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# Dataset on the isotopic ( $\delta^{13}$ C, $\delta^{15}$ N) and elemental (C, N) composition of estuarine primary producers in the subtropical Southwestern Atlantic coast.

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#### ABSTRACT

Benthic and pelagic primary producers had their isotopic ( $\delta^{13}$ C,  $\delta^{15}$ N) and elemental (C, N) composition monitored in the Patos Lagoons estuary, in southern Brazil. The present dataset comprises temporal data obtained through seasonal samplings of C<sub>3</sub> (*Scirpus* spp.) and C<sub>4</sub> (*Spartina densiflora*) salt marsh plants, ephemerous bloom-forming drift macroalgae (Ulvophyceae), the widgeon grass *Ruppia maritima*, particulate (POM) and sedimentary (SOM) organic matter in shallow waters (< 2m) of a subtropical estuary from austral summer 2010 to autumn 2016. POM and SOM were collected as proxies of phytoplankton and microphytobenthos, respectively. Salt marsh plants were randomly sampled (N = 126) at a regularly flooded low marsh area, whereas submerged drift macroalgae (N = 29) and *Ruppia plants* (N = 14) were collected in adjacent mudflats. POM was collected (N = 33) by filtering water samples using glass fiber filter. SOM was obtained (N = 35) by removing superficial sediment. In laboratory, samples were processed and further analyzed for total organic carbon (TOC), total nitrogen (TN) and carbon (<sup>13</sup>C/<sup>12</sup>C) and nitrogen (<sup>15</sup>N/<sup>14</sup>N) stable isotopes ratios. With a total of 237 samples analyzed, this dataset provides key information on the isotopic and elemental composition of distinct estuarine primary producers and sources of particulate organic matter (POM and SOM) and their temporal variability in a highly variable aquatic environment. Such knowledge may add to ecological studies investigating food webs, biogeochemical cycles and sources tracking in coastal systems.

**Keywords:** Stable isotopes; Long-term monitoring; Seasonal trends; Aquatic primary production; Isotope mixing models.

# **PRIOR PUBLICATIONS**

CLAUDINO, M.C., ABREU, P.C., GARCIA, A.M. Stable isotopes reveal temporal and between-habitat changes in trophic pathways in a southwestern Atlantic estuary. Marine Ecology Progress Series, 489, 29–42. 2013. DOI: https://doi.org/10.3354/meps10400.

GARCIA, A.M. et al. Temporal variability in assimilation of basal food sources by an omnivorous fish at Patos Lagoon Estuary revealed by stable isotopes (2010–2014). Marine Biology Research, 13, 98–107. 2017. DOI: https://doi.org/10.1080/17451000.2016.1206939.

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LANARI, M. et al. Dynamics of estuarine drift macroalgae: growth cycles and contributions to sediments in shallow areas. Marine Ecology Progress Series, 570, 41–55. 2017. DOI: https://doi.org/10.3354/meps12116.

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POSSAMAI, B., HOEINGHAUS, D.J., GARCIA, A.M. Environmental factors drive interannual variation in estuarine food-chain length. Estuarine, Coastal and Shelf Science, 252: 107241. 2021. DOI: https://doi.org/10.1016/j.ecss.2021.107241.

POSSAMAI, B., HOEINGHAUS, D.J., GARCIA, A.M. Shifting baselines: Integrating ecological and isotopic time-lags improves trophic position estimates in aquatic consumers. Marine Ecology Progress Series, 666, 19. 2021. DOI: https://doi.org/10.3354/meps13682.

