Transport of marine-derived nutrients to subtropical freshwater food webs by juvenile mullets: a case study in southern Brazil

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ABSTRACT: In higher latitudes of the northern hemisphere, anadromous fishes can act as biological vectors, moving marine-derived nutrients into freshwater ecosystems during their reproductive migration. Much less information is available on the potential role of marine and estuarine-related fishes as biological vectors in subtropical latitudes. Here, we investigated whether mullet juveniles might transport marine nutrients into the freshwater food webs of coastal streams in southern Brazil (32°17'S, 52°15'W). To test this hypothesis, we analyzed the stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) in 10 basal production sources and 10 consumer species, including juvenile mullets, at 3 sites (surf zone, lower stream reach, headwater reach) along a marine–freshwater gradient. Our results revealed a significant decreasing trend in δ^{15}N and δ^{13}C values from the sea to the freshwater sites, suggesting an importation and incorporation of marine-derived nutrients into the coastal stream. A mixing model showed a marked shift in assimilation from marine to freshwater production sources by mullet *Mugil liza* juveniles as they moved from the surf zone into the coastal stream (lower stream and headwater reaches). Our mixing model also indicated that material of marine origin is assimilated by freshwater predators, particularly those found at the lower stream reach. Further experimental and field work is needed to evaluate the implications of marine-derived carbon and nitrogen transport by juvenile mullets for the structure and dynamics of food webs in coastal streams.

KEY WORDS: Brazil · Food web spatial subsidies · Freshwater piscivore · *Mugil liza* · Sea–land connectivity · SIAR mixing models

INTRODUCTION

Understanding the factors that influence the exchange of matter and energy between ecosystems is one of the central themes in landscape ecology (Risser et al. 1983, Polis et al. 2004). The magnitude and frequency of energy flows between ecosystems depend on a number of factors (Turner 1989, Dunning et al. 1992), but the dynamics of many ecosystems are known to be strongly influenced by the inflow of organisms and matter (nutrients and detritus) from outside their boundaries (Carpenter et al. 1998). In this context, animals that move between ecosystems, located adjacent to each other or far apart, may play key roles in the transport of nutrients (Vanni 2002). One of the most well-known examples of how animals can carry nutrients for long distances involves the upstream reproductive migration of

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anadromous fish (Naiman et al. 2002, Walters et al. 2009). As demonstrated by Kline et al. (1990), nutrients released as a result of the movement of Pacific salmon from the marine environment where they grow, to the river headwaters where they reproduce and usually die, may be responsible for up to 100% of the allochthonous production in Alaskan rivers. Anadromous fishes (e.g. alewife or Atlantic salmon) that do not die immediately after spawning also contribute substantial amounts of marine-derived nutrients (e.g. nitrogen) into freshwater ecosystems via excretion (Post & Walters 2009). Much less evidence, however, is available on the potential role of marine estuarine-dependent fish (Elliott et al. 2007) as biological vectors in subtropical latitudes. These fish enter into estuarine shallow water habitats at high abundances, most likely seeking protection against predators or feeding grounds (Garcia et al. 2012).

One technique for investigating the exchange of materials and energy between ecosystems and food webs involves the use of stable isotopes (Schindler & Lubetkin 2004). The ratios between heavier and lighter isotopes (e.g. $^{13}C:^{12}C$ or $^{15}N:^{14}N$) of elements can be used to identify sources of organic matter for primary producers and to provide information on how matter passes through consumers in the food web (Peterson & Fry 1987). This technique has been successfully used in investigations of nutrient exchanges between ecosystems, particularly between marine, estuarine and freshwater environments (e.g. Garcia et al. 2007, Walters et al. 2009, Winemiller et al. 2011).

A remarkable ecological feature of the southernmost state of Brazil is the presence of coastal streams or washouts (locally known as 'sangradouros') that physically interconnect the marine environment with freshwater wetlands located behind the foredune ridge. These streams pass through the coastal foredunes towards the marine surf zone and serve as an important drainage system for the wetlands located behind the dune fields (Figueiredo & Calliari 2006). The number and distribution of coastal streams along the Rio Grande do Sul (RS) coastline (~600 km long) vary throughout the year, being much more common in the winter, when it is possible to observe 9 streams for each 10 km of coastline, compared to ~3 streams in 10 km during summer months (Pereira da Silva 1998). This pattern is associated with the higher precipitation and low evaporation rate (Krusche et al. 2003) that increase water levels in the wetlands during the winter. Water accumulation in these freshwater systems favors the appearance of coastal streams with ephemeral and intermittent connections with the sea (Figueiredo & Calliari 2006). Despite this variability, some coastal streams along the RS coastline have permanent connections with the sea (Figueiredo & Calliari 2006).

