taxa, location, and time.

A basic statistical analysis of $\delta^{13}$C values in different macroalgae groups was applied to distribute and calculate the arithmetic mean, standard deviation, and 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. We compared taxa and morphofunctional groups 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 in $\delta^{13}$C macroalgae in specimens of the same morphofunction and taxon collected in different habitats were also investigated with a Kruskal–Wallis test.

The relationships between $\delta^{13}$C with the inherent macroalgae properties (taxon and morphology), biogeographical collection zone (GC coastline and coastal sector), habitat features (substrate, hydrodynamic, protection, and emersion level), and environmental conditions (temperature, $\text{pH}$, and salinity) were examined through simple and multiple linear regression analyses. Excluding temperature, $\text{pH}$, and salinity, most of the independent variables are categorical independent variables. Simple linear regression analyses were performed to establish the relationships between $\delta^{13}$C macroalgae with each environmental parameter analyzed as possible driving factors (e.g., temperature, salinity, and $\text{pH}$). 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 macroalgae. In the multivariable regression model, the dependent variable, $\delta^{13}$C macroalgal values, is described as a linear function of the independent variables $X_i$, as follows:

$$\delta^{13}C_{\text{macroalgal}} = a + b_1(X_1) + b_2(X_2) + \ldots + b_n(X_n),$$

where $a$ is regression constant (it is the value of intercept, and its value is zero), $b_1, b_2, \ldots, b_n$ 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, the $p$ value for the test (prob $> F$), coefficients of determination ($R^2$), and the adjusted $R^2$ statistics (Stroup et al., 2018). All regression coefficients were used as indicators of the quality of the regression (Burnham and Anderson, 2002; Draper and Smith, 1998). The 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 using JMP 14.0 software (SAS Institute Inc.).
3 Results

3.1 Taxonomy and morphofunctional groups

Sampled specimens belong to 3 phyla, 63 genera, and 170 species. The phyla were identified as Chlorophyta (25%), Ochrophyta (22%), and Rhodophyta (53%). The most representative genera (and their species) were Ulva (U. lactuca, U. lobata, U. flexuosa, and U. intestinalis), Codium (C. amplivesiculatum and C. simulans), Chaetomorpha (C. antennina), Padina (P. durvillei), Dictyota (D. dichotoma), Colpomenia (C. tuberculata and C. sinuosa), Sargassum (S. sinicola and S. horridum), Amphiorea (Amphiroa spp.), Spyridia spp., Polysiphonia spp., Gymnogongrus spp., Gracilaria (G. verruculophylla, G. pacifica, and G. crispa), Hypnea (H. pannosa and H. johnstonii), Grateloupia (G. filicina and G. versicolor), and Laurencia (L. papillosa and L. pacifica). The endemic species included Codium amplivesiculatum, Rhodypha successfully (strategy 3) were observed only in Rhodophyta. Moreover, most species showed large δ13C variabilities, which is evidence of a mechanism that uses a mix of HCO3- and CO2 for photosynthesis (strategy 2).

Multiple comparison analyses revealed significant differences in the δ13C macroalgal values among genera, ordered as Schizymenia < Polysiphonia < Ulva, Gracilaria and Spyridia (<16.1 ± 0.6‰ to −15.1 ± 0.2‰) < Gymnogongrus, Laurencia, Hypnea, Cladophora, Dictyota, Sargassum, Chaetomorpha, and Grateloupia (from −15.4 ± 0.7‰ to −13.8 ± 0.8‰) < Codium and Padina (<12.5 ± 2.4‰ to −12.4 ± 2.5‰) < Colpomenia and Amphiroa (<9.2 ± 0.3‰ to −7.8 ± 0.7‰) (F = 16.81, p < 0.001).

Aggregation of δ13C values based on morphofunctional features is displayed in Fig. 4. The most representative groups in the phylum Chlorophyta varied from −15.8 ± 0.3‰ for C-Tubular to −12.4 ± 0.5‰ for C-Thallus erect. The phylum Ochrophyta includes O-Thick leathery macrophytes with the lowest mean (−14.8 ± 0.3‰) and O-Hollow with a spherical or subspherical shape with the highest values (−9.2 ± 0.3‰). The lowest and highest δ13C values for Rhodophyta were observed for R-flattened macrophytes (−24.0 ± 9.6‰) and R-Larger-sized articulated coralline (−7.9 ± 0.8‰), 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-Larger-sized corticated < C-Blade-like and C-Filamentous uniseriate < C-Thallus erect and O-Compressed with branch < O-Hollow with spherical < R-Larger-sized articulated coralline.

High intraspecific variability in δ13C signal for the more representative genera of each taxon is shown in Tables 1–3. For Codium, C. brandegeei (11.8 ± 1.2‰) and C. simulans (−11.4 ± 2.2‰) showed higher δ13C values than C. amplivesiculatum (−14.4 ± 2.7‰). Colpomenia species
had higher $\delta^{13}$C values than the other genera, with higher values for $C.\ tuberculata$ (−8.7 ± 3.2‰) than $Colpomenia$ sp. (−10.9 ± 3.6‰) and $C.\ sinuosa$ (−10.2 ± 2.9‰). $Gracilaria$ showed comparable $\delta^{13}$C values in the four species (from −16.4 ± 1.6‰ for $G.\ pacifica$ to −15.5 ± 2.4‰ for $Gracilaria$ sp.). $Hypnea$ showed non-significant $\delta^{13}$C differences in three representative species (−16.4 ± 1.7‰ for $H.\ spinella$ to −14.9 ± 2.3‰ for $Hypnea$ sp.). $Laurencia$ sp. (−12.9 ± 1.2‰) was higher than $L.\ pacifica$ (−14.9 ± 2.2‰), while $Padina$ sp. (−11.1 ± 1.5‰) higher than $P.\ durvillei$ (−13.2 ± 2.6‰). $Sargassum$ was one of the most diverse genera studied with six representative species, with $\delta^{13}$C values ordered as follow: $S.\ horridum$ = $S.\ sinicola$ = $S.\ johnstonii$ (−15.5 ± 2.9 to −15.1 ± 2.4‰) < $S.\ lapazeanum$ (−14.5 ± 1.6‰) = $Sargassum$ sp. (−14.2 ± 2.3‰) < $S.\ herpitorium$ (−13.6 ± 1.6‰). $S.\ phorizum$ (−17.0 ± 1.2‰) and $S.\ filamentosa$ (−15.8 ± 3.8‰) showed non-significant differences. The six representive species of $Ulva$ were divided into two morphological groups, filamentous and laminates. Filamentous species averaged −16.3 ± 2.0‰ for $U.\ clathrata$, −16.0 ± 3.6‰ for $U.\ flexuosa$, −15.7 ± 1.7‰ for $U.\ acanthophora$, and −15.3 ± 2.5‰ for $U.\ intestinals$, and $Ulva$ laminates included $U.\ linza$ (−15.5 ± 2.4‰) and $U.\ lactuca$ (−14.1 ± 3.1‰). Non-significant differences were observed between morphological groups and among species. A high intra-specific variability, 11%–28%, explains average overlapping.

### 3.3 $\delta^{13}$C macroalgal variability in coastal sectors

A diversity of macroalgal assemblages were documented along the GC coastlines, with differences in the taxonomic composition according to their fico-floristic region. Multiple comparison analyses of $\delta^{13}$C signals evidenced significant differences between the most common genera and species of macroalgae between and within assemblages grouped by coastal sector, season, and collecting year (Tables S2–S3).

