

Stable isotopes reveal temporal and between-habitat changes in trophic pathways in a southwestern Atlantic estuary

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ABSTRACT: Studies on food web structure in estuaries based on stable isotopes have usually not taken spatiotemporal variations in the isotopic variability of food web components into account. We investigated temporal and between-habitat variations in the isotopic composition ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) of basal food sources—i.e. primary producers, particulate organic matter (POM) in the sediment or in suspension, and biofilm—and consumers and explored their implications for food web structure and dynamics. Samples of 9 basal sources ($N = 224$) and 6 representative invertebrates and fish ($N = 303$) were collected monthly over 1 yr in a marsh creek and a mudflat of the Patos Lagoon, a large southwestern Atlantic estuary. The relative contributions of food sources to consumers were estimated by mixing models. Overall, $\delta^{13}\text{C}$ values of food sources and consumers were higher ($p < 0.05$) in the mudflat, whereas $\delta^{15}\text{N}$ values were enriched ($p < 0.05$) in the marsh creek for most primary producers and consumers. C and N isotope values varied on both monthly and seasonal scales, with several food sources and consumers exhibiting higher values ($p < 0.05$) during the spring and summer. The primary food sources were POM in the sediment, C_4 plants (e.g. widgeon grass), and, to a lesser extent, suspended POM. Biofilm and macroalgae were also food sources for consumers in the marsh creek and mudflat, respectively, especially during warmer seasons. Our findings corroborate the hypothesis that the Patos lagoon food web is spatially structured and supported by local basal sources and detritus of different origins, which can be seasonally important for consumers in each particular habitat.

KEY WORDS: Estuarine food web · Salt marsh detritus · Mixing model · SIAR · Widgeon grass · Biofilm · Fish · Decapod crustaceans · Temporal variability

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INTRODUCTION

Estuaries are among the most productive ecosystems in the world and are characterized by complex trophic dynamics associated with their diverse habitats and basal food sources—primary producers, particulate organic matter (POM) and biofilm (Day et al. 1989), which in turn are influenced by variability in hydrodynamics and physicochemical conditions at various spatiotemporal scales (Kennish 1986). Estuary hydrodynamics is usually influenced by several regional factors (e.g. rainfall, freshwater discharge, and wind patterns) that can have local effects on

physicochemical conditions and water levels (Möller & Fernandes 2010). Such temporal variability in hydrodynamics and physicochemical parameters is usually associated with seasonal changes in species composition, as well as primary and secondary production (Seeliger et al. 1998). Seasonal pulses in secondary production have historically been exploited by artisanal and coastal fisheries on fish (e.g. mullets and sciaenids) and invertebrates (e.g. shrimp and blue crabs) with high commercial value (Day et al. 1989).

Earlier studies of trophic dynamics in estuaries were usually based on stomach content analysis of consumers (Whitfield 1988). Although this can reveal

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consumers' diets and trophic relationships with higher taxonomic resolution, the method has limitations (Bennemann et al. 2006). For example, (1) it is not feasible to apply this approach to microconsumers because of the difficulty or impossibility of dissecting their digestive apparatus; (2) some food items found inside stomachs can be refractory to digestion, hindering a precise determination of their nutritional value (Jepsen & Winemiller 2002); and (3) detritus can dominate the stomach contents of some estuarine species, and the identification of the origin or taxonomic status of such detritus is usually troublesome (Whitfield 1988).

A more inclusive approach to the investigation of trophic dynamics and the relationships among consumers in different ecosystems involves the analysis of the stable isotope ratios of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) in basal food sources and in consumers (Fry 2006). Isotope ratios are used to infer the relative contributions of food sources to consumers and the consumer's trophic position in the food chain (Fry 2006). Nitrogen stable isotope ratios, in particular, have also been used to evaluate the eutrophication level and the changes in aquatic biota in response to inputs of domestic and industrial effluents (Abreu et al. 2006, Fry 2006).

However, most previous stable isotope studies of food web structure in estuaries have not taken into account the spatiotemporal variations in the isotopic composition of basal food sources and consumers. Rather, most studies constitute 'snapshots' of patterns associated with between-site differences (e.g. Connolly et al. 2005, Pasquaud et al. 2008, Hoeninghaus et al. 2011) or changes along salinity gradients (e.g. Garcia et al. 2007, França et al. 2011). In some cases, spatiotemporal variability is considered, but it is restricted to a specific food web component such as invertebrates (Lebreton et al. 2012), suspended POM (Sato et al. 2006), or aquatic and terrestrial plants (Cloern et al. 2002). The few studies encompassing multiple food web components at both spatial and temporal scales have shown that significant variations may (e.g. Vizzini & Mazzola 2003, Faye et al. 2011) or may not (e.g. Rodríguez-Graña et al. 2008) occur on both scales. The extent to which isotopic variability in estuarine organisms occurs at different spatial (e.g. between-habitat) and temporal (e.g. seasonal) scales is largely unknown, which diminishes our ability to describe trophic dynamics in estuaries. For instance, prior studies in estuaries using stable isotopes have claimed that consumers reflect mainly assimilation of autochthonous primary producers (e.g. Garcia et al. 2007). However, they do not take into

consideration if temporal and among-habitat variations in the distribution and isotopic variability of estuarine primary producers (e.g. blooms of free-floating macroalgae) could affect their observed patterns and conclusions.

We investigated temporal (monthly and seasonal) and spatial (between-habitat) variations in the isotopic composition ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) of basal food sources and consumers (invertebrates and fish) in a large southwestern Atlantic estuary and explored the implications of such variations for our understanding of food web structure and dynamics. We investigated the following hypotheses: (1) consumers rely mainly on the autochthonous primary production of local estuarine food webs, (2) a seasonal rather than monthly temporal scale of variation in the isotopic composition of basal food sources and consumers is the most prevalent, and (3) estuarine fish show low variation in trophic levels across spatiotemporal scales.

MATERIALS AND METHODS

Study area

The Patos Lagoon is located in the coastal plain of the southernmost region of Brazil (Fig. 1A) and constitutes one of the largest coastal lagoons in the world (Kjerfve 1986). The lagoon is connected to the sea through a channel (4 km long, 800 m wide at its narrowest section, and 18 m deep) bordered by rock jetties constructed in the early 1900s to keep the entrance to the channel sufficiently deep for navigation (Möller & Fernandes 2010). Its estuarine zone represents approximately 10% (1022.7 km²) of the entire lagoon (10360 km²), and most of this area is characterized by shallow waters (<2 m). The lagoon has a microtidal regime (<0.47 m), and the variations in its hydrodynamics are controlled primarily by freshwater runoff and wind circulation, which together influence both the water level and salinity in the estuarine zone (Möller & Fernandes 2010). Overall, higher salinity values are common during the summer (mean salinity 22.5) because freshwater inflow is low. In contrast, lower salinity values are common during the winter (mean salinity 8.5), when the freshwater inflow increases because of high rainfall. The seasonal fluctuations in hydrodynamics and abiotic factors such as temperature and salinity are associated with seasonal variations in the species composition and abundance of primary producers and consumers in the estuarine zone (Seeliger et al. 1998). Seagrass beds (primarily widgeon grass *Ruppia mari-*