Mullets *Mugil liza* are highly prized in southern Brazil by artisanal and industrial fisheries (Reis & D'Incao 2000), and the entrance of large numbers of juvenile mullets into the estuarine regions of southern Brazil has been well documented (e.g. Ramos & Vieira 2001, Hoeinghaus et al. 2011). This species spawns offshore along the southern Brazilian coast, and their recruits (<50 mm) use net upstream circulation at the bottom of the water column of the estuary's mouth for transport into estuaries. At Patos Lagoon estuary (32°S), one of the largest estuarine regions in southern Brazil (971 km^2), mullet recruits remain in the shallow waters of the estuary until reaching maturity, after which (April to May) they join the adult mullet population and start a reproductive migration towards offshore spawning grounds (Vieira 1991, Vieira & Scalabrin 1991). *Mugil liza* juveniles are common year-round both inside Patos Lagoon estuary and along the surf zone in the marine coastal adjacent area (Vieira 1991, Garcia et al. 2001).

In addition to entering major estuaries, large schools of juvenile mullets are found along the entire course of permanent coastal streams, where they constitute one of the dominant species in the fish assemblages of these systems (Bastos et al. in press). However, no information is available about the ecological importance of the large numbers of mullets entering into these coastal streams. Due to the high abundance of marine estuarine-dependent fish in these environments, we hypothesized that they promote the transport of marine-derived nutrients from the surf zone to freshwater wetland ecosystems. To evaluate this hypothesis, we investigated the stable isotope ratios of carbon and nitrogen in basal production sources and macroconsumers along one of these coastal streams, extending from the marine surf zone adjacent to its mouth towards its upstream reaches.

MATERIALS AND METHODS

Study area

This study was conducted in the southern coastal plain of RS in southern Brazil (Fig. 1A), which primarily consists of extensive sandy beaches and dune fields. We established 3 sampling sites along a coastal stream (32° 17′ S, 52° 15′ W) with a permanent connection with the sea: (1) the surf zone adjacent to the stream, hereafter referred to as the surf zone; (2)
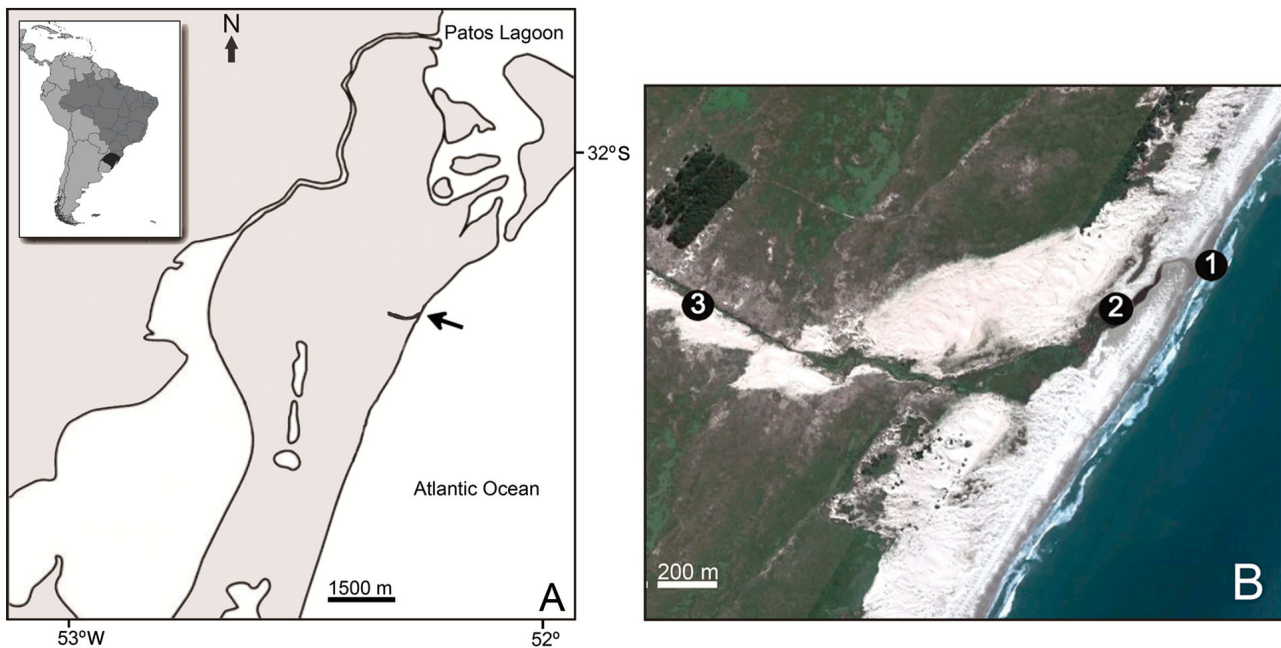
Fig. 1. (A) The coastal stream (arrow) in southern Brazil, and (B) the 3 sampling sites along the marine–freshwater gradient: (1) surf zone, (2) lower stream reach and (3) headwater reach. White areas: dunes