For example, genera $Padina$ (e.g., $P.\ durvillei$) and $Ulva$ (e.g., $U.\ lactuca$), collected in C1 sector during the rainy season, showed lower $\delta^{13}$C values than in other sectors. Differences in the $\delta^{13}$C signal are mainly related to the carbon uptake strategies of the macroalgae (Fig. 5). Even though most species inhabiting the GC coastal sectors dominated strategies based on active CCMs, the tendencies differed between taxa and coastal regions. Strategy 2 with mixing DIC sources...
Figure 3. Variability in $\delta^{13}$C values for the genera collected along the coastline of the Gulf of California according to their taxon: (a) Chlorophyta, (b) Ochrophyta, and (c) Rhodophyta. Genera with $n = 1$ are not shown, and genera with $n = 2$ were not considered in the statistical comparison. Different letters indicate significant differences ($P < 0.05$): $a > b > c > d > e$. Shaded background represents the cutoff limits for using CO$_2$ only users and HCO$_3^-$ only users, according to Raven et al. (2002a). For Chlorophyta: Bry = Bryopsis, Cau = Caulerpa, Cha = Chaetomorpha, Cla = Cladophora, Cod = Codium, Phy = Phyllodictyon, Str = Struveopsis, Ulv = Ulva. Ochrophyta: Col = Colpomenia, Dic = Dictyota, Ect = Ectocarpus, End = Endarachne, Hyd = Hydroclathrus, Pad = Padina, Ros = Rosenvingea, Sar = Sargassum, Spa = Spatoglossum, Zon = Zonaria. Rhodophyta: Aca = Acanthophora, Ahn = Ahnfeltiopsis, Amp = Amphiroa, Cen = Centroceras, Cer$^1$ = Ceramium, Cer$^2$ = Ceratodictyon, Cho$^1$ = Chondracanthus, Cho$^2$ = Chondria, Das = Dasya, Digi = Digenia, Euc = Eucheuma, Gel = Gelidium, Gig = Gigartina, Gra$^1$ = Gracilaria, Gra$^2$ = Grateloupia, Gra$^3$ = Gracilariopsis, Gym = Gymnogongrus, Hal = Halymenia, Hyp = Hypnea, Jan = Jania, Lau = Laurencia, Lom = Lomentaria, Neo = Neosiphonia, Pol = Polysiphonia, Pri = Prionitis, Rho$^1$ = Rhodoglossum, Rho$^2$ = Rhodymenia, Sch = Schizymenia, Spy = Spyridia, Tac = Tacanoosca.

Figure 4. Variability in $\delta^{13}$C values for morphofunctional groups by taxa along the coastline of the Gulf of California.
is dominant in all regions and taxa (60–90%). Exceptions were observed in the P1 (68%) and C1 (37%) regions for Ochrophyta, in which the specialized strategy 1 (the HCO₃⁻ user) was significant. Strategy 3 based on the use of CO₂ was observed in the peninsular coast in P2 and P3 for Rhodophyta with 2–3.3%. Overall, more negative δ¹³C values were observed at continental (C2) compared to the peninsular coastline (P1–P3) and southward than northward.

3.4 The δ¹³C macroalgal variability as a function of taxonomy, habitat features, and environmental conditions

Variability in δ¹³C values for the most representative genera was evaluated by multiple comparative analyses as a function of habitat features, including the substrate, hydrodynamic, and emersion level. Large δ¹⁵C variability observed between specimens of the same genus collected in the different habitats do not show any significant pattern, and non-significant differences were observed. An exception was observed with the emersion level (shown in Fig. 6), in which intertidal specimens recorded less negative values than subtidal in most macroalgae genera, for example, for Hydroclathrus (intertidal −5.7 ± 0.9‰, subtidal −11.4 ± 5.9‰), Amphiroa (intertidal −6.9 ± 1.5, subtidal −9.9 ± 6.1‰), Hypnea (intertidal −13.5 ± 2.5‰, subtidal −18.6 ± 1.8‰), and Laurencia (intertidal −13.5 ± 1.3‰, subtidal −17.1 ± 1.8‰). Exceptions were observed for Polysiphonia (intertidal −19.7 ± 2.2‰, subtidal −14.9 ± 6.7‰), Spyridia (intertidal −16.9 ± 3.3‰, subtidal −13.2 ± 0.7‰), and Colpomenia (intertidal −9.4 ± 3.4‰, subtidal −7.7 ± 1.3‰).

Non-significant differences were observed for the same genera at different temperature ranges except for Grateloupiopsis (cold, −19.2 ± 4.7‰, typical −14.4 ± 2.2‰, warm −14.5 ± 2.2‰) and Polysiphonia (cold, −21.0 ± 0.4‰, typical −18.1 ± 5.5‰, warm −17.9 ± 2.3‰) with more negative values in colder than warmer waters (F = 6.42, p < 0.001). Neither significant difference was observed in δ¹³C values in macroalgae specimens from the different genera in the same temperature range (Fig. 7a).

Significant differences were observed among the genera related to the pH level in seawater (Fig. 7b). Under typical pH seawater, Amphiroa and Colpomenia were 1‰–2‰ more negatives than in alkaline waters, while Ulva and Spyridia were 3‰–5‰ less negative than in acidic waters. Amphiroa and Colpomenia were not collected in acidic water, and neither was Spyridia in alkaline waters to compare. Another genus also showed extremes values between alkaline (Tacanoosca −7.6 ± 1.0‰) and acidic waters (Schizymenia −32.9 ± 2.0‰). The following order was observed in the genera collected at the three pH ranges: alkaline > typical > acidic. Significant differences were observed for genera Ahnfeltiopsis, Caulerpa, Gymnogongrus, Padina, and Ulva, with higher values in alkaline than in acidic waters. Values of δ¹³C for species of the same genus collected in typical pH waters are mostly overlapped between alkaline and acidic seawaters. Non-significant differences in δ¹³C values were observed for Grateloupiopsis, Hypnea, and Polysiphonia concerning pH-type waters.

We analyzed the carbon uptake strategies on macroalgal assemblages as a function of environmental factors like temperature, pH, and salinity (Fig. 8). The temperature and salinity non-significantly explained the δ¹³C macroalgal variability. A poor but significant correlation was observed be-
between $\delta^{13}$C and pH ($R^2 = 0.04$) (Table 4). The proportion of specimens with a strategy of only $\text{HCO}_3^-$ use was different between environmental factors and taxa (previously described). For example, Ochrophyta showed the highest proportion (35 %) in colder temperatures, in pH alkaline (31 %), and in a typical salinity regime (27 %). Chlorophyta was enhanced to 30 % in acid pH, and Rhodophyta recorded 21 % in normal seawater. The opposite strategy (only use of dissolved CO$_2$) was observed only in Rhodophyta. The highest percentage was observed in the estuarine salinity regimen (10 %).

3.5 Variation latitudinal of $\delta^{13}$C macroalgae

The $\delta^{13}$C macroalgal variation in the GC biogeography was evaluated by linear regression analysis between $\delta^{13}$C values along the 9° latitude of both GC coastlines. A non-significant latitudinal trend was observed for datasets, but for the three phyla’s most representative genera, $\delta^{13}$C values correlated with latitude (Fig. 9). In Chlorophyta, with the higher genera number, $\delta^{13}$C values increased with latitude, with low but significant correlation. Contrarily, in Ochrophyta and Rhodophyta specimens, the $\delta^{13}$C values decreased non-significantly with latitude.

In the most representative morphofunctional groups, significant correlations ($p < 0.001$) were observed for $\delta^{13}$C macroalgae versus latitude (Fig. 10). Representative morphofunctional groups of Chlorophyta (e.g., C-Tubular, C-Filamentous uniseriate) showed a positive correlation, while those belonging to Ochrophyta (e.g., O-Thick leathery) and Rhodophyta (e.g., R-Larger-sized corticated) showed a negative trend with latitude.