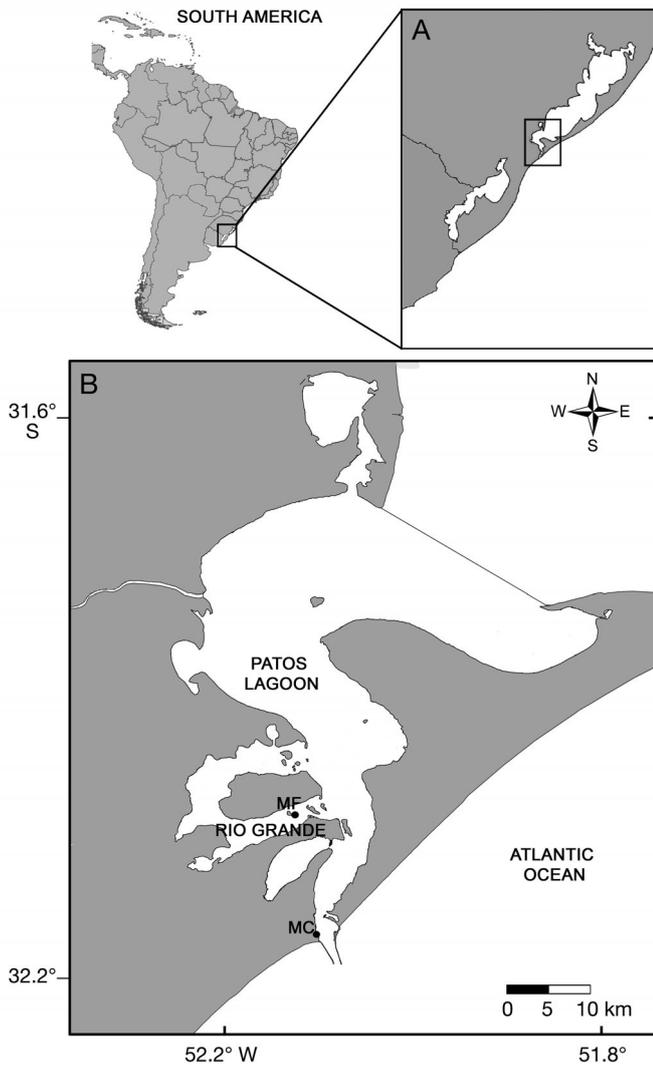


Fig. 1. (A) Patos Lagoon and (B) its estuarine zone with the locations of the habitats studied: a marsh creek near the connection of the estuary with the sea (MC) and a mudflat in the central portion of the estuary (MF)

tima) are commonly found in these shallow embayment areas, whereas salt marsh plants (e.g. dense-flowered cordgrass *Spartina densiflora* and alkali bulrush *Scirpus maritimus*) are abundant along their margins (Seeliger et al. 1998). The phytoplankton (diatoms, dinoflagellates, and cyanobacteria) displays seasonal patterns in biomass, productivity, and community composition driven by meteorological conditions (Odebrecht & Abreu 1998). The marsh intertidal zone is dominated by the omnivorous crab *Neohelice granulata*, which dwells in burrows located between the roots and stalks of plants. This species contributes to the fragmentation and remobilization of buried plant biomass and plays an important role in the cycling of organic matter derived from marsh

plants (Costa 1998a). The shallow waters of both marsh creeks and mudflats harbor commercially valuable species such as the pink shrimp *Farfantepenaeus paulensis* and juvenile mullet (primarily *Mugil liza*) (Seeliger et al. 1998).

Field collection and sample processing

Samples were collected monthly from March 2010 to February 2011 in 2 shallow water areas of the estuary: a marsh creek (MC) near the estuary's connection to the sea and a mudflat (MF) located in the middle of the estuary (Fig. 1B). Each month, duplicate samples were obtained to characterize basal food sources and consumers. We collected marsh plants (*Spartina densiflora*, *Scirpus maritimus*, *Scirpus olneyi*, and *Juncus acutus*); seagrass (*Ruppia maritima*); macroalgae (*Rhizoclonium riparium*); and other potential basal food sources such as biofilm, suspended POM, and POM in the sediment (SOM). Samples of the primary producers were collected by hand using scissors. Biofilm was carefully scratched from the submerged surface of *Scirpus maritimus* stalks and later inspected with a stereoscopic microscope to remove stalk fragments. POM was obtained by filtering approximately 0.25 to 1 l of water with a pre-combusted (450°C for 4 h) Whatman glass-fiber filter (0.75 µm). To obtain a sample of SOM, we removed the top 2 cm from the surface of the sediment using a plastic core (10 cm diameter).

We sampled representative species of the dominant macrofauna in the estuary (Seeliger et al. 1998) such as fish (the Brazilian silverside *Atherinella brasiliensis*, one-sided livebearer *Jenynsia multidentata*, and mullet *Mugil liza*), decapod macrocrustaceans (*Neohelice granulata*, blue crab *Callinectes sapidus*, and *Farfantepenaeus paulensis*), and infaunal organisms (the microcrustacean *Kalliapseudes schubartii* and polychaete *Laeonereis acuta*). Fish and decapod macrocrustaceans were collected with beach seine hauls, and infaunal organisms were collected with plastic cores (10 cm diameter, 20 cm depth, 0.0078 m² area) and retained on 500 µm mesh nets. All samples were stored in sealed plastic bags on ice and transported to the laboratory, where they were kept frozen and later processed (Garcia et al. 2007, Hoeninghaus et al. 2011).

After thawing, tissues were dissected from each primary producer and consumer: leaves and filaments were dissected from widgeon grass *Ruppia maritima* and the free-floating green macroalgae *Rhizoclonium riparium*, respectively, and anterodor-

sal and abdominal muscular tissues (approximately 5 g per sample) were dissected from fish and decapod macrocrustaceans, respectively. Infaunal organisms <1 mm were processed whole. Each tissue was rinsed with distilled water, placed in a sterile Petri dish, and dried in an oven at 60°C to a constant weight (minimum of 48 h). Dried samples were ground to a fine powder with a mortar and pestle and stored in Eppendorf tubes. Subsamples were weighed (1 to 3 mg for animal tissues, 25 to 30 mg for SOM, and approximately 3 mg for other basal sources), pressed into ultrapure tin capsules (Costech Analytical Technologies), and sent to the Analytical Chemistry Laboratory of the Institute of Ecology, University of Georgia, for analysis of carbon and nitrogen isotope ratios. The carbon standard was Pee Dee Belemnite limestone, and the nitrogen standard was atmospheric nitrogen. Based on the standard deviation of the internal standard replicates, the analytical precision was 0.17 for carbon and 0.18 for nitrogen.

Acidification and lipid content

$\delta^{13}\text{C}$ values can be lower in samples with higher levels of inorganic carbon (e.g. carbonate), and acidification can be used to correct this effect. We divided our SOM samples into 2 treatments: one receiving acidification (AC) and the other not receiving acidification (N-AC). AC subsamples were acidified with approximately 2 ml of 1 M HCl, and after bubbling had ceased, they were dried at 60°C for 18 h (Ryba & Burgess 2002). There were no significant differences between the $\delta^{13}\text{C}$ values in AC and N-AC samples (Student's *t*-test, $n = 28$, $p > 0.30$); however, there were statistically significant differences in their $\delta^{15}\text{N}$ values ($p < 0.01$). We concluded that our estuarine SOM and POM samples had low inorganic carbon content, and we used only the non-acidified SOM and POM samples.

Samples with higher lipid contents can have lower $\delta^{13}\text{C}$ values because this tissue is likely to be less enriched in ^{13}C compared with other tissues (DeNiro & Epstein 1977). In such cases, chemical lipid extraction or mathematical normalization can be used to control this effect. According to Post et al. (2007), when samples of aquatic animals have a lipid content <5%, which corresponds to a C:N ratio <3.5, it is not necessary to correct this effect. This was the case for the great majority of our consumers (>90%) (see Table S1 in the supplement, available at www.int-res.com/articles/suppl/m489p029_suppl.pdf).