a location near the stream mouth where the stream creates a pool that receives the direct influence of marine spray and occasional saline water intrusion during storm surges, hereafter referred to as the lower stream reach; and (3) an upstream location near the freshwater wetlands behind the dune fields, hereafter referred to as headwater reach (Fig. 1B).

Food web sampling

Food web components were sampled in the austral fall between 22 March and 08 April 2011. At each sampling site, we collected the most abundant and representative components of the food web. Macrophytes were collected by hand and scissors. Suspended particulate organic matter (POM) samples were obtained by filtering water from each sampling site into a pre-combusted glass-fiber filter (porosity: 1.2 µm). Samples of particulate organic matter in the sediment (SOM) were obtained by removing the top 2 cm surface of the sediment using a plastic core and a knife. Fish and decapod crustaceans were sampled with beach seine hauls, dip nets and gill nets. All field samples were placed in labeled Ziplock plastic bags, immediately placed on ice and then frozen upon arrival in the laboratory.

Stable isotope sample processing

Stable isotope samples were processed following Garcia et al. (2007). Briefly, all plant and animal muscle samples were dried in an oven at 60°C for at least 48 h. Dried samples were ground to a fine powder with a mortar and pestle, stored in clean vials and sent to the Analytical Chemistry Laboratory of the Institute of Ecology, University of Georgia, for analysis of carbon and nitrogen stable isotope ratios. The results were expressed in delta notation (parts per thousand deviation from a standard material): $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$, where $R = {}^{13}C{:}^{12}C$ or ${}^{15}N{:}^{14}N$ ratios. Both carbon and nitrogen stable isotope ratios were analyzed for each sample, and their values were determined relative to standards of PeeDee Belemnite and atmospheric nitrogen, respectively.

Stomach contents analyses

In order to investigate potential aquatic predators inside the studied coastal stream who might have been feeding on juvenile mullets, we analyzed the stomach content of 3 carnivorous fish inhabiting the lower and headwater stream reaches: the thraira

Hoplias malabaricus, the pike characin *Oligosarcus jenynsii*, and the pike cichlid *Crenicichla lepidota*. We chose these species based on studies carried out in other ecosystems where they were shown to be usually carnivorous, feeding upon insects and crustaceans (Gurgel et al. 1998, Silveira et al. 2011), and in some cases, with tendency to piscivory (Winemiller 1989, Hartz et al. 1996, Corrêa et al. 2012). These species were caught monthly from December 2010 to March 2011 at the lower and headwater reaches of the studied coastal streams using multiple fishing gears: beach seine (9 × 2.4 m long and high, mesh size: 13 mm wings, 5 mm center and bag), beam trawl (mouth of 1 × 1 m, size mesh: 5 mm) and dip net (diameter: 39 cm, mesh size: 5 mm). The monthly sampling effort was comprised of 3 beach seine hauls, 3 beam trawl hauls and dip net fishing for 30 min (Bastos et al. in press).

In laboratory, each individual was weighed (*W*, in g) and measured (total length, TL, in mm) and, after being surgically dissected, had their stomach contents analyzed. Food items found in the stomachs were identified to the lowest possible taxonomic level using a stereoscopic microscope. Food resources found in non-empty stomachs had their frequency of occurrence (%*F*) calculated as the percentage of the total number of stomachs in which a particular food item was found (Hyslop 1980).