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

The $\delta^{13}$C macroalgal variability was analyzed as a function of the life-form and environmental factors. Firstly, simple linear regression analyses were performed to evaluate the de-
Table 4. Summary of the estimated regression coefficients for each simple linear regression analysis and of the constant of fitted regression models. Estimated regression coefficients include degree of freedom for the error (DFE), root-mean-square error (RMSE), coefficient of determination ($R^2$) and the adjusted $R^2$ statistics, Mallow’s $C_p$ criterion ($C_p$), Akaike information criterion (AIC), Bayesian information criterion (BIC) minimum, $F$ ratio test, and $p$ value for the test ($p < F$). Model information includes value of the constant $a$ ($\delta^{13}C$, ‰), standard error ($SE$), $t$ ratio, and prob $>|t|$ (values * are significant).

| Independent variables | DFE | RMSE | $R^2$ | Adjust $R^2$ | Cp | AIC | BIC | $F$ ratio | Prob > $F$ | $\delta^{13}C$ (‰) | SE | $t$ ratio | Prob > $|t|$ |
|------------------------|-----|------|-------|-------------|----|-----|-----|----------|-----------|----------------|-----|----------|----------|
| **Inherent macroalgae properties** | | | | | | | | | | | | | |
| Phyla | 806 | 3.66 | 0.08 | 0.07 | 3 | 4401 | 4420 | 33.1 | < 0.0001*** | −13.98 | 0.13 | −107.4 | < 0.0001*** |
| Morphofunctional | 788 | 3.10 | 0.35 | 0.34 | 21 | 4149 | 4251 | 21.6 | < 0.0001*** | −14.21 | 0.35 | −40.80 | < 0.0001*** |
| Genus | 746 | 2.92 | 0.46 | 0.41 | 63 | 4104 | 4393 | 10.1 | < 0.0001*** | −14.71 | 0.23 | −62.64 | < 0.0001*** |
| Species | 641 | 2.79 | 0.57 | 0.46 | 168 | 4419 | 4898 | 5.2 | < 0.0001*** | −14.60 | 0.16 | −93.22 | < 0.0001*** |
| **Biogeographical collection zone** | | | | | | | | | | | | | |
| GC coastline | 807 | 3.79 | 0.01 | 0.01 | 2 | 4456 | 4470 | 7.4 | 0.0067* | −13.97 | 0.13 | −104.5 | < 0.0001*** |
| Coastal sector | 803 | 3.73 | 0.05 | 0.04 | 6 | 4433 | 4465 | 7.9 | < 0.0001* | −14.12 | 0.16 | −90.85 | < 0.0001*** |
| Latitude | 807 | 3.80 | 0.00 | 0.00 | 2 | 4462 | 4476 | 1.5 | 0.23 | −12.25 | 1.41 | −8.71 | < 0.0001*** |
| Longitude | 807 | 3.81 | 0.00 | 0.00 | 2 | 4463 | 4477 | 0.1 | 0.80 | −15.44 | 5.83 | −2.65 | 0.0002* |
| **Habitat features** | | | | | | | | | | | | | |
| Substrate | 807 | 3.80 | 0.00 | 0.00 | 2 | 4460 | 4474 | 3.2 | 0.08 | −13.82 | 0.15 | −92.06 | < 0.0001* |
| Hydrodynamic | 807 | 3.80 | 0.00 | 0.00 | 2 | 4462 | 4476 | 1.3 | 0.26 | −13.88 | 0.15 | −95.00 | < 0.0001*** |
| Emersion level | 807 | 3.69 | 0.06 | 0.06 | 2 | 4412 | 4427 | 52.2 | < 0.0001** | −14.05 | 0.13 | −107.6 | < 0.0001*** |
| **Environmental conditions** | | | | | | | | | | | | | |
| Temperature | 802 | 3.70 | 0.01 | 0.01 | 2 | 4390 | 4404 | 5.4 | 0.0207* | −16.11 | 0.96 | −16.78 | < 0.0001* |
| pH | 807 | 3.73 | 0.04 | 0.04 | 2 | 4430 | 4444 | 33.4 | < 0.0001** | −32.45 | 3.21 | −10.13 | < 0.0001*** |
| Salinity | 806 | 3.80 | 0.00 | 0.00 | 2 | 4456 | 4470 | 9.0 | 0.34 | −15.77 | 1.91 | −8.27 | < 0.0001*** |

* $p < 0.05$, ** $p < 0.0001$. 

Figure 7. Variability in $\delta^{13}C$ values in macroalgal specimens for the most representative genera as a function of temperature (a) and pH (b) ranges in samples collected along the Gulf of California coastline.

Independent variable’s prediction power ($\delta^{13}C$ macroalgal variable) as a 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 (Burnham and Anderson, 2002; Draper and Smith, 1998) as was described in Methods; however, the description of our results focused on the coefficients of determination ($R^2$ and adjusted $R^2$). The coefficient $R^2$ describes the relationship between the independent variables $X_i$ with the dependent variable $Y$ ($\delta^{13}C$ macroalgal values). $R^2$ is interpreted as the percent of contribution to the $\delta^{13}C$ variability. In comparison, 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. Phyla explain only 8% variability regarding the inher-
ent macroalgae properties, the morphofunctional properties 35 %, genera 46 %, and species 57 %.

The biogeographical collection zone, featured by coastline (continental versus peninsular) and coastal sectors (C1–C3 and P1–P3), explained a maximum of 5 % variability. Only the emersion level (6 %) contributed to the $\delta^{13}$C variability related to the habitat features. 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 it was significant for genera (5 %) and species (10 %).

Multiple regression analyses were also performed to interpret the complex relationships among $\delta^{13}$C macroalgae, considering the life-forms (morphofunctional properties and taxa by genus) and their responses to environmental parameters. Results for the fitted regression models performed for morphofunctional groups (Table 5) and genera (Table 6) evidenced that the effect of the coastal sector and pH ranges on the $\delta^{13}$C macroalgae increased the contribution by 9 %–10 % for each one. The emersion level increased by 5 %–6 %, the contribution with respect to the individual effect of morphofunctional group and genus, and the temperature and pH by 1 % and 3 %, respectively, while salinity decreased by 1 %–2 %. The combined effect of the biogeographical collection zone (e.g., coastline sector) and morphofunctional group (Table 5) and genus (Table 7) increased in 11 %–12 %.

Considering the combined effect of the coastline sector + habitat features for morphofunctional group or genus (Table 7), the full model showed $R^2$ values 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 $\delta^{13}$C variability; however, these contributions were significantly lower than those explained by life-forms, such as the morphofunctional properties and taxa by genus and species.

The combined effect of environmental conditions on the $\delta^{13}$C variability was tested for the best-represented genera and morphological groups. Results evidenced that 9 of 21 morphological groups showed significant effects on the $\delta^{13}$C variability (Table 8), five increasing and four decreasing the model constant of $\delta^{13}$C$=-14.2%$. For example, for the O-Hollow with spherical or subspherical shape (+4.9‰) and R-Larger-sized articulated corallines (+6.3‰), the predicted values are $-7.9 \pm 0.8%$ and $-9.2 \pm 0.4%$. For R-Filamentous uniseriate and pluriseriate with erect thallus ($-2.1%$) and C-Tubular ($-1.6%$), the predicted values are $-16.3 \pm 0.5%$ and $-15.8 \pm 0.5%$, respectively. Regarding taxon, a significant effect was observed only in 13 genera, including Colpomenia (+5.4‰), Amphiroa (+6.8‰), and Padina (+2.2‰) increasing the signal and Polysiphonia ($-3.7%$), Gracilaria ($-0.9%$), and Spyridia ($-1.4%$) decreasing the signal of the model constant (Table 9). In 33 species a significant effect on the $\delta^{13}$C variability was observed, including C. tuberculata (+5.9‰), C. sinuosa (+4.4‰), H. pannosa (+4.4‰), H. johnstonii (+4.4‰), and Amphiroa spp. (+4.4‰ to 8.2‰) increasing the model constant $\delta^{13}$C$=-14.6%$, and Spyridia sp. ($-2.5%$), G. filicina ($-2.3%$), P. mollis ($-5.2%$), and S. pacifica ($-19.2%$) decreasing the model constant (Table 10).