#### DATA IMPORTANCE

- Stable isotopes and elemental analyses have been used as natural biomarkers to investigate biogeochemical cycles, source tracking and food webs based on the assumption that the assimilation of distinct basal sources in mixtures can be distinguished and estimated through different isotopic values;
- The present dataset provides information on carbon (<sup>13</sup>C/<sup>12</sup>C) and nitrogen (<sup>15</sup>N/<sup>14</sup>N) stable isotope ratios, and total organic carbon (TOC) and total nitrogen (TN) contents of distinct estuarine primary producers and their temporal scales of variation in a subtropical coastal system;
- Such data provide insights on the isotopic and elemental content among distinct basal sources and the extent to which they may change and overlap over time due to heterogeneous environmental conditions;
- The isotopic and elemental data presented here may subsidize other ecological studies investigating food webs, nutrient cycling and carbon stocks in coastal systems based on the use of stable isotopes mixing models;
- Furthermore, the knowledge on the temporal scales of variation (both seasonal and interannual) of distinct basal sources is crucially important to establish appropriate sampling delineations in highly variable aquatic systems.

#### **MATERIALS AND METHODS**

## Study área

The Patos Lagoon estuary (PLE) comprises an area of 971 km<sup>2</sup> in southern Brazil (Fig. 1). It is a funnel-shaped estuary, and its connection to the ocean ranges from 3 km to 0.5 km wide at the end nearest to the ocean. Therefore, there is a substantial influence of freshwater in this estuary,

once the entrance of seawater is reduced due to its shape.

Estuarine primary producers were collected in a mudflat and a salt marsh edge in a shallow embayment off Pombas Island, in the mesomixohaline region of the PLE (Fig. 1, highlighted). The sampling area is highly representative of the spatio-temporal dynamics of local estuarine primary production and environmental conditions (i.e., water salinity,

temperature, turbidity, level, and nutrient concentrations; LANARI et al., 2017).

Figure 1. The Patos Lagoon estuary (PLE) in southern Brazil with the sampling area indicated (red dot). Map obtained from Lanari et al. (2021).



## **Field sampling**

From March 2010 (austral summer) to June 2016 (autumn), primary producers were collected at the end of each season (i.e., March, June, September, December). Estuarine primary producers were: suspended particulate organic matter (POM; a proxy for phytoplankton and organic matter in the water column), particulate organic matter in the sediment (SOM; a proxy for microphytobenthos and organic detrital matter in sediments), opportunistic drift macroalgae, the widgeon grass Ruppia maritima, and saltmarsh C<sub>3</sub> (Scirpus spp.) and C<sub>4</sub> (Spartina densiflora) plants. The number of replicates for each producer varied from 2 up to 6 per season throughout the study period. Salt marsh plants were randomly sampled at a regularly flooded low marsh, whereas submerged drift macroalgae and Ruppia plants were collected in adjacent mudflats. POM was obtained by filtering one liter of water onto a precombusted (450°C for 4 h) Whatman glass fiber filter (0.75 µm). SOM was obtained by removing the top 2 cm from the surface of the sediment using a core (10 cm diameter). Macroalgal thalli and seagrass and salt marsh plant leaves were manually collected, using scissors when necessary. All samples were stored in plastic bags and preserved on ice for transportation to the laboratory, where they were stored in a freezer until processing. Some temporal gaps in data sampling occurred due to logistical and financial constraints.

All plants were identified to the lowest taxonomic level possible based on morphological and anatomical features. *Scirpus* spp. were comprised of *Scirpus maritimus* and *Scirpus olneyi*, but isotopic and elemental data did not distinguish them. Macroalgae were drift mats of algae identified as *Ulva* spp. and *Rhizoclonium* spp. based on morphological attributes, although molecular analyses are necessary for taxonomic confirmation at species level. Macroalgae were thus grouped as Ulvophyceae.