Data analysis

Spatial patterns in the carbon and nitrogen stable isotope ratios of basal production sources and consumers at the surf zone, lower stream reach and headwater reach sites were primarily investigated using biplots (Fry 2006). Sources of organic carbon assimilated by consumers were indicated by the relative positions of taxa on the *x*-axis (δ^{13}C values), whereas trophic level was indicated by the relative position on the *y*-axis (δ^{15}N) (Fry 2006). Possible spatial differences in the δ^{13}C and δ^{15}N values of SOM, macrophytes and fish between the lower stream reach and headwater reach sites were evaluated using the non-parametric Mann-Whitney *U* test. In the case of POM, the analysis was done using the non-parametric Kruskal-Wallis test (Zar 1999); For both statistical tests, we used a 5% level of significance.

To estimate the relative contributions of marine and freshwater basal production sources to the consumers occurring within the coastal stream, we employed the Bayesian stable isotope mixing model in R (SIAR, version 3.0.1) (Parnell et al. 2010, R

Development Core Team 2012). Based on the protocol proposed by Phillips et al. (2005), suggesting *a posteriori* pooling of basal production sources with similar isotopic composition as a measure to achieve higher resolution in mixing models, we considered the average value of POM collected in the surf zone as the marine basal production source and the average values of POM, SOM and macrophytes collected at each site of the coastal stream as the freshwater basal production source. We also employed the mixing model to analyze the relative contributions of basal production sources to representative fish found in the coastal stream, which were pooled into 3 groups: (1) migratory prey fish, represented by juvenile mullet *Mugil liza*; (2) non-migratory prey fish, represented by characid *Astyanax eigenmanniorum*, cichlid *Australoheros facetus* and cyprinodontifoms *Cnesterodon decemmaculatus* and *Jenynsia multidentata* species; and (3) predatory fish, represented by thraira *Hoplias malabaricus*, pike characin *Oligosarcus jenynsii* and pike cichlid *Crenicichla lepidota*, which are among the main top predators in the studied coastal stream (Bastos et al. in press). In order to correct for the trophic enrichment factors of consumers in the mixing models, we calculated their trophic position (TP) using the following equation: TP $= \lambda + (\delta^{15}N_{predator} - \delta^{15}N_{producers})/F$, where λ is the trophic level in the food web, $\delta^{15}N_{predator}$ is the average δ nitrogen value of the consumer being evaluated, $\delta^{15}N_{producers}$ is the average δ nitrogen value of the producers and *F* is the per-trophic-level fractionation of nitrogen. We used the *F* value (2.54 ± 0.11) as suggested by Vanderklift & Ponsard (2003), and in this case, $\lambda = 1$ because producers are the first trophic level.

RESULTS

A total of 28 samples of 10 basal production sources (POM = 5, SOM = 4, macrophytes = 19) and 50 samples of 10 consumer species (crab = 1, fish = 9) were collected at 3 sampling sites (surf zone, lower stream reach and headwater reach), and their δ^{13}C and δ^{15}N were analyzed (Table 1). Stable isotope ratios could not be measured for SOM samples collected at the surf zone due to the low content of organic material in the sediment. Macrophyte δ^{13}C values ranged from −19.33 to −32.02‰, with no significant differences between samples collected at the lower stream reach and headwater reach sites (*U* = 17.0, p = 0.63). In contrast, the macrophyte δ^{15}N values were significantly higher (*U* = 5.0, p = 0.02) at the lower stream reach

Table 1. Sample number (n), total length range (TL), mean values (±1 SD) of carbon and nitrogen isotope ratios and C:N ratios of consumers and basal sources collected at each sample site along the coastal stream gradient. POM: particulate organic matter; SOM: sediment organic matter