Figure 8. Proportion of species using different DIC sources according to their carbon assimilation strategies: HCO$_3^-$ only users (CO$_2$ concentrating mechanism active), users of both sources (HCO$_3^-$ and CO$_2$), and CO$_2$ only users (non-CO$_2$ concentrating mechanism active) as a function of (a) pH ranges, (b) temperature ranges, and (c) salinity ranges.

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3.7 Preliminary estimations of $\Delta^{13}$C macroalgae

Concurrent analysis of surface seawater for alkalinity, proportions of the chemical species of DIC ($\text{CO}_2$, $\text{HCO}_3^-$, and $\text{CO}_3^{2-}$), and $\delta^{13}$C DIC evidenced that $\delta^{13}$C DIC in GC seawater averages $1.4 \pm 0.4 \%e$ ($-1 \%e$ to $4.9 \%e$) (Fig. S1). In our preliminary data, the $\delta^{13}$C-DIC seawater slightly (in $0.5 \%e$) decreased during the rainy season in those zones influenced by river discharges along the continental coastline. Non-significant differences were observed among coastal sectors. The $\delta^{13}$C-DIC values in GC seawater are comparable to the averages $1.4 \%e$–$1.6 \%e$ reported for the surface seawaters in the eastern North Pacific in the 1970s–2000s (Hinger et al., 2010; Quay et al., 2003; Santos et al., 2011).

Based on the subtraction of $\delta^{13}$C macroalgae to $\delta^{13}$C-DIC seawater, the integrative discrimination factor against $^{13}$C averaged $16.0 \pm 3.1 \%e$, $16.8 \pm 4.3 \%e$, and $14.0 \pm 3.8 \%e$ for phyla Chlorophyta, Rhodophyta, and Ochrophyta, re-
Figure 10. Trends in the $\delta^{13}$C macroalgae in specimens for morphofunctional groups by taxa along the coastline of the Gulf of California as a function of latitudinal gradient.
spectively. Five groups were identified as a function of the Δ¹³C values: one for Chlorophyta (Δ¹³C = 16.0 ± 3.1‰), two for Rhodophyta (16.6 ± 3.8‰ and 34.6 ± 1.8‰), and two for Ochrophyta (9.1 ± 1.7‰ and 15.7 ± 2.7‰) (Fig. S2). Values of Δ¹³C were comparable to δ¹³C of the thallus of macroalgae. Thus, δ¹³C macroalgae reflect mainly the discrimination during carbon assimilation. Like δ¹³C macroalgae, the Δ¹³C values were subject to considerable variation.

4 Discussions

4.1 Explaining the δ¹³C macroalgal variability

A high variability in the δ¹³C values was revealed in the large inventory of macroalgae collected along the GC coastline. A linear regression analysis of the effects of life-forms revealed that the δ¹³C variability in the macroalgal community is mainly explained by taxonomic (genus 46%, species 57%) and morphofunctional group (35%). This result is consistent with the report of Lovelock et al. (2020), which found that 66% of δ¹³C variability was explained by taxonomy. Even so, the variability associated with each genus is not the same and can be classified in three groups: (1) high variability (e.g., Schizymenia = ±19.1%), moderate variability (e.g., Hydroclathrus = ±7.3%; Amphipora = ±6.8%), and low variability (e.g., Gracilaria = ±0.89; S. pyridia = ±1.46%). The observed δ¹³C variability in this study is comparable with those reported in the literature, compiled in Table S4.

Most authors studying the isotopic composition of C in macroalgae have reported the high isotopic variability, which has been attributable to the taxon-specific photosynthetic DIC acquisition properties (Díaz-Pulido et al., 2016; Lovelock et al., 2020; Marconi et al., 2011; Mercado et al., 2009; Raven et al., 2002a; Stepien, 2015). Our study observed that the intrinsic characteristics of each morphofunctional group of macroalgae (e.g., thallus structure, growth form, branching pattern, and taxonomic affinities) also influence the δ¹³C macroalgal signals. The thallus thickness influences the diffusion boundary layer on the surface of the macroalga, where they carry out the absorption of essential ions and dissolved gases (Hurd, 2000; Sanford and Crawford, 2000). Thus, morphology can modulate the photosynthesis rates.

Table 5. Summary of the estimated regression coefficients for each multivariate linear regression analysis and of their constant of fitted regression models performed in individuals binned by genus. Estimated regression coefficients include degree of freedom for the error (DFE), root-mean-square error (RMSE), coefficient of determination (R²) and the adjusted R² statistics, Mallows’ 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 (Δ¹³C, ‰), standard error (SE), t ratio, and prob > |t| (values * are significant).

| Independent variables | DFE | RMSE | R² | Adjust R² | Cp | AIC | BIC | F ratio | Prob > F | Δ¹³C (‰) | SE | t ratio | Prob > |t|
|-----------------------|-----|------|---|----------|----|-----|-----|--------|---------|----------|----|--------|---|------|
| Coastal sector        | 652 | 2.78 | 0.57 | 0.47      | 157 | 4169| 4834| 20.0   | < 0.0001* | -17.52  | 0.64| -27.24 | < 0.0001* |
| Substrate             | 711 | 2.90 | 0.49 | 0.42      | 98  | 4140| 4577| 0.4    | 0.52     | -16.35  | 0.62| -26.20 | < 0.0001* |
| Hydrodynamic          | 714 | 2.87 | 0.50 | 0.43      | 95  | 4120| 4545| 0.1    | 0.78     | -16.43  | 0.64| -25.95 | < 0.0001* |
| Emersion level        | 713 | 2.77 | 0.53 | 0.47      | 96  | 4060| 4489| 153.0  | < 0.0001* | -16.65  | 0.60| -27.85 | < 0.0001* |
| Temperature           | 695 | 2.81 | 0.50 | 0.43      | 109 | 4083| 4564| 98.4   | < 0.0001* | -14.60  | 0.92| -15.91 | < 0.0001* |
| Temperature ranges    | 686 | 2.87 | 0.49 | 0.40      | 118 | 4128| 4645| 97.7   | < 0.0001* | -12.91  | 0.40| -31.97 | < 0.0001* |
| pH                    | 701 | 2.86 | 0.51 | 0.43      | 108 | 4134| 4611| 156.6  | < 0.0001* | -28.57  | 2.69| -10.64 | < 0.0001* |
| pH ranges             | 697 | 2.67 | 0.57 | 0.51      | 112 | 4028| 4522| 152.2  | < 0.0001* | -16.39  | 0.58| -28.05 | < 0.0001* |
| Salinity              | 697 | 2.89 | 0.50 | 0.42      | 111 | 4151| 4640| 162.2  | < 0.0001* | -17.75  | 1.63| -10.88 | < 0.0001* |
| Salinity ranges       | 721 | 2.91 | 0.47 | 0.41      | 86  | 4117| 4504| 167.8  | < 0.0001* | -17.64  | 0.74| -23.68 | < 0.0001* |

Table 6. Summary of the estimated regression coefficients for each multivariate linear regression analysis and of their constant of fitted regression models performed in individuals binned by coastline sector and genus. Estimated regression coefficients include degree of freedom for the error (DFE), root-mean-square error (RMSE), coefficient of determination (R²) and the adjusted R² statistics, Mallows’ 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 (Δ¹³C, ‰), standard error (SE), t ratio, and prob > |t| (values * are significant).