# **Elemental and isotopic analyses**

In laboratory, samples were washed with distilled water to debris removal, placed in sterile Petri dishes and dried until constant weight (at 60°C for 48h). The tissues analyzed were leaves and thalli filaments for macroscopic plants. The dried samples were ground to a fine powder with a mortar and pestle and stored in clean Eppendorf tubes. Aliquots of each dried sample were weighted (25-30 mg for SOM and ~ 3 mg for other basal sources) and pressed into ultrapure tin capsules (Costech, Valencia, CA). Samples were sent for analyses of total organic carbon (TOC), total nitrogen (TN) and carbon (<sup>13</sup>C/<sup>12</sup>C) and nitrogen (<sup>15</sup>N/14N) stable isotopes in the Analytical Chemistry Laboratory (University of Georgia, USA) from 2010 through 2015 and in the Stable Isotope Ecology Laboratory (University of North Texas, USA) in 2016. SOM samples were not acidified prior to the analyses given the low carbonate content found in the sediment samples (average < 1%; maximum of 1.5%) compared to other benthic estuarine/marine environments (CLAUDINO et al., 2013). The stable isotope results were expressed in delta notation (the parts-perthousand deviation from a standard material):  $\delta^{13}$ C or  $\delta^{15}$ N = [(Rsample/Rstandard) - 1] \* 1000, where R =  ${}^{13}C/{}^{12}C$  or  ${}^{15}N/{}^{14}N$ . Standards were carbon in the Vienna Pee Dee Belemnite calibrated using certified reference materials and nitrogen in air. TOC and TN values were expressed as percentages of plant dry weight.

# **Quality control**

Material sampling and processing for isotopic and elemental were performed by researchers following the same procedures since the beginning of the time series. Samples from 2010-2015 and 2016 were analyzed in distinct laboratories and the standard deviation betweenlaboratory revealed to be low and acceptable for comparing the isotope ratios for both carbon (SD=0.03‰, t=0.008, d.f.=8, p-value= 0.993) and nitrogen (SD= 0.08‰; t=0.004, d.f.= 7.988, pvalue=0.996). Samples taxonomic validity was verified using the World Register of Marine Species (WoRMS; www.marinespecies.org).

# **DATA DESCRIPTION**

A total of 237 samples were collected during the study period, resulting in a total of 948 measurement values of  $\delta^{13}$ C,  $\delta^{15}$ N, TOC and TN. Vegetal tissue from macroscopic producers (salt marsh plants, widgeon grass and macroalgae) comprised 169 samples. Salt marsh plants represented a total of 126 samples (Spartina densiflora, N = 54; Scirpus spp., N = 72), R. maritima had 14 samples and the Ulvophyceae macroalgae had 29 samples. A total of 69 samples of water (POM; N = 33) and sediments (SOM; N = 35) were taken. A variable number of replicates of each primary producer were obtained throughout the study period (Fig. 2). Temporal gaps in data sampling occurred in Spring 2011- Summer 2012 and Summer and Autumn 2015. Moreover, sampling gaps occurred for R. maritima and Ulvophyceae due to their high biomass interannual variability and for POM, SOM and salt marsh plants due to logistical constraints.



Figure 2. Number of samples seasonally collected for each primary producer from summer 2010 to autumn 2016. S: summer; A: autumn; W: winter; Sp: spring.

Mean isotopic ( $\delta^{13}$ C,  $\delta^{15}$ N) and elemental (TOC, TN) values of each producer were calculated for the whole sampling period (i.e., 2010 to 2016; Table 1). Mean  $\delta^{13}$ C values across primary producers varied from -26.80 (C<sub>3</sub> plant) up to -12.18 (Widgeon grass; Table 1).  $\delta^{15}$ N mean values were less variable among distinct primary sources. TOC mean values varied from 0.74% (SOM) up to 40.90% ( $C_3$  plant), whereas TN mean values ranged from 0.23 (SOM) up to 3.65 (Widgeon grass). Seasonal mean values (+ SE) of the isotopic and elemental composition of estuarine primary producers per year are not presented here but can be found in Lanari and collaborators (2021).

**Table 1.** Minimum, maximum and mean ( $\pm$  standard error) isotopic ( $\delta^{13}$ C and  $\delta^{15}$ N) and elemental (TOC and TN) values in C<sub>3</sub> (*Scirpus* spp.) and C<sub>4</sub> (*Spartina densiflora*) salt marsh plants, the widgeon grass *Ruppia maritima*, Ulvophyceae macroalgae, particulated (POM) and sedimentary organic matter (SOM), proxies for phytoplankton and microphytobenthos, respectively, during the sampling period (2010 to 2016). Isotopic values are expressed in delta notation (the parts-per-thousand deviation from a standard material) and elemental compositions are percentages of plant dry weight.