	Surf zone					Lower stream reach					Headwater reach				
	n	TL (mm)	$\delta^{15}N$	$\delta^{13}C$	C:N	n	TL (mm)	$\delta^{15}N$	$\delta^{13}C$	C:N	n	TL (mm)	$\delta^{15}N$	$\delta^{13}C$	C:N
CONSUMERS															
Migratory prey fish															
Mugil liza	3	23–36	8.6 ± 0.3	−17.7 ± 1.3	3.4	2	25–82	8.2 ± 0.9	−20.3 ± 1.8	3.3	1	122	0.1	−23	3.1
Non-migratory prey fish															
Astyanax eigenmanniorum						3	41–45	9.4 ± 0.5	−26.2 ± 0.7	3.3					
Australoheros facetus						1	26	7.4	−19.7	3.4					
Cnesterodon decemmaculatus											1	33	5.2	−27.4	3.3
Jenynsia multidentata						3	47–68	9.8 ± 0.5	−22.3 ± 2.5	3.4	5	21–41	6.8 ± 0.3	−22.7 ± 1.1	3.3
Predatory fish															
Hoplias malabaricus						1	260	10.2	−23.7	3.1	9	78–128	7.7 ± 1.3	−22.7 ± 1.1	3.2
Crenicicla lepidota						1	40	7.7	−26	3.4	5	52–173	5.3 ± 1.5	−23.9 ± 0.8	3.2
Characidium rachovii						3	30–39	8.3 ± 0.4	−25.8 ± 0.8	3.5					
Oligosarcus jenynsii						4	113–176	11.1 ± 0.4	−23 ± 1.5	3.1	6	91–135	6.6 ± 1.1	−22.5 ± 1	3.1
Crabs															
Callinectes sapidus						2	31–101	8.2 ± 0.0	−22.9	3.2					
BASAL FOOD SOURCES															
POM	2		7.3 ± 3.7	−18.4 ± 0.2	11.9	1		1.1	−25.7	13.2	2		−1.2 ± 3.1	−27.9 ± 0.5	11.5
SOM						2		5.2 ± 0.3	−23.6 ± 0.3	8.1	2		0.3 ± 2.0	−25.6 ± 2	9.3
Macrophytes															
Schoenoplectus californicus						2		2 ± 1.6	−26.8 ± 0.3	37.1	1		−1	−27.2	40.4
Androtrichum trigynum											2		1.5 ± 0.4	−27.1 ± 0.3	45.7
Myriophyllum aquaticum						1		4.2	−32	12	1		2.2	−31.7	8.1
Ceratophyllum sp.						1		6.5	−27.9	9.5	2		−6.3 ± 2.3	−31.3 ± 0.8	8.6
Limnobium laevigatum											4		2.2 ± 1	−26.7 ± 0.5	10.1
Lemna sp.						2		11 ± 0.2	−26.3 ± 0.1	15.1	2		4.7 ± 0.3	−26.5 ± 0.0	12.2
Wolffia sp.											1		4.6	−19.3	9.5
Azolla filiculoides											1		−0.4	−27.4	8

(median = 5.31) relative to the headwater reach site (median = −0.36) (Fig. 2). POM samples showed differences in $\delta^{13}C$ (H_2 = 6.1, p = 0.01) and $\delta^{15}N$ (H_2 = 6.0, p = 0.01) values across the 3 sampling sites, with higher values at the surf zone ($\delta^{13}C$: −18.29 to −18.52‰; $\delta^{15}N$: 9.90 to 4.72‰), intermediate values at the lower stream reach ($\delta^{13}C$: −25.66‰; $\delta^{15}N$: 1.05‰) and lower values at headwater reach ($\delta^{13}C$: −28.82 to −27.62‰; $\delta^{15}N$: −1.09 to −7.42‰). SOM $\delta^{13}C$ and $\delta^{15}N$ values ranged from −23.41 to −27.31‰ and 5.43 to 0.81‰, respectively, with no significant differences in $\delta^{13}C$ (U = 0.0, p = 0.20) or $\delta^{15}N$ (U = 1.0, p = 0.40) values between the headwater reach and lower stream reach sites (Fig. 2).