Data analysis

Temporal (monthly and seasonal) and spatial (MC and MF sites) patterns in the carbon and nitrogen stable isotope ratios of basal food sources and macroconsumers (decapod macrocrustaceans and fish) were initially investigated using biplot diagrams (Fry 2006). To examine the spatial and temporal differences in the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N values, we used a 3-way nested permutational multivariate analysis of variance (PERMANOVA). The statistical model consisted of 3 factors: Season (4 levels, fixed); Location (2 levels, fixed and crossed with Season); and Month (12 levels, random, nested in Season). PERMANOVA was performed on the Euclidean distance matrices, and a square root transformation was performed on the data. The statistical significance of the PERMANOVA results was assessed using a permutation procedure ($n = 9999$) of the residuals under a reduced model (Anderson 2001). Calculations and tests were conducted using the PERMANOVA+ for PRIMER-E software program (Anderson et al. 2008). The seasons were defined as follows: austral autumn (March, April, and May); winter (June, July, and August); spring (September, October, and November); and summer (December, January, and February).

We employed the Bayesian stable isotope mixing model in the Stable Isotope Analysis in R (SIAR) package (Parnell et al. 2010) to estimate the relative contributions of basal food sources to macroconsumers (decapod macrocrustaceans and fish). The SIAR mixing model allows for the incorporation of uncertainties associated with sample variability and trophic enrichment even in undetermined systems, which was not possible with previous approaches (e.g. IsoSource). SIAR assumes that the variability associated with food sources and trophic enrichment is normally distributed (Parnell et al. 2010). To better restrict our model, we used elemental concentrations (%C and %N) measured for each organic basal source in this study.

Nitrogen isotopic distributions were used to estimate the trophic positions of consumers following the equation $T_p = \lambda + (\delta^{15}\text{N}_{\text{predator}} - \delta^{15}\text{N}_{\text{mullet}})/F$, where λ is the trophic level of consumers estimating the base of the food web (in this case, $\lambda = 2$, because mullets are primary consumers that feed mainly on microalgae), $\delta^{15}\text{N}_{\text{predator}}$ is the nitrogen signature of the consumer being evaluated, and F is the per-trophic-level fractionation of nitrogen (Post 2002). We used nitrogen fractionation values of 2.54 ± 0.11 to distinguish between trophic levels (Vanderklift & Ponsard 2003).

To characterize the fish diet of *Jenynsia multidentata* and *Atherinella brasiliensis*, we removed their stomachs and analyzed stomach content using a stereoscopic binocular microscope. Each food item found in the stomach was identified to the lowest taxonomic level possible, and its frequency of occurrence (%F) was calculated (Hyslop 1980). We pooled the food items into 9 categories: decapod macrocrustaceans, gastropods, planktonic microcrustaceans (mainly copepods), fish, polychaetes, insects, vegetation, ostracods, and tanaidaceans (only the tube-dwelling microcrustacean *Kalliapseudes schubartii*). The diet composition and %F of food items for the mullet *Mugil liza* and decapod macrocrustaceans *Neohelice granulata*, *Farfantepenaeus paulensis*, and *Callinectes sapidus* were obtained from prior studies (see Table 3).

RESULTS

Spatiotemporal changes in sample composition

A total of 224 samples of 9 basal food sources, 303 samples of 6 consumer species (decapod crustaceans, 140; fish, 163), and 90 samples of 2 infaunal species from sites MC and MF were analyzed (see Table S1). Some basal food sources (e.g. the dense-flowered cordgrass *Spartina densiflora* [C₄], the alkali bulrush *Scirpus maritimus* [C₃], POM, and SOM) were collected from both habitats. Other food sources were observed only in MC near the estuary's mouth (the spiny rush *Juncus acutus* [C₃] and biofilm) or at MF in the central portion of the estuary (the three-square *Scirpus olneyi* [C₃], the widgeon grass *Ruppia maritima*, and the free-floating green macroalgae *Rhizoclonium riparium*). The basal food sources in each habitat varied over time (Fig. 2). At MC, most basal food sources were available and were collected throughout the year. POM was also collected monthly at this site; however, its stable isotopic composition could not be determined from June to December because of insufficient material. At MF, in contrast, only 3 basal food sources occurred year-round (alkali bulrush, dense-flowered cordgrass, and SOM); widgeon grass and macroalgae were not present during autumn. Similarly, the stable isotopic composition of POM samples could not be analyzed from June to December because of the low amount of material. All macro-consumer species of fishes, decapod crustaceans, and infaunal organisms were obtained at each habitat (MC and MF) (see Table S1).

Variability in isotopic composition

In general, there were relatively low monthly variations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for each basal food source in both MC and MF (Fig. 2). Primary producers with C₃ (alkali bulrush and spiny rush) and C₄ (dense-flowered cordgrass and widgeon grass) photosynthetic pathways had different average $\delta^{13}\text{C}$ values, with <2‰ variation in their monthly average values during the year (Fig. 2). SOM and POM also exhibited relatively low monthly variation in their $\delta^{13}\text{C}$ values. In contrast, biofilm exhibited more pronounced monthly variation, with $\delta^{13}\text{C}$ values ranging from -27.2 in June to -16.6 in November. Compared with carbon stable isotope ratios, the monthly variation in $\delta^{15}\text{N}$ values of basal food sources was higher during the year. The average $\delta^{15}\text{N}$ values of the alkali bulrush, in particular, showed the most pronounced variation among the basal food sources, with average values varying from 3‰ in August to 9‰ in November at both habitats. POM and widgeon grass also exhibited marked $\delta^{15}\text{N}$ variation during the year, with higher values in January to February and in October. The spiny rush exhibited the highest variation in C:N ratios during the year, whereas SOM, POM, and widgeon grass had the lowest variation. The highest average C:N ratios were observed for marsh plants (dense-flowered cordgrass, alkali bulrush, spiny rush), whereas the lowest values were observed for SOM (Fig. 2) and POM.

Overall, consumers exhibited temporal variations similar to those of the basal food sources, with most species exhibiting <3‰ monthly variation in their average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Fig. 3). Exceptions included the one-sided livebearer, mullet, and pink shrimp, which had more pronounced temporal variations (especially in their $\delta^{13}\text{C}$ values) that tended to increase from September to February in both habitats. Most consumers had an average C:N ratio of approximately 3.5 throughout the year with the exception of the tube-dwelling tanaidaceans and the polychaetes, which had ratios ranging from 4 to 6 (Fig. 3).