	C <sub>3</sub>			<b>C</b> 4			Widgeon grass		
	Min.	Max.	Mean ( <u>+</u> SE)	Min.	Max.	Mean ( <u>+</u> SE)	Min.	Max.	Mean ( <u>+</u> SE)
δ13C	-29.65	-23.09	-26.80 (0.18)	-13.99	-11.24	-12.77 (0.09)	-17.09	-9.16	-12.18 (0.62)
$\delta^{\rm 15}N$	2.71	11.44	6.98 (0.23)	3.41	15.39	6.54 (0.32)	4.21	8.46	6.50 (0.34)
тос	33.94	50.03	40.90 (0.36)	32.54	54.56	40.45 (0.42)	36.93	43.41	40.28 (0.54)
TN	0.86	3.15	1.68 (0.05)	0.48	2.64	1.20 (0.06)	2.81	4.42	3.65 (0.17)
	Ulvophyceae			РОМ			SOM		
	Min.	Max.	Mean ( <u>+</u> SE)	Min.	Max.	Mean ( <u>+</u> SE)	Min.	Max.	Mean ( <u>+</u> SE)
δ <sup>13</sup> C	-19.25	-8.88	-12.84 (0.52)	-23.31	-18.04	-20.13 (0.24)	-17.71	-12.59	-15.71 (0.19)
$\delta^{{}^{15}}N$	2.61	8.64	6.29 (0.27)	0.91	20.3	7.00 (0.81)	1.69	23.96	8.51 (0.85)
тос	18.99	54.57	31.78 (1.32)	0.76	2.50	1.49 (0.08)	0.13	3.53	0.74 (0.15)
TN	1.21	4.92	3.06 (0.19)	0.17	0.77	0.31 (0.02)	0.02	2.78	0.23 (0.10)

The dataset was formatted using the Darwin Core standard (DwC; WIECZOREK et al., 2012), the most popular standard for sharing and integrating biodiversity information worldwide (TANHUA et al., 2019). Data was organized in the OBIS-ENV-DATA structure (DE POOTER et al., 2017) which allows the publication of biotic and associated environmental data. Three spreadsheets were used: an Event Core containing information pertaining sampling events; an Occurrence extension with qualitative biological information; and an extended Measurement or Fact extension (extMoF) with quantitative biotic and environmental information (Table 2). Field terms followed the DwC vocabulary (DARWIN CORE MAINTENANCE GROUP, 2020) and unique identifiers in the DwC fields eventID and occurrenceID linked the information among the core and extensions. The dataset is available for download as a Darwin Core archive (DwC-A) zipfile.

**Table 2.** Field terms used in the dataset according to the Darwin Core vocabulary. Detailed information is also available in the Darwin Core Maintenance Group (2020) webiste.

Spreadsheet	Field	Definition
	datasetName	The name identifying the dataset.
	institutionCode	The name in use by the institution having custody of the dataset.
	rightsHolder	A person or organization owning or managing the data rights.
	parentEventID	An identifier for the broader Event that groups this and potentially other Events.
	eventID*	A unique identifier, specific to the dataset, providing information associated with the sampling events (i.e., something that occurs at a place and time). Constructed based on a combination of information country,
		institution, water body and data of sampling events.
	eventDate	The date during which a sampling event occurred. Dates are presented in the Year-Month format in accordance with the ISO 8601-1:2019.
	habitat	The habitat in which sampling events occurred.
	country	The country in which sampling events occurred.
	countryCode	The standard code for the country in accordance with the ISO 3166-1- alpha-2 country code.
Event Core	stateProvince	The state in which sampling events occurred.
	county	The full, unabbreviated name of the next smaller administrative region than stateProvince (city, municipality, etc.) in which sampling events occurred.
	waterBody	The name of the water body where sampling occurred.
	decimalLatitude	The geographic latitude (in decimal degrees, using the spatial reference system given in geodeticDatum) of the geographic center of the sampling area.
	decimalLongitude	The geographic longitude (in decimal degrees, using the spatial reference system given in geodeticDatum) of the geographic center of the sampling area.
	geodeticDatum	The ellipsoid, geodetic datum, or spatial reference system upon which the geographic coordinates given in decimalLatitude and decimalLongitude are based.
	eventRemarks	In the present dataset, this field presents information on which season/year the sampling event represents.