Although the low sample size precluded statistical test, it was possible to observe that migratory fish (*Mugil liza*), showed an apparent upstream depletion in $\delta^{13}C$ and $\delta^{15}N$ values. *Mugil liza* had higher values at the surf zone ($\delta^{13}C$: −16.19 to −8.50‰; $\delta^{15}N$: 8.87 to 8.30‰), intermediate values at the lower stream reach ($\delta^{13}C$: −18.52 to −22.01‰; $\delta^{15}N$: 9.07 to 7.30‰) and a lower value at the headwater reach ($\delta^{13}C$: −22.01‰; $\delta^{15}N$: 0.09‰) (Fig. 2). $\delta^{13}C$ values of non-migratory prey fish ranged from −19.68 to −27.35‰, with no significant differences between the lower stream reach and headwater reach sites (U = 22.0, p = 0.55). In contrast, their $\delta^{15}N$ values were significantly higher (U = 60.0, p = 0.001) at the lower stream reach (median = 8.94) relative to the headwater reach site (mean = 6.65) (Fig. 2). Similar to most basal production sources and consumers, predatory fishes showed significant differences in $\delta^{15}N$ (U = 4.0, p < 0.001), with higher values at the lower stream reach ($\delta^{15}N$: 11.54 to 7.70‰) and lower values at the headwater reach (9.17 to 3.51‰). On the other hand, predatory fish $\delta^{13}C$ values ranged from −20.69 to −24.79‰, with no significant differences between the lower stream reach and headwater reach (U = 76.0, p = 0.35). Crabs were sampled
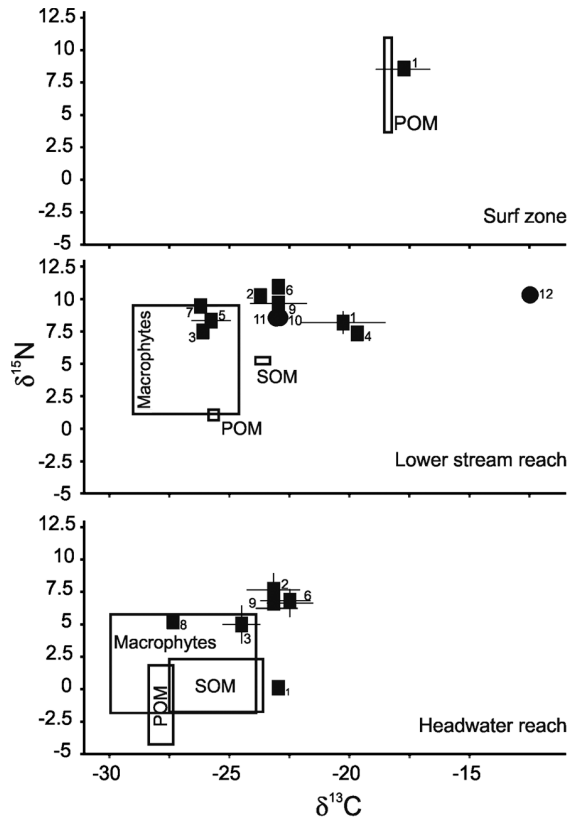
Fig. 2. Mean (±SD) carbon (δ^{13}C) and nitrogen (δ^{13}N) stable isotope values of consumers and basal resources at 3 locations along the coastal stream gradient (surf zone, lower stream reach and headwater reach). Basal resources (open boxes): particulate (POM) and sediment (SOM) organic matter and macrophytes. Consumer species: fish (■) and crabs (●). Species codes: 1: *Mugil liza*, 2: *Hoplias malabaricus*, 3: *Crenicichla lepidota*, 4: *Australoheros facetus*, 5: *Characidium rachovii*, 6: *Oligosarcus jenynsii*, 7: *Astyanax eigenmanniorum*, 8: *Cnesterodon decemmaculatus*, 9: *Jenynsia multidentata*, and 10,11,12: *Callinectes sapidus*

only at the lower stream reach site, precluding any statistical test due to the lack of spatial replication, with 2 individuals showing similar low values of δ^{13}C (−22.7 and −23.0‰) and δ^{15}N (8.2 and 8.2‰), and one individual with higher values of δ^{13}C (−12.5 ‰) and δ^{15}N (10.1‰) (Fig. 2).

The mixing model suggested a steadily decreasing trend in the contribution of marine production sources to juvenile mullets from the sea to the coastal stream: 0.83 to 1 (95% credible interval) at the surf zone, 0.43 to 0.83 at the lower stream reach and 0 to 0.46 at the headwater reach site (Fig. 3A). The contribution of freshwater production sources to juvenile mullets showed the opposite trend, with a marked increase from the lower stream reach (0.17 to 0.57) to the headwater reach (0.54 to 1). Although to a lesser extent, marine production sources also contributed

nutrients to freshwater non-migrant prey fish at the lower stream reach (0.11 to 0.50) and headwater reach (0.38 to 0.65) (Fig. 3B). The same contribution of marine production sources was observed for the predatory fish, particularly in the lower stream reach (0.19 to 0.50) relative to the headwater reach (0.11 to 0.36) (Fig. 3C)

The stomach content analyses of 94 freshwater carnivorous fish in the coastal stream (thraira: 22, pike characin: 36 and pike cichlid: 50) revealed a diet comprised of 21 food items of both animal and plant origins. As predicted, juvenile mullets constituted a conspicuous food item in the diet of these predators at both sites inside the coastal stream, particularly in the lower stream reach (%F = 31.58) (Fig. 4). The average size (TL) of the juvenile mullets found within the stomach contents, which were not too digested and could be measured, was 26.4 (SD = ±11.1, n = 18) and 25.8 mm (±3.8, 6) at the lower stream and headwater reaches, respectively.