PERMANOVA revealed statistically significant differences for basal food sources and consumers in both habitats (Tables 1 & 2). Significant differences in the average $\delta^{13}\text{C}$ values between habitats were noted for SOM, *Scirpus maritimus* (Table 1), decapod crustaceans, and polychaetes (Table 2). Overall, food sources and consumers had significantly enriched average $\delta^{13}\text{C}$ values in MF compared with MC. Only the macrophytes (dense-flowered cordgrass and alkali bulrush) and 2 fish species (*Atherinella brasiliensis* and *Jenynsia multidentata*) exhibited statistically

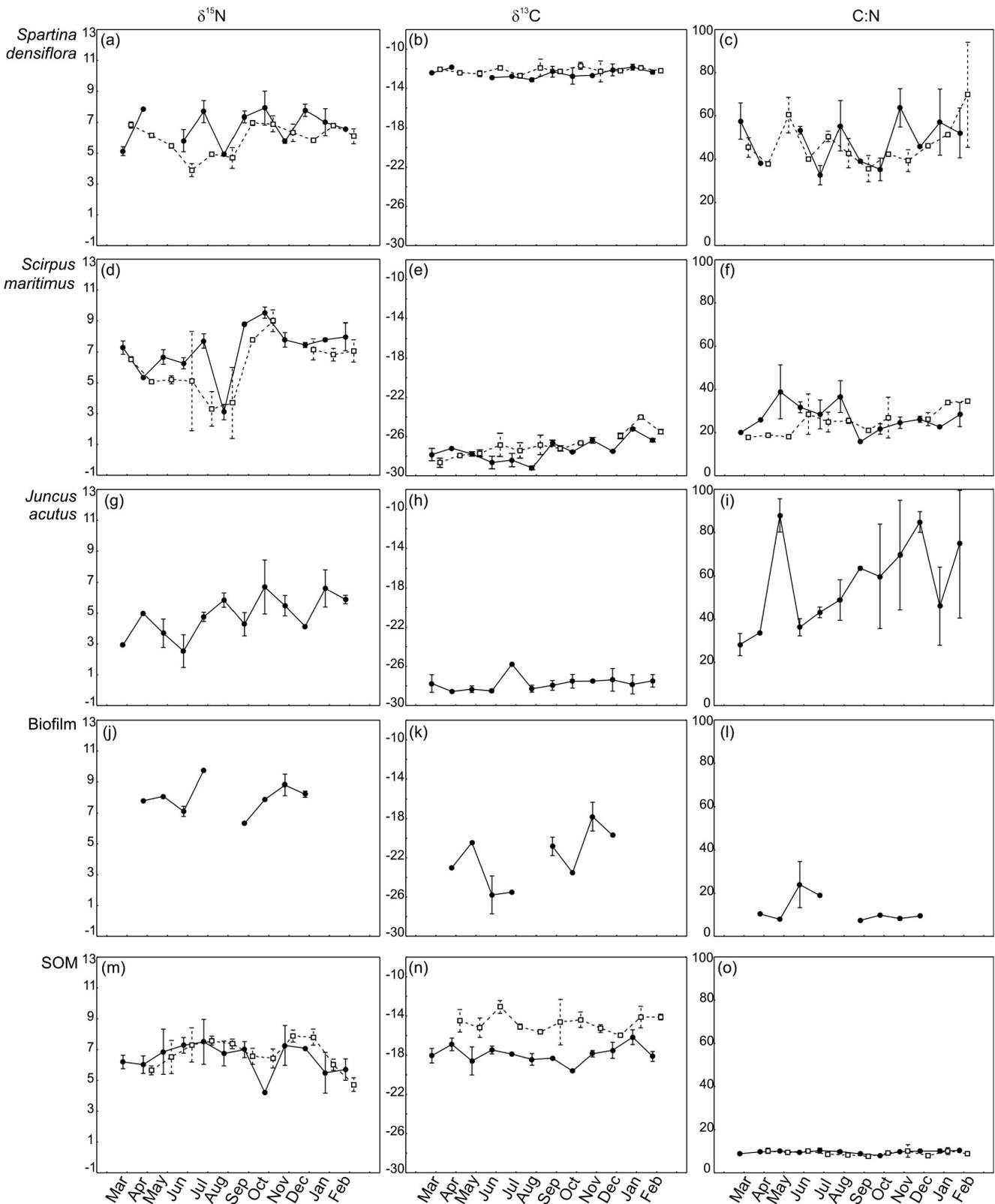


Fig. 2. Monthly mean values (\pm SE) of nitrogen ($\delta^{15}\text{N}$), carbon ($\delta^{13}\text{C}$), and the C:N ratio of basal food sources collected in the marsh creek (MC, solid lines) and mudflat (MF, dotted lines) of the Patos Lagoon estuary. SOM: sediment organic matter

significant ($p < 0.05$) temporal variations in average $\delta^{13}\text{C}$ values, with enrichment during warmer seasons (spring and summer).

There were also significant differences in average $\delta^{15}\text{N}$ values between sites. With the exception of mullet and pink shrimp, macroconsumers (Table 2) had enriched $\delta^{15}\text{N}$ values in MC compared with MF. Basal food sources (except SOM and *Spartina densiflora*) exhibited higher $\delta^{15}\text{N}$ values during the warmer seasons.

There were no significant differences in the C:N ratio of most basal food sources and consumers across spatiotemporal scales. Exceptions were POM, infaunal invertebrate species, and blue crab, which exhibited between-site differences, and one-sided livebearer, pink shrimp, and tanaidacean, which exhibited temporal variations in their average C:N values (Tables 1 & 2).

Contribution of basal food sources to consumers

The $\delta^{13}\text{C}$ values of primary producers with a C_3 photosynthetic pathway (*Scirpus maritimus*, *Juncus acutus*, and *Scirpus olneyi*) varied the most from those of consumers, with average differences from 9.1‰ at MC to 13.4‰ at MF (Fig. 4). POM, SOM, and C_4 plants (and in some seasons biofilm and macroalgae) had $\delta^{13}\text{C}$ values that were more similar to those of consumers. Moreover, the amplitude of variation in the average $\delta^{13}\text{C}$ values of consumers increased 2-fold between colder (autumn to winter) and warmer (spring to summer) seasons, which appeared to be related to seasonal differences in the relative importance of the basal food sources to these consumers.

The main basal food sources sustaining consumers at both sites included a mixture of SOM, C_4 plants, and, to a lesser extent, POM. A substantial habitat-dependent contribution from biofilm and macroalgae in particular seasons (autumn and winter, respectively) was also observed. In contrast, C_3 salt marsh plants contributed the least to consumers (Fig. 5).

Overall, the trophic position of consumers was similar among seasons and sites, with the exception of blue crab and one-sided livebearer. Statistically significant differences in trophic level between sites occurred during the autumn and winter for blue crab ($p < 0.01$) and only during the winter for one-sided livebearer ($p < 0.01$). The pink shrimp moved to a higher trophic level in the winter at both sites ($p < 0.05$). The mullet and burrowing crab belonged to the lowest trophic levels, whereas Brazilian silver-side had the highest trophic level (Fig. 6).