| Independent variables | DFE | RMSE | R² | Adjust R² | Cp | AIC | BIC | F ratio | Prob > F | Δ¹³C (‰) | SE | t ratio | Prob > |t|
|-----------------------|-----|------|---|----------|----|-----|-----|--------|---------|----------|----|--------|---|------|
| Substrate             | 590 | 2.76 | 0.62 | 0.47      | 219 | 4287| 5155| 15.8   | < 0.0001* | -17.08  | 0.66| -25.72 | < 0.0001* |
| Hydrodynamic          | 592 | 2.73 | 0.62 | 0.49      | 217 | 4266| 5128| 18.6   | < 0.0001* | -17.18  | 0.67| -25.70 | < 0.0001* |
| Protection level      | 590 | 2.75 | 0.62 | 0.48      | 219 | 4285| 5153| 20.0   | < 0.0001* | -17.51  | 0.64| -27.22 | < 0.0001* |
| Emersion level        | 603 | 2.69 | 0.63 | 0.50      | 206 | 4217| 5045| 18.6   | < 0.0001* | -17.47  | 0.64| -27.49 | < 0.0001* |
| Temperature ranges    | 569 | 2.74 | 0.61 | 0.46      | 235 | 4293| 5202| 28.0   | < 0.0001* | -13.73  | 0.45| -30.32 | < 0.0001* |
| pH ranges             | 580 | 2.50 | 0.69 | 0.57      | 229 | 4155| 5051| 9.7    | 0.0019*  | -16.88  | 0.62| -27.15 | < 0.0001* |
| Salinity ranges       | 631 | 2.76 | 0.58 | 0.47      | 176 | 4183| 4913| 21.2   | < 0.0001* | -18.30  | 0.79| -23.05 | < 0.0001* |
Table 7. Summary of the estimated regression coefficients for each multivariate linear regression analysis and of their constant of fitted regression models performed in individuals binned in coastline sector, habitat features, environmental conditions, and physiological state separately by morphofunctional group and genus. Estimated regression coefficients include degree of freedom for the error (DFE), root-mean-square error (RMSE), coefficient 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).

| Term | Estimated | SE | Razón $t$ | $prob > |t|$ |
|------|-----------|----|-----------|-------------|
| Model constant | -14.2 | 0.4 | -40.80 | $< 0.0001^{* *}$ |
| R-Larger-sized articulated corallines | 4.5 | 1.7 | 2.58 | 0.0100* |
| O-Compressed with branched or divided thallus | 1.2 | 0.5 | 2.66 | 0.0079* |
| C-Erect thallus | 1.8 | 0.6 | 2.84 | 0.0046* |
| R-Larger-sized articulated corallines | 6.3 | 0.8 | 7.95 | $< 0.0001^{*}$ |
| O-Hollow with spherical or subspherical shape | 5.0 | 0.5 | 10.51 | $< 0.0001^{*}$ |
| R-Blade-like with one of the few layers of cells | -5.9 | 3.0 | -1.98 | 0.0476* |
| C-Tubular | -1.6 | 0.5 | -3.26 | 0.0012* |
| R-Filamentous uni-pluriseriate with erect thallus | -2.2 | 0.6 | -3.92 | $< 0.0001^{*}$ |
| R-Flattened macrophytes with cortication | -8.9 | 1.3 | -7.10 | $< 0.0001^{*}$ |

* $p < 0.05$, ** $p < 0.0001$.

However, a non-biological or ecological explanation of the $\delta^{13}C$ variability, and therefore carbon use physiology, can be given in terms of morphology.

The $\delta^{13}C$ macroalgae depend on the carbon source ($\delta^{13}C$ DIC in seawater), the isotope discrimination during carbon assimilation in the photosynthesis ($\Delta^{13}C_p < 29 \, ‰$ in a variable degree), and the plant respiration ($\Delta^{13}C_p$ average $\pm 2.3 \, ‰$) (Carvalho et al., 2009a, b, 2010a; Carvalho and Eyre, 2011; Rautenberger et al., 2015). Comparatively, the $\Delta^{13}C_p$ value is relatively small regarding $\Delta^{13}C_p$. Thus, $\delta^{13}C$ macroalgal value is an integrative value of the isotope discrimination during DIC seawater assimilation ($\Delta^{13}C = (\delta^{13}C - \delta^{13}C$ DIC seawater $- \delta^{13}C$ macroalgae)) (Carvalho et al., 2009a). Based on the $\Delta^{13}C$ values, five groups were identified in our study: one for Chlorophyta ($\Delta^{13}C = 16.0 \pm 3.1 \, ‰$), two for Rhodophyta ($16.6 \pm 3.8 \, ‰$ and $34.6 \pm 1 \, ‰$), and two for Ochrophyta ($9.1 \pm 1.7 \, ‰$ and $15.7 \pm 2.7 \, ‰$). Values of $\Delta^{13}C$ were comparable to $\delta^{13}C$ of the thallus of macroalgae. The $\delta^{13}C$ macroalgal values reflect the discrimination during carbon assimilation attributable to the taxon-specific photosynthetic DIC acquisition properties. $\Delta^{13}C$ macroalgal variability, captured in the $\delta^{13}C$ macroagal signals, is related to the thickness of the boundary layer around the thallus (Raven et al., 1982), the leakage during carbon uptake (Maberly et al., 1992; Sharkey and Berry, 1985), photosynthetic intensity (Kühler and Raven, 1995, 1996; Wienecke and Fischer, 1990), and respiration rates (Carvalho et al., 2010a; Carvalho and Eyre, 2011; Rautenberger et al., 2015). All intrinsic properties are related to the life-form.

Many species that recorded high $\delta^{13}C$ values (and low $\Delta^{13}C$ values) were fleshy macroalgae that are characterized to be bloom-forming macroalgae belonging to genera Ulva, Gracilaria, Cladophora, Spyridia, and Sargassum.
genera with significant effects are enlisted.

switching from C\textsubscript{3} Ulva, Gracilaria, Sargassum-Bloom-forming macroalgae (e.g.,
prising that species with high photosynthetic activity and
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Changes in the habitat features and environmental conditions, such as light intensity and DIC availability, influencing the growth rate and photosynthetic intensity, have a strong influence on \(\delta^{13}\text{C}\) signal (Carvalho et al., 2007, 2009a; Carvalho and Eyre, 2011; Mackey et al., 2015; Rautenberger et al., 2015; Stepien, 2015). The light intensity is the external factor with more influence on the \(\Delta^{13}\text{C}\) macroalgal due to the regulation of carbon assimilation intensity (Carvalho et al., 2009a, b; Cooper and DeNiro, 1989; Grice et al., 1996). Experimental studies found the light levels to be a critical factor affecting the \(\delta^{13}\text{C}\) values. For example, under saturating light conditions, Ulva switched from a carbon uptake of HCO\textsubscript{3}\textsuperscript{−} and CO\textsubscript{2} to increased HCO\textsubscript{3}\textsuperscript{−} use (Rautenberger et al., 2015). Furthermore, field studies have shown that species growing in low-light habitats like deep subtidal zones tend to have more negative \(\delta^{13}\text{C}\) values than those in higher-light environments (Cornwall et al., 2015; Díaz-Pulido et al., 2016; Hepburn et al., 2011; Marconi et al., 2011; Mercado et al., 2009; Stepien, 2015). In this study, intertidal specimens recorded less negative values than subtidal in most macroalgal genera. However, our study did not record the vertical effect in the \(\delta^{13}\text{C}\) signal related to the light limitation because only shallow habitats (non-light limited) were studied.

The \(\delta^{13}\text{C}\)-DIC seawater is reasonably uniform in surface seawater (−4.8‰ to 3.6‰, median 1.5‰), with \(\delta^{13}\text{C}\) values for CO\textsubscript{2}, HCO\textsubscript{3}\textsuperscript{−}, and CO\textsubscript{2}\textsuperscript{−} nearly −10‰, −0.5‰, and 2‰, respectively (Kroopnick, 1985; Mook et al., 1974). Exceptions can be expected where variations in the salinity, alkalinity, and proportions of the chemical species of DIC (CO\textsubscript{2}, HCO\textsubscript{3}\textsuperscript{−}, or CO\textsubscript{2}\textsuperscript{−}) occur (e.g., in coastal environments influenced by river and groundwater discharges) (Carvalho et al., 2015; Chanton and Lewis, 1999; Hinger et al., 2010; Mook et al., 1974). Regarding DIC sources for macroalgae in the GC surface seawater, the availability, chemical proportions, and \(\delta^{13}\text{C}\) DIC were also relatively constant and uniform. Thus, the influence of the \(\delta^{13}\text{C}\)-DIC variations on the \(\delta^{13}\text{C}\) macroalgal variability is negligible in the GC.