DISCUSSION

The δ^{13}C values of the basal production sources at the 3 sampling sites showed a gradual decrease from the surf zone towards the freshwater sites in the coastal stream. These findings could be explained by differences in the isotopic composition of marine phytoplankton, which usually have average δ^{13}C values of −20‰, in contrast to the organic matter derived from sources with lower δ^{13}C values, such as plants that use the C$_3$ photosynthetic pathway, which exhibit values from −29.38 to −23.91‰ (Deegan & Garritt 1997, Garcia et al. 2007, Marshall et al. 2007). Accordingly, the main basal production source in the surf zone is marine phytoplankton (Brown & McLachlan 1990, Odebrecht et al. 2010), which seems to explain the average δ^{13}C value of −18.40‰ we found for POM collected in this area. In contrast, all primary producers and basal production sources collected within the stream had lower average δ^{13}C values (macrophytes: −27.11‰, POM: −28.45‰, SOM: −25.56‰). Therefore, the differences in the availability of primary producers at the marine versus freshwater sites, associated with the contrasting isotopic composition of marine phytoplankton and freshwater macrophytes, may explain the differences we observed between the δ^{13}C values of organisms collected in the surf zone and the coastal stream.

However, the mechanisms described above cannot explain the significant differences found in the average isotopic values of many components of the food
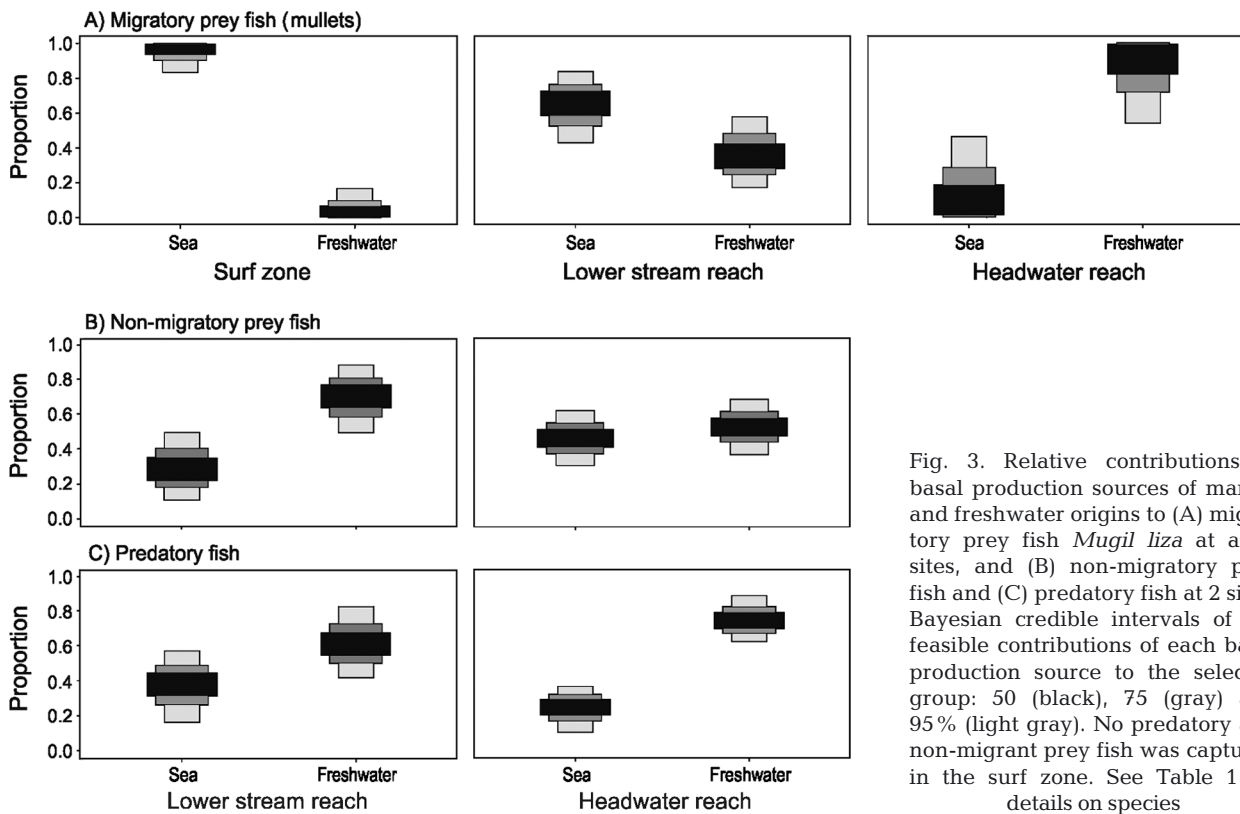
Fig. 3. Relative contributions of basal production sources of marine and freshwater origins to (A) migratory prey fish *Mugil liza* at all 3 sites, and (B) non-migratory prey fish and (C) predatory fish at 2 sites. Bayesian credible intervals of the feasible contributions of each basal production source to the selected group: 50 (black), 75 (gray) and 95% (light gray). No predatory and non-migrant prey fish was captured in the surf zone. See Table 1 for details on species