DISCUSSION

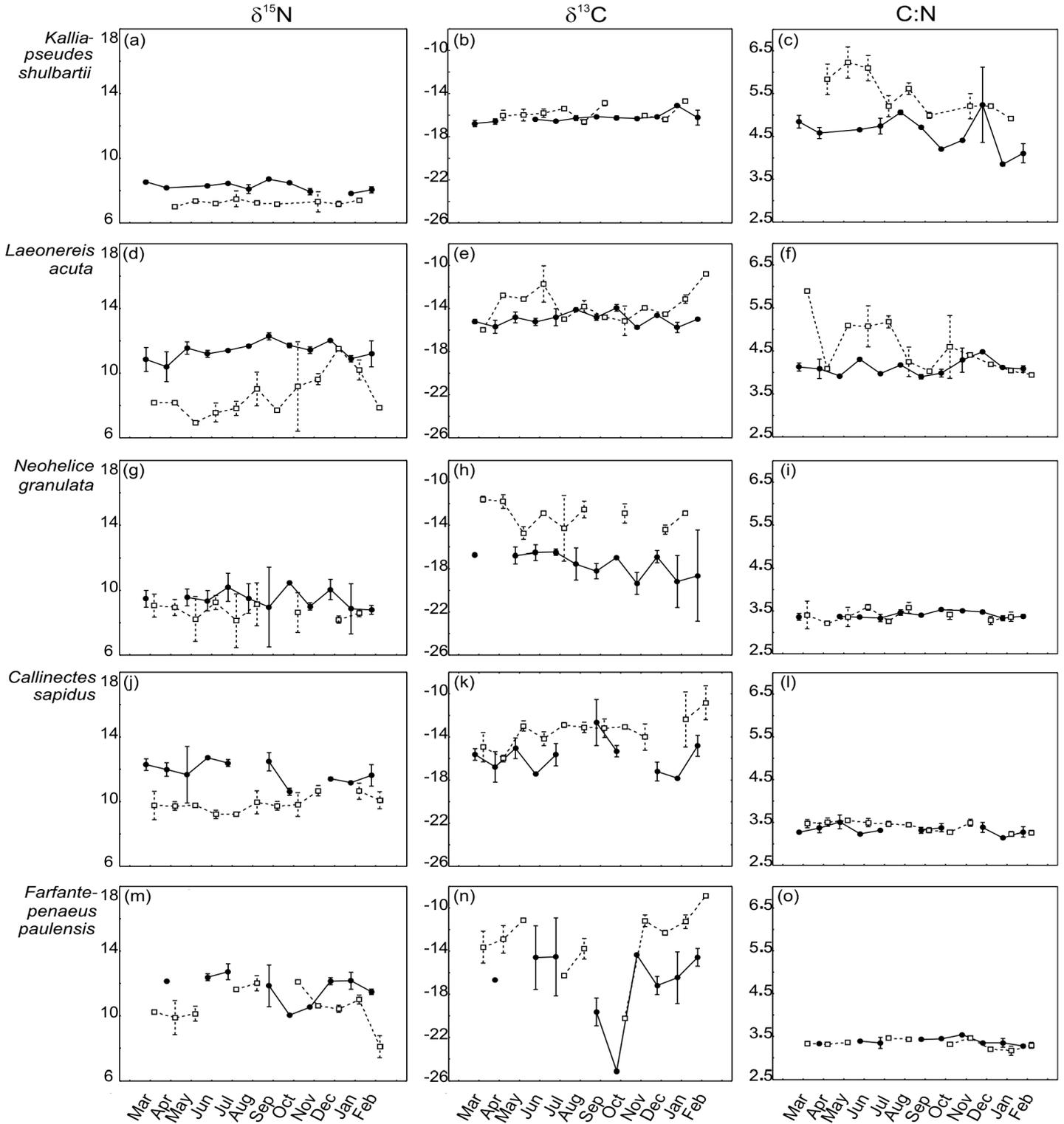
Spatiotemporal variability in carbon and nitrogen ratios

Between-habitat differences in the $\delta^{13}\text{C}$ values of basal food sources could be explained by the presence of primary producers with higher $\delta^{13}\text{C}$ values such as widgeon grass (−10.7‰) and free-floating green macroalgae (12.9‰) in the mudflat, but not in the marsh creek, where primary producers with lower $\delta^{13}\text{C}$ values predominated (e.g. alkali bulrush −27.4‰ and biofilm −21.8‰). Such differences could explain differences in SOM, which consists of a pool of organic matter and detritus of pelagic and benthic origin that has accumulated over the substrate (Day et al. 1989). Other studies show that the isotopic composition of SOM reflects the dominant local vegetation (Kennedy et al. 2010).

Such between-habitat differences in the carbon isotope ratios of basal food sources appear to have influenced the isotopic compositions of both decapod crustaceans and fish. The greater diversity of fish trophic guilds depending on both the benthic and pelagic trophic pathways most likely resulted in higher variance and, consequently, fewer between-site differences in isotope ratios than in decapod crustaceans, which feed primarily on benthic prey (Table 3). These patterns highlight the importance of autochthonous primary production in structuring estuarine food webs. Garcia et al. (2007) compared the isotopic variability in food web components between estuarine and freshwater regions of the Patos Lagoon and found also that consumers rely mainly on the autochthonous primary production of each subsystem.

Temporal variations in carbon and nitrogen isotope ratios were expected because of the high hydrodynamic variability of the Patos Lagoon estuarine zone (Möller & Fernandes 2010) and the annual senescence process that estuarine plants undergo (Lillebø et al. 2003). Cloern et al. (2002) also found high variability (approximately 5 to 10‰) in monthly $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within some plant groups, associated with annual cycles of growth and senescence in the San Francisco Bay estuary.

The biofilm in the marsh creek exhibited the greatest seasonal variation in carbon isotope ratios, related to temporal differences in biofilm composition because of biotic (e.g. colonization by microorganisms) and/or abiotic (e.g. salinity, temperature, flow, and light) factors. Macroalgae in the mudflat also exhibited variability in carbon isotope ratios, as they colo-



(Fig. 3 continued on next page)

Fig. 3. Monthly mean values (\pm SE) of nitrogen ($\delta^{15}\text{N}$), carbon ($\delta^{13}\text{C}$), and the C:N ratio of decapod crustaceans and fish collected in the marsh creek (MC, solid lines) and mudflat (MF, dotted lines) of the Patos Lagoon