The effect of other environmental factors, such as salinity and pH, on \(\delta^{13}\text{C}\) macroagal signals was evaluated. Regarding salinity, the influence of freshwater discharge by rivers and groundwater decreases the \(\delta^{13}\text{C}\) signal, which could be explained by the reduction in the salinity regimen that follows a decrease in \(\delta^{13}\text{C}\) DIC in water (Hinger et al., 2010; Santos et al., 2011). In our study, a non-significant correlation between \(\delta^{13}\text{C}\) macroalgae and salinity was observed.

Based on pH, differences in \(\delta^{13}\text{C}\) were found only for a few genera (e.g., Amphiroa, Colpomenia, Ulva, Spyridia), with an increasing trend in the \(\delta^{13}\text{C}\) values with pH increase, such as was reported by Maberly et al. (1992) and Raven et al. (2002b). Similar results were reported for Cornwall et al. (2017) in the field study, with the differential response of

| Term           | \(\delta^{13}\text{C}, \%e\) estimated | SE  | \(t\) value | Prob > \(|t|\) |
|----------------|-------------------------------------|-----|-------------|----------------|
| Model constant | −14.7                               | 0.2 | −62.64      | < 0.0001**     |
| Amphiroa       | 6.8                                 | 0.8 | 9.05        | < 0.0001**     |
| Codium         | 2.3                                 | 0.6 | 4.08        | < 0.0001**     |
| Colpomenia     | 5.4                                 | 0.4 | 14.02       | < 0.0001*      |
| Corallina      | 6.4                                 | 2.9 | 2.22        | 0.0269*        |
| Gracilaria     | −0.9                                | 0.4 | −2.18       | 0.0294*        |
| Hydrodictyon   | 7.3                                 | 1.1 | 6.59        | < 0.0001**     |
| Jania          | 5.1                                 | 1.7 | 2.97        | 0.0031*        |
| Padina         | 2.2                                 | 0.5 | 4.8         | < 0.0001**     |
| Polysiphonia   | −3.7                                | 0.8 | −4.82       | < 0.0001**     |
| Schizymenia    | −19.1                               | 2.1 | −9.33       | < 0.0001**     |
| Spyridia       | −1.5                                | 0.7 | −2.10       | 0.0361*        |
| Struveopsis    | 4.1                                 | 1.3 | 3.15        | 0.0017*        |
| Tacanoosca     | 3.5                                 | 1.3 | 2.71        | 0.0070*        |

\(\ast p < 0.05, \ast\ast p < 0.001.\)
Table 10. Constants of fitted regression model explaining the δ¹³C variability by species. Model information includes value of the constant a (δ¹³C, ‰), standard error (SE), t ratio, and prob > |t|. Only genera with significant effects are enlisted.

| Term                  | δ¹³C, ‰ estimated | SE  | t value | Prob > |t| |
|-----------------------|------------------|-----|---------|---------|---|
| Model constant        | −14.6            | 0.2 | −93.22  | < 0.0001** |
| Amphiroa misakiensis  | 7.1              | 2.8 | 2.55    | 0.0110*  |
| Amphiroa sp.          | 8.1              | 0.9 | 8.67    | < 0.0001** |
| Amphiroa sp.2         | 6.6              | 1.6 | 4.1     | < 0.0001** |
| Amphiroa sp. 3        | 8.2              | 2.8 | 2.95    | 0.0033**  |
| Caulerpa peltata      | 3.9              | 1.6 | 2.4     | 0.0165*  |
| Cladophora microchaedoides | −7.2     | 2   | −3.64   | 0.0003** |
| Codium brandegeei     | 2.8              | 1.1 | 2.63    | 0.0088**  |
| Codium simulans       | 3.2              | 0.9 | 3.41    | 0.0007**  |
| Codium sp.            | 3                | 1.3 | 2.4     | 0.0167*  |
| Colpomenia ramosa     | 3.2              | 1.4 | 2.27    | 0.0237*  |
| Colpomenia sinuosa    | 4.4              | 1.1 | 4.17    | < 0.0001** |
| Colpomenia sp.        | 3.6              | 0.9 | 4.27    | < 0.0001** |
| Colpomenia tuberculata| 5.9              | 0.4 | 15.45   | < 0.0001** |
| Corallina vancouverensis | 6.3        | 2.8 | 2.27    | 0.0238*  |
| Grateloupa filicina   | −2.4             | 1.1 | −2.08   | 0.0382*  |
| Halymenia actinophyta | −9.9             | 2.8 | −3.57   | 0.0004** |
| Hydroclathrus clathratus | 7.2        | 1.1 | 6.82    | < 0.0001** |
| Hymena johnstonii     | 3.4              | 1.3 | 2.74    | 0.0063** |
| Hymena pannosa        | 2.8              | 1.3 | 2.24    | 0.0256* |
| Jania sp.             | 5                | 2   | 2.56    | 0.0106*  |
| Padina durvilli       | 1.4              | 0.5 | 2.87    | 0.0043** |
| Padina sp.            | 3.5              | 0.7 | 4.77    | < 0.0001** |
| Polysiphonia mollis   | −5.2             | 1.1 | −4.93   | < 0.0001** |
| Polysiphonia sp.      | −4.8             | 1.4 | −3.44   | 0.0006** |
| Pyropia thareti       | −5.5             | 2.8 | −1.98   | 0.0480* |
| Rhizoclonium riparium | −5.1             | 1.6 | −3.15   | 0.0017** |
| Rhodymenia sp.        | −4.1             | 2   | −2.08   | 0.0380* |
| Schizymenia pacifica  | −19.2            | 2   | −9.76   | < 0.0001** |
| Spyrida sp.           | −2.5             | 1.3 | −1.97   | 0.0496* |
| Straveopsis sp.       | 4                | 1.4 | 2.86    | 0.0044** |
| Taconoosca uncinata   | 3.4              | 1.3 | 2.74    | 0.0062** |
| Ulva acanthophora     | −1.2             | 0.6 | −2.06   | 0.0399*  |
| Ulva compressa        | −3.2             | 1.4 | −2.33   | 0.0203* |

* p < 0.05, ** p < 0.001.

the δ¹³C signals to pH among 19 species, in which only four species were sensitive to pH changes. A very weak but significant positive linear regression was observed between δ¹³C and pH. Also, a decreasing trend in the δ¹³C was recorded in the following order: alkaline > typical > acidic. According to Stepien (2015), the result of meta-analyses between pH drift experiments and δ¹³C thresholds was positive only for Rhodophyta and Ochrophyta but not for Chlorophyta. About 86% of the Stepien metadata met the theoretical CCM assignment based on both parameters, with exceptions for species with δ¹³C < −30‰ that have been capable of raising pH to > 9. A strong association between pH compensation point and δ¹³C was reported by Iñiguez et al. (2019) in three taxa of polar macroalgae. Environmental conditions may influence the δ¹³C macroalgal values but not change the carbon use physiology in the macroalgae, which is most likely inherently species-specific.

4.2 Using δ¹³C macroalgae to indicate the presence of an active CCM

In our study, the δ¹³C macroalgal signals were used to evidence the presence of an active CCM. This tool was first used in macroalgal shallow communities of the GC. Most macroalgae species displayed δ¹³C values that exhibit active CCMs. Then, macroalgae were classified into three strategies for DIC uptake, in agreement with the Maberly et al. (1992) and Raven et al. (2002a) thresholds: (1) CCM-only by active uptake HCO₃⁻ (δ¹³C > −10‰), (2) CCM active uptake HCO₃⁻ and diffusive uptake CO₂ (δ¹³C < −11‰ to −30‰), and (3) non-CCM CO₂ by diffusion only (δ¹³C < −30‰).
About 84% of the analyzed specimens showed the facultative uptake of \( \text{HCO}_3^- \) and \( \text{CO}_2 \), the most common strategy identified in macroalgal shallow communities (Cornwall et al., 2015; Díaz-Pulido et al., 2016; Hepburn et al., 2011; Stepien, 2015). Based on the carbon uptake strategies, the most abundant macroalgae were those able to use both \( \text{HCO}_3^- \) and \( \text{CO}_2 \) using active uptake plus passive diffusion (strategy 2).