Occurrence extension	eventID*	Idem to Event Core description.
	occurrencelD*	A unique identifier, specific to the dataset, providing information on the occurrence of biological material. Constructed based on the eventIE
		information.
	scientificName	The full scientific name of an organism at the lowest possible taxonomic level.
	organismQuantity	A number for the quantity of individual organisms sampled (i.e., the number of replicates).
	organismQuantityType	The type of quantification system used for the quantity of organisms. In our case, the number of individual organisms from which samples (i.e. leaves and filaments) were obtained.
	scientificNameAuthorship	The authorship information for the scientificName.
	scientificNameID	An identifier for the nomenclatural (not taxonomic) details of a scientific name.
	kingdom	The full scientific name of the kingdom in which the taxon is classified.
	phylum	The full scientific name of the phylum or division in which the taxon is classified.
	class	The full scientific name of the class in which the taxon is classified.
	order	The full scientific name of the order in which the taxon is classified.
	family	The full scientific name of the family in which the taxon is classified.
	genus	The full scientific name of the genus in which the taxon is classified.
	specificEpithet	The name of the first or species epithet of the scientificName.
	taxonRank	The taxonomic rank of the most specific name in the scientificName field
	occurrenceStatus	A statement about the presence or absence of a Taxon in a sampling event
	basisOfRecord	The specific nature of the data record.
	eventID*	Idem to Event Core description.
	occurrenceID*	Idem to Occurrence extension description. For SOM and POM, this fiel was left blank due to the no taxonomic identification of phytoplankton an microphytobenthos.
	measurementType	The nature of the measurement. In our case, $\delta^{13}$ C, $\delta^{15}$ N, TOC and TN.
	measurementValue	The value of the measurement.
eMoF	measurementID	A unique identifier of information pertaining measurements. Different codes with information regarding sampling year, month, and replicate number were used to label the distinct replicates obtained for each producer. For salt marsh plants, widgeon grass and macroalgae measurementID codes were constructed based on the occurrenceID.
extension	measurementUnit	The units associated with the measurementValue. Parts per thousand for isotopic values and percentage of dry weight for elemental analyses.
	measurementValueID	An identifier for facts stored in the in the column measurementValue. A it refers to a value, was left empty according to De Pooter et al., 2017.
	measurementTypeID	A unique identifier for the measurementType according to the Britis Oceanographic Data Center.
	measurementUnitID	A unique identifier for the measurementUnit according to the British Oceanographic Data Center.
	measurementAccuracy	The description of the potential error associated with the measurementValue.
	measurementRemarks	Comments or notes on the measurements.

\*Unique identifiers linking core and extension spreadsheets.

Data presented in the Event Core (year-month) were not the exact sampling date, but an approximation representing each seasonal sampling event (see eventRemarks field, Table 2). Due to the lack of taxonomic identification, POM and SOM data were presented as environmental measurements in the eMoF extension with no associated occurrenceID term. Distinct replicates for plants, SOM and POM were labelled using the measurementID field in the extMoF extension.

## SUPPLEMENTARY MATERIALS

Repository: Global Biodiversity Information Facility (GBIF). | DOI: https://doi.org/10.15468/ntbfcg Link de access: https://www.gbif.org/dataset/35014baf-5683-4267-b19d-8953f1fd3383

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