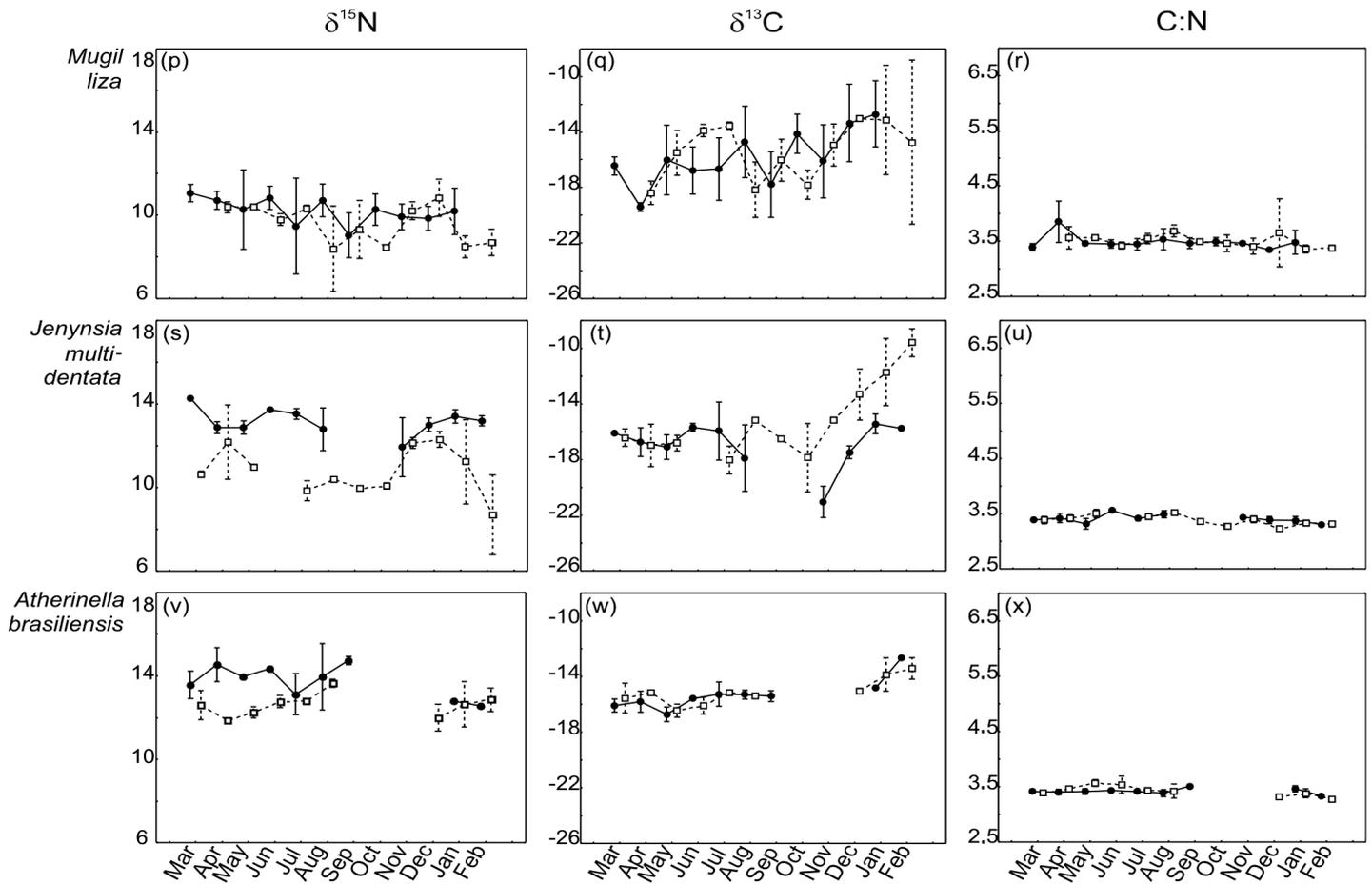


Fig. 3 (continued)

nized the mudflat in the winter and showed decay and senescence during spring and summer (M. C. Claudino pers. obs.). Therefore, the observed seasonal variation in the $\delta^{13}\text{C}$ value of macroalgae at this site could be related to shifts in their composition, epiphyte colonization, or senescence (Seeliger et al. 1998).

Between-habitat differences in nitrogen isotope ratios did not appear to be associated with differences in the species composition of primary producers. First, ^{15}N -enriched organic matter could come from a channel located 150 m from the entrance of the marsh creek, which drains water from a nearby partially flooded pasture. Animal waste products can be significantly enriched in ^{15}N because of the volatilization of ^{15}N -depleted ammonia, and the subsequent oxidation of much of the residual waste material may produce nitrate with enriched $\delta^{15}\text{N}$ values. Thus, when animal waste with a typical $\delta^{15}\text{N}$ value of approximately +5‰ is converted to nitrate, the $\delta^{15}\text{N}$ values are usually notably enriched (+10 to +20‰) (Kendall et al. 2007). A second hypothesis

Table 1. PERMANOVA for differences in the $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C:N ratios of basal food sources based on a Euclidean resemblance matrix. Significant values in **bold**

	$\delta^{15}\text{N}$		$\delta^{13}\text{C}$		C:N ratios	
	df	p	df	p	df	p
Organic matter in sediment						
Location	1	0.491	1	<0.001	1	0.227
Season	3	0.348	3	0.743	3	0.366
Month	8	0.003	8	0.053	8	0.42
Scirpus maritimus						
Location	1	0.064	1	0.013	1	0.581
Season	3	0.001	3	0.002	3	0.131
Month	8	0.089	8	0.028	8	0.089
Spartina densiflora						
Location	1	0.107	1	0.104	1	0.559
Season	3	0.065	3	0.043	3	0.278
Month	8	0.001	8	0.822	8	0.026
Particulate organic matter						
Location	1	0.656	1	0.083	1	0.302
Season	1	0.096	1	0.700	1	0.094
Month	3	0.053	3	<0.001	3	0.174

Table 2. PERMANOVA for differences in $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C:N ratios of consumers based on a Euclidean resemblance matrix. Significant values in **bold**

	$\delta^{15}\text{N}$		$\delta^{13}\text{C}$		C:N ratios	
	df	p(perm)	df	p(perm)	df	p(perm)
<i>Atherinella brasiliensis</i>						
Location	1	0.022	1	0.588	1	0.620
Season	3	0.063	3	0.086	3	0.273
Month	6	0.713	6	0.022	6	0.071
<i>Jenynsia multidentata</i>						
Location	1	0.029	1	0.009	1	0.913
Season	3	0.352	3	0.003	3	0.013
Month	8	0.179	8	0.127	8	0.120
<i>Mugil liza</i>						
Location	1	0.413	1	0.748	1	0.806
Season	3	0.159	3	0.012	3	0.595
Month	8	0.495	8	0.673	8	0.174
<i>Farfantepenaeus paulensis</i>						
Location	1	0.186	1	0.020	1	0.617
Season	3	0.266	3	0.228	3	0.002
Month	8	0.001	8	<0.001	8	0.605
<i>Callinectes sapidus</i>						
Location	1	0.001	1	0.002	1	0.044
Season	3	0.876	3	0.398	3	0.152
Month	8	0.415	8	0.007	8	0.017
<i>Neohelice granulata</i>						
Location	1	0.054	1	0.008	1	0.706
Season	3	0.253	3	0.161	3	0.371
Month	8	0.949	8	0.626	8	0.244
<i>Kalliapseudes shulbartii</i>						
Location	1	0.111	1	0.144	3	0.064
Season	3	0.931	3	0.469	8	0.007
Month	8	<0.001	8	<0.001	3	0.746
<i>Laonereis acuta</i>						
Location	1	<0.001	1	0.048	1	0.021
Season	3	0.130	3	0.525	3	0.285
Month	8	0.261	8	0.010	8	0.009

could be associated with the natural bioturbation of sediment by invertebrates. Botto et al. (2005) showed that the burrowing crab *Neohelice granulata*, which was abundant and widespread in MC (M. C. Claudino pers. obs.), increases the denitrification process on the aerobic-anaerobic surfaces of its burrows, leading to a preferential volatilization of $^{14}\text{N}_2$. These hypotheses are not mutually exclusive.