Macroalgae collected in GC also involved only \( \text{HCO}_3^- \) users (strategy 1) and those relying on diffusive CO\(_2\) uptake (strategy 3). Photosynthesis that relies on CO\(_2\) uptake (lack of CCM), the most primitive mechanism (Carling et al., 1993), has fewer energy costs than \( \text{HCO}_3^- \) uptake, which requires complex machinery with a high operational cost (Giordano et al., 2005; Hopkinson et al., 2011, 2014; Raven and Beadall, 2016). The energy for macroalgae to uptake \( \text{HCO}_3^- \) cross the plasma membrane, and convert to CO\(_2\) for photosynthesis is obtained through irradiance (Cornelissen et al., 2007). Based on our sampling effort, focused on intertidal and shallow subtidal habitats featured by high light intensities, we expected high proportions of species with the carbon uptake strategy that use only \( \text{HCO}_3^- \). Results evidenced that strategy 1 was recorded in specimens belonging to 58 species of 170 total species. The higher proportions of CCM species (\( \text{HCO}_3^- \) users) with high energetic requirements are explained by those elevated irradiances (Cornwall et al., 2015; Hepburn et al., 2011). Ochrophyta showed the highest proportion of species that depend only on \( \text{HCO}_3^- \) uptake on both coastlines in the southern region of GC (P1, C1). The low solubility of CO\(_2\) is related to high temperatures in subtropical waters (Zeebe and Wolf-Gladrow, 2001) that impede the development of CCM (Raven et al., 2002b) and by the high affinity to DIC by Ochrophyta, as such has been described before by Díaz-Pulido et al. (2016).

Only three non-calculifying species (\( \text{Schizymenia pacifica}, \text{Halymenia sp.}, \text{Gigartina sp.} \)) belonging to Rhodophyta were \( \text{CO}_2 \)-exclusive users (\( \delta^{13}\text{C} = -33.2 \pm 1\%_o \)). Based on measurements of pH drift, Murru and Sandgren (2004) reported \( \text{Schizymenia pacifica} \) and two species of \( \text{Halymenia} \) (e.g., \( \text{H. schizymenioides} \) and \( \text{H. gardneri} \)) as restricted \( \text{CO}_2 \) users. Measurements of \( \delta^{13}\text{C} \) in \( \text{Halymenia dilatata} \) confirmed the \( \text{CO}_2 \)-restricted photosynthesis in specimens collected offshore in deep reefs of the Great Barrier Reef (Díaz-Pulido et al., 2016). Red macroalgae that lack CCM tend to inhabit low-light habitats like subtidal or low intertidal zones and are abundant in cold waters (Cornwall et al., 2015; Raven et al., 2002a). According to these authors, approximately 35% of the total red algae tested globally are strictly \( \text{CO}_2 \) dependents. The percentage of macroalgae species representative of Arctic and Antarctic ecosystems that lack CCM is 42%–60% (Iñiguez et al., 2019; Raven et al., 2002b), 50% for temperate waters of New Zealand (Hepburn et al., 2011), and up to 90% found for a single site of Tasmania, Australia (Cornwall et al., 2015). Our study sampled 91 red macroalgae species (of 453 red macroalgae species reported in the GC; Pedrero and Sentíes, 2003), of which <3% were \( \text{CO}_2 \) dependents. This low percentage could be related to the fact that deep habitats (>2 m depth low tide) were not explored in our surveys.

Few calcifying macroalgae species using \( \text{HCO}_3^- \) were also collected, including the genera \( \text{Amphiphora} (-7.8 \pm 3.7\%_o) \) and \( \text{Jania} (-9.4 \pm 0.7\%_o) \), both Rhodophyta with articulated form. \text{Padina}, a genus with less capacity to precipitate \( \text{CaCO}_3 \) (Iluz et al., 2017), displayed relatively high \( \delta^{13}\text{C} \) values (−12.5 ± 2.4\%_o), suggesting the presence of CCM using \( \text{HCO}_3^- \). Some species of \text{Padina} can use \( \text{HCO}_3^- \), but their efficiency may differ from species to species (Enríquez and Rodríguez-Román, 2006; Raven et al., 2002a). Stepien (2015) reported a global mean of −14.8 ± 1.0\%_o for calcifying species compared to −20.1 ± 0.3\%_o for non-calcifying species. Calcifying macroalgae species showed a \( \delta^{13}\text{C} \) signal indicative of \( \text{HCO}_3^- \) use, the same source described as the substrate for calculation (Digby, 1977; Roleda et al., 2012), and other sources like respiratory CO\(_2\) for the calcifying process (Borowitzka and Larkum, 1976). Also, the boundary layers acidified by an excess of H\(^+\) released as residual products of the calcification benefit the \( \text{HCO}_3^- \) uptake (Comeau et al., 2012; McConnaughey et al., 1997). Another possibility to explain high \( \delta^{13}\text{C} \) values can also be related to the highly efficient light properties enhanced by the carbonate skeleton, resulting in an optimization of photosynthetic activity (Vásquez-Elizondo et al., 2017). Hofmann and Heesch (2018) reported high \( \delta^{13}\text{C} \) values in eight rhodolith species (calcifying species) for the organic matter thallus and for thallus, including \( \text{CaCO}_3 \) structure collected in deep habitats (25–40 m) where light availability is limited. Because of the ocean acidification in progress, negative impacts are expected on calcifying organisms, and more attention as ecological sentinels is warranted in the GC.

Measurements of \( \delta^{13}\text{C} \) signal are evidence of the presence or absence of CCMs in macroalgae and indicate carbon use physiology (Giordano et al., 2005). However, the isotopic signature may be inconclusive in determining the efficient use of one or more DIC species (\( \text{CO}_2 \) and \( \text{HCO}_3^- \)) (Roleda and Hurd, 2012). The preferential DIC uptake of macroalgae is assessed by pH drift experiments (Fernández et al., 2014, 2015; Hepburn et al., 2011; Narvarte et al., 2020; Roleda and Hurd, 2012). Also, it can be determined by simultaneously measuring the CO\(_2\) uptake and \( \text{O}_2 \) production rates using membrane-inlet mass spectroscopy (MIMS) (Burlacot et al., 2020; Douchi et al., 2019). Macroalgae that are unable to raise the seawater pH to \( \geq 9.0 \) are primarily \( \text{CO}_2 \) users, while those that can raise the seawater pH \( > 9.0 \) (absence of \( \text{CO}_2 \) are \( \text{HCO}_3^- \) users (Roleda and Hurd, 2012). Those differences in the carbon uptake strategies can be easily deduced by pH drift experiments, which were not done in our study but are reported in the literature (Table S4). Also, the change in \( \delta^{13}\text{C} \) signature within the range specific to a carbon use strategy (e.g., mixed \( \text{HCO}_3^- \) and \( \text{CO}_2 \) user) can be com-

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4.3 Variability in δ13C macroalgae between the GC bioregions

Changes in the δ13C signal with latitude, mainly related to the light and temperature, have been reported in the literature (Hofmann and Heesch, 2018; Lovelock et al., 2020; Marconi et al., 2011; Mercado et al., 2009; Stepien, 2015). For example, a negative correlation between latitude and δ13C macroalgae was described by Stepien (2015). The authors concluded that the δ13C signal increased by 0.09‰ for each latitude degree from the Equator. Hofmann and Heesch (2018) showed a robust decreasing latitudinal effect in δ13C signals (\(R^2 = 0.43\delta^{13}C_{total}\) and 0.13, for \(\delta^{13}C_{organic-tissue}, p = 0.001\)) for rhodolite and macroalgae from coral reefs in Australia. In both cases, the latitude range is higher than what we tested (30 to 80° and from 10 to 45°, respectively). These differences on a large scale tend to be associated with a temperature effect (Stepien, 2015) and their effect on CO2 solubility in seawater (Zeebe and Wolf-Gladrow, 2001). However, in our study, no geographical pattern in the δ13C macroalgae was observed. Our linear regression analyses for latitudes showed a low but significant correlation for the dataset classified by morphofunctional group and genus – negative in the cases of Rhodophyta and Ochrophyta groups and positive for Chlorophyta.

Light is not limited along the GC latitudes. Most of the shallow habitats occupied by macroagal communities in the GC were high-light environments. In agreement with the literature, the surface seawater temperature across the GC varies by only 1°C annual mean (Escalante et al., 2013; Robles-Tamayo, 2018). However, larger temperature variations of 5–10°C were recorded in the coastal waters across the GC bioregions in both climatic seasons. The combined effect of the coastline sector, habitat feature, and environmental condition for morphofunctional group or genus explained 60%–62% and 71%–72% of the δ13C variability, respectively. Our analysis of variability for the best-represented morphological groups (e.g., R-Filamentous uniseriate and pluriseriate with erect thallus and C-Tubular) and genera (e.g., Colpomenia, Padina, Polysiphonia, and Gracillaria) revealed that certain life-forms are better monitors explaining the variability in δ13C macroalgae (and Δ13C values) than others. The δ13C variability in morphological groups refers to change within a specific carbon use strategy but not change in the carbon use physiology that is inherently species-specific. The biological or ecological relevance of the δ13C variability as a function of the morphology, in terms of the efficiency in the use of DIC and the isotopic discrimination during carbon assimilation and respiration, must be investigated in species of the same genus but which are morphologically different or between the same morphological structures belonging to a different taxon.

The proportion of specimens with different carbon uptake strategies also showed regional variations. For example, the facultative uptake of HCO3− and CO2 was dominant in the macroagal shallow communities in the GC (60% to 90% of specimens). Exceptions were observed for Ochrophyta in the P1 (68%) and C1 (37%) regions, where the strategy using only HCO3− dominated, while the strategy based on the use of only CO2 was observed in the peninsular coast in P2 and P3 for Rhodophyta with 2%–3.3%. Finally, the coastal sector C2 showed more negative δ13C values in macroalgae specimens of the same genus compared to the peninsular coastline (P1–P3). Small but detectable changes were observed in the phylum distribution based on environmental conditions. For example, Ochrophyta showed the highest proportion (35%) in colder temperature, in pH alkaline (31%), and in typical salinity regime (27%), while Chlorophyta enhanced to 30% in acid pH, and Rhodophyta recorded 21% in normal seawater. The opposite strategy (only use of dissolved CO2) was observed only in Rhodophyta. The highest percentage was observed in the estuarine salinity regimen (10%). Again, more research is required to obtain valuable information on the physiological and environmental status of macroalgae.

5 Conclusions

In conclusion, we observed high δ13C macroagal variability in macroalgae communities in the Gulf of California, such
as reported in other worldwide marine ecosystems. The life-form is the principal cause of δ¹³C macroalgal variability, which explains up to 57%. Changes in habitat characteristics and environmental conditions also influence the δ¹³C macroalgal variability within a specific carbon use strategy. Considering the combined effect of the life-form, coastline sector, and environmental conditions, the full model explains up to 72% (genus) of the variability. The effects of the coastal sector, pH ranges, and emersion level were significant, while for salinity and temperature they were negligible.

Most macroalgae inhabiting in GC displayed the presence of CO₂ concentrating mechanisms to uptake HCO₃⁻ for photosynthesis, and 84% of the total analyzed specimens were able to use both HCO₃⁻ and/or CO₂ employing active uptake plus passive diffusion (strategy 2: −10 < δ¹³C < −30‰). Specimens belonging to 58 species of 170 total species showed carbon uptake strategy 1 that uses only HCO₃⁻. A higher proportion of CCM species (HCO₃⁻ users) was expected because we focused on intertidal and shallow subtidal habitats featured by high light intensities. Only three non-calciﬁng species (Schizymenia pacifica, Halymenia sp., Gigartina sp.) belonging to Rhodophyta (3%) were CO₂-exclusive users (strategy 3: δ¹³C < −30‰). The low percentage of CO₂ dependents versus 40%–90% reported for temperate regions could be related to the shallow habitat sampled in our surveys (<2 m depth low tide). The calcifying macroalgae genera Amphiroa and Jania using HCO₃⁻ (high δ¹³C values) were present in the macroalgal communities in the GC. Because of the ongoing ocean acidification, these calcifying organisms constitute excellent ecological sentinels in the GC.

Finally, diverse authors have reported signiﬁcant correlations between δ¹³C signal and latitude, mainly related to the light and temperature. However, in our study’s latitude range (21°–31°N), the linear regression analyses showed a low correlation for the δ¹³C macroalgal dataset classiﬁed by morphofunctional group and genus, which was negative for Rhodophyta and Ochrophyta and positive for Chlorophyta. Non-clear δ¹³C macroalgal patterns occur along the GC latitudes. However, detectable changes were observed in the δ¹³C macroalgae and the proportion of specimens with different carbon uptake strategies among coastal sectors. For example, the facultative uptake of HCO₃⁻ and CO₂ was dominant in the macroalgal shallow communities in the GC (60% to 90% of specimens), but in the P1 (68%) and C1 (37%) the use of only HCO₃⁻ was the dominant strategy.

Our research is the ﬁrst approximation to understand the δ¹³C macroalgal variability in one of the most diverse marine ecosystems in the world, the Gulf of California. We did not pretend to resolve the intricate processes controlling the variations in δ¹³C or Δ¹³C macroalgae during carbon assimilation and respiration and determine the isolated inﬂuence of each environmental factor. Despite the large dataset and corresponding statistical analyses, our study faces limitations due to research design and because no research on δ¹³C macroalgal analysis was developed previously in the GC. The primary deﬁciency is the lack of pH drift experiments to discriminate δ¹³C signal variations in the carbon uptake strategies to determine preferential DIC uptake of macroalgae (CO₂ or HCO₃⁻). The second limitation concerns the lack of controlled experiments to discern what type of CCM is expressed in macroalgae (e.g., direct HCO₃⁻ uptake by the anion-exchange protein AE, types of mitochondrial AC, or the co-existence of different CCMs). Also, more research is required to assess the biological or ecological relevance of the δ¹³C variability as a function of the morphology (e.g., DIC uptake efﬁciency and isotope discrimination during carbon assimilation and respiration). Future studies assessing the ability of macroalgae to use CO₂ and/or HCO₃⁻ can be assessed by pH drift experiments and MIMS in the cosmopolitan’s species and within genera with differences in the δ¹³C values between species (e.g., Ulva and Sargassum).

Finally, controlled experiments in laboratory and mesocosms can be conducted with ﬁeld studies are required to elucidate what type of CCM is expressed in macroalgae. Even so, the δ¹³C macroalgae were a good indicator to infer the presence or absence of CCMs, to identify the macroalgae lineages that could be in a competitive advantage based on their carbon uptake strategy, and to identify their geographical distribution along with GC. Under the current climate change conditions and their effects as ocean acidification progresses and the bloom-forming macroalgae events increase in Mexico and worldwide, the analysis of δ¹³C macroalgae constitutes an excellent tool to help to predict the prevalence and shift of species in macroalgal communities which are focused on carbon metabolism. However, to obtain the maximum beneﬁt from isotopic tools in the carbon-use strategy study, diverse and species-speciﬁc, it is necessary to use them in combination with other techniques referred to herein.

Data availability. Datasets are each permanently deposited. Further information can be found at https://www.proquest.com/openview/2060de588b217ca47495469b53ae2f347/1?pq-origsite=gscholar&cbl=4882998 (Soto-Jimenez et al., 2020).

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