Trophic levels of consumers

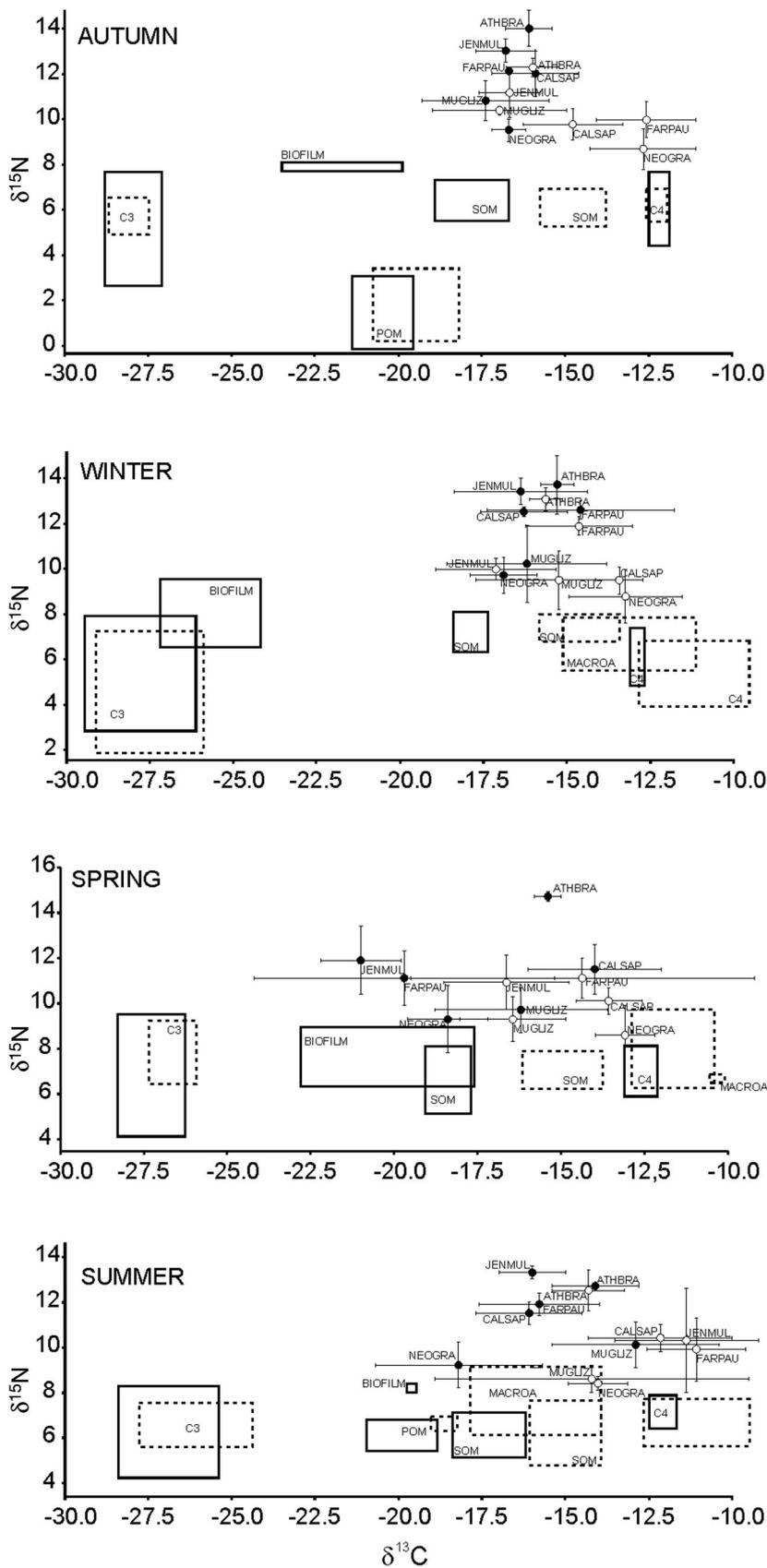
The variation in consumer trophic levels was greater between sites than among seasons, and higher trophic levels occurred in MC near the connection of the estuary with the sea, where variations in trophic level were noted only for blue crab and one-sided livebearer. Such between-habitat differ-

ences could reflect the fact that the $\delta^{15}\text{N}$ ratios of basal sources were higher at MC, as discussed above. The lower trophic levels were occupied by mullet and burrowing crab, which corresponds to their illiophagous (i.e. mud-eating) and detritivorous habits, respectively (D'Incao et al. 1990, Oliveira & Soares 1996). The mullet feeds primarily on microalgae (diatoms) and detritus (Oliveira & Soares 1996), whereas the burrowing crab feeds on animal and vegetal detritus associated with the sediment (Barutot et al. 2011). Thus, both the stable isotope and the stomach content data indicated that these species are primary consumers.

Importance of basal food sources to consumers

The stable isotope SIAR mixing model demonstrated that SOM, C_4 plants (e.g. widgeon grass), and, to a lesser extent, POM are the primary carbon sources for representative decapod crustaceans and fish in the Patos Lagoon estuary. Biofilm and macroalgae also made significant contributions to consumers in MC and MF, respectively, especially during the warmer seasons. Notably, C_3 marsh plants (e.g. alkali bulrush *Scirpus maritimus*) do not appear to be important carbon sources for consumers, despite their high biomass. The nutritional value of marsh plants for estuarine consumers is controversial (Fry 2006). Rodríguez-Graña et al. (2008) and Kang et al. (2007) reported that C_3 marsh plants do not represent important carbon sources for estuarine consumers, but C_3 plants could be important carbon sources in low-salinity habitats (Stribling & Cornwell 1997), in the diets of invertebrates (e.g. amphipods) (Créach et al. 1997), and in the formation of detritus (Botto et al. 2011).

According to Kendall et al. (2007), higher C:N ratios (15 to >50) indicate material derived from terrestrial vascular plants (e.g. C_3 salt marsh plants), whereas lower ratios (5 to 8) indicate material derived from algae and/or heterotrophic aquatic microorganisms that colonize particulate matter or otherwise contribute to POM. Such differences arise from the absence of cellulose in algae and its abundance in vascular plants, as well as the consequent relative richness of proteinaceous material in algae, and these differences are largely preserved in SOM. We found that the average C:N values for POM (6.4:1) and SOM (8.5:1) were much lower compared with those observed for C_3 plants (18:1 to 69:1), which suggests that these vascular plants apparently do not contribute significantly to the formation of detritus.



Rather, our observed C:N values for POM and SOM are more similar to those observed for biofilm (8.9:1), widgeon grass (11.3:1), and macroalgae (12.6:1). Microalgae, such as the benthic diatom *Coscinodiscus*, also have low C:N values (6.6:1, based on 2 samples) in this estuary (M. C. Claudino unpubl. data). Hence, our C:N results corroborate the hypothesis that these basal food sources contribute to the formation of estuarine detritus and have higher nutritional value for consumers in the Patos Lagoon estuary (32° S) compared with C₃ salt marsh plants.

Most of the primary production and detritus derived from marsh plants is trapped and consumed by terrestrial organisms (Gaona et al. 1996, Costa 1998b). However, the stable isotopic composition of C₃ marsh plants could be altered during the decomposition process, resulting in enriched $\delta^{13}\text{C}$ values more similar to those in consumers. In this scenario C₃ marsh plants would have indirect nutritional value for these species. To our knowledge, no experimental work has yet determined what happens to the stable isotope composition of C₃ marsh plants during their decomposition. Studies on C₄ marsh plants (*Spartina alterniflora*; Currin et al. 1995) and freshwater macrophytes (Fellerhoff et al. 2003) have not reported substantial changes. Further studies are needed to evaluate whether the stable isotopic ratios of the consumers reflect those of the microorganisms on the decomposing plants rather than those of the plants

Fig. 4. Carbon and nitrogen stable isotope biplots (mean \pm SD) for basal food sources (squares) and consumers (circles) collected in the marsh creek (MC, continuous line for sources and filled circles for consumers) and in the mudflat (MF, dashed line for sources and open circles for consumers) of the Patos Lagoon estuary across seasons (autumn, winter, spring, and summer). Fish species codes: ATHBRA, *Atherinella brasiliensis*; JENMUL, *Jenynsia multidentata*; MUGLIZ, *Mugil liza*. Decapod crustaceans species codes: CALSAP, *Callinectes sapidus*; FARPAU, *Farfantepenaeus paulensis*; NEOGRA, *Neohelice granulata*. MACROA: macroalgae; POM: particulate organic matter; SOM: sediment organic matter

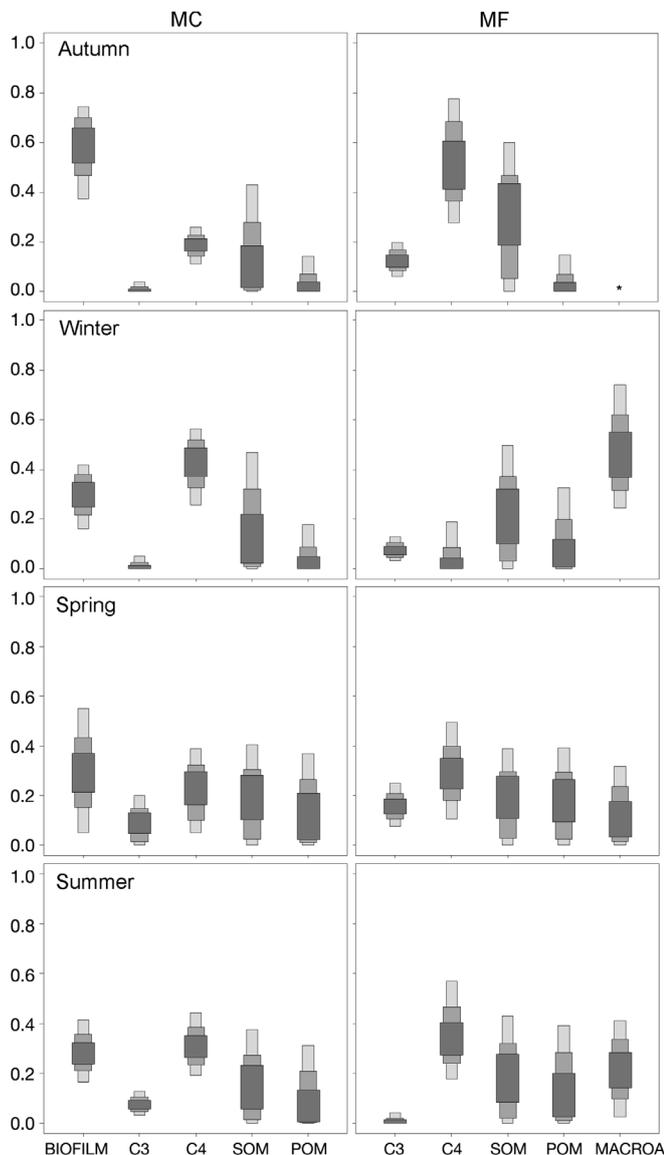


Fig. 5. Results of the stable isotope analysis in R showing estimated contribution (with 95, 75, and 50% credibility intervals) of basal sources to consumers collected in the marsh creek (MC) and mudflat (MF)

themselves, because consumers may select for fungi and bacteria when feeding on detritus.

In conclusion, our findings corroborate the hypothesis that the food webs of the studied subtropical ecosystems are spatially structured and supported by basal sources and detritus of different origins, which can be seasonally important for consumers in each particular habitat. More specifically, the primary food sources for representative decapod crustaceans and fish in this subtropical estuary appeared to be SOM, C₄ plants (e.g. widgeon grass), and, to a lesser

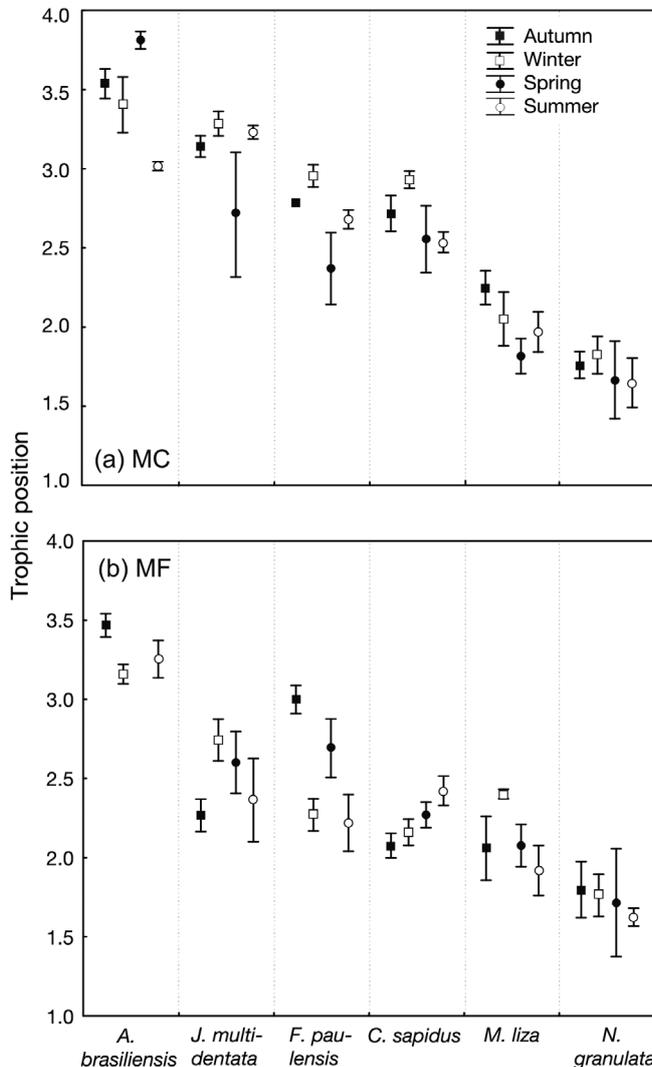


Fig. 6. Trophic levels (mean ± SE) of decapod crustaceans (*Callinectes sapidus*, *Farfantepenaeus paulensis*, and *Neohelice granulata*) and fish (*Atherinella brasiliensis*, *Jenynsia multidentata*, and *Mugil liza*) collected in the marsh creek (MC) and in the mudflat (MF) of the Patos Lagoon estuary

extent, POM. Biofilm and macroalgae can also make a significant contribution to consumers in the marsh creek and mudflat, respectively, especially during warmer seasons. We observed enriched $\delta^{13}\text{C}$ values in the mudflat because of the predominance of ^{13}C -enriched sources such as the C₄ plants, which were found year-round, and the seasonal macroalgae. The $\delta^{15}\text{N}$ ratio also differed among habitats but showed an opposite pattern with enriched values in the marsh creek, which could be the result of anthropogenic and/or natural phenomena. Notably, despite the high abundance of C₃ marsh plants in the estuary, they appeared to have little importance as a

Table 3. Food categories and frequency of occurrence (%F) of consumers

Taxon	%F	Taxon	%F
<i>Callinectes sapidus</i> ^a			
n = 997			
Detritus	80.6	<i>Atherinella brasiliensis</i> ^d	
Bivalve	67.6	n = 151	
Crustacea	45.2	Tanaidacea	47.0
Plant detritus	43.1	Insecta	28.3
Sand	32.8	Polychaeta	25.6
Annelida	32.7	Organic matter	24.8
Gastropoda	24.2	Crustacea	20.9
Tanaidacea	16.5	Gastropoda	9.1
Decapods	16.4	Plant detritus	8.4
Fish	6.5	Fish	7.7
Insecta	4.3	<i>Jenynsia multidentata</i> ^d	
n = 183			
<i>Farfantepenaeus paulensis</i> ^b			
n = 997			
Detritus	100.0	Tanaidacea	41.1
Sand	97.7	Polychaeta	28.5
Tanaidacea	95.5	Plant detritus	26.6
Polychaeta	64.4	Crustacea	19.7
Ostracoda	46.7	Organic matter	18.4
Foraminifera	33.3	Insecta	15.4
Organic matter	20.0	Fish	7.1
Nematoda	17.7	Decapods	6.0
Plant	4.4	<i>Mugil liza</i> ^e	
n = 15			
<i>Neohelice granulata</i> ^c			
n = 236			
Sediment	50.0	Diatom	59.5
Plant	50.0	Sand	39.6
Crustacea	50.0	Cyanophyceae	0.8
Plant detritus	50.0	Dinoflagellate	0.1
Animal detritus	50.0	^a Oliveira et al. (2006)	
Organic matter	37.5	^b Soares et al. (2005)	
Molluscs	12.5	^c Barutot et al. (2011)	
		^d This study	
		^e Vieira (1985)	

basal food source for the studied consumers in the studied food web. Further investigations of the stable isotopic variability of C₃ salt marsh plants during their decomposition using laboratory and field experiments as well as a triad isotopic approach (C, N, S) are needed to evaluate the real contribution of C₃ marsh plants to estuarine consumers.

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