web at the 2 freshwater stream sites. The downstream and upstream sites of the studied coastal stream had similar compositions of primary producers (i.e. C_3 macrophytes were dominant at both sites), and both experienced low salinity (near zero) throughout the year (Bastos et al. in press), which possibly excludes the presence of marine phytoplankton. However, they showed significant differences in
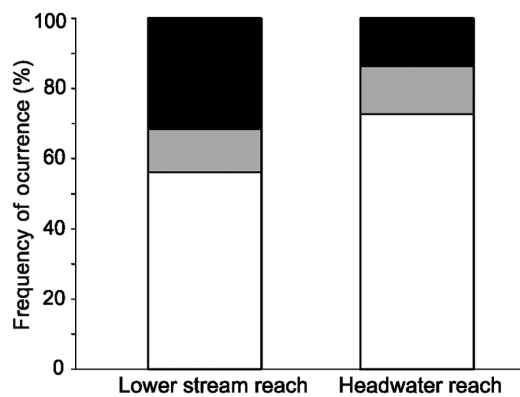

Fig. 4. Frequency of occurrence of juvenile mullets (black), insects (gray) and others (white) in the stomach contents of predatory fish

average $\delta^{13}C$ values for most components of the food web. A possible explanation for this finding could be related to the effects of water velocity and the boundary layer phenomenon on the fractionation of the $^{13}C{:}^{12}C$ ratio during their uptake by primary producers (Fry 2006). The lower stream reach is approximately two-fold larger and has lower current velocities than the headwater reach (3200 versus 1800 m^2) (Bastos et al. in press). Slower waters can create a thicker diffusive layer around the primary producers (e.g. algae and submerged macrophytes) and thereby inhibit the diffusion of CO_2, causing a greater build-up of $^{13}CO_2$ (Michener & Lajtha 2007). This effect results in higher $\delta^{13}C$ values in primary producers and consumers (Finlay et al. 2002). This mechanism could therefore explain the higher $\delta^{13}C$ values found for consumers and submerged macrophytes (*Ceratophyllum* sp.) at the lower stream reach.

The studied coastal stream also revealed a downstream enrichment in $\delta^{15}N$ values. In some cases, spatial differences in nitrogen ratios in continental systems can be attributed to anthropogenic nitrogen sources (e.g. domestic wastewater), which can lead to the $\delta^{15}N$ enrichment of the biota (McClelland et al. 1997, McClelland & Valiela 1998, Steffy & Kilham

2004). However, we believe it is unlikely that anthropogenic loading of enriched $\delta^{15}N$ is driving the among-sites differences in $\delta^{15}N$ in our studied coastal stream because it is located in a relatively well-preserved and uninhabited area. In fact, this coastal stream has significantly lower values of total nitrogen (0.63 mg l^{-1}) compared with other coastal streams in the same coastal plain that receive domestic sewage loads from resort beaches like Hermenegildo (1.18 mg l^{-1}) and Cassino (4.06 mg l^{-1}) (R. F. Bastos unpubl. data).

A more feasible hypothesis to explain the downstream enrichment in $\delta^{15}N$ values is that the food web of the coastal stream is incorporating marine-derived nitrogen, as marine organic matter and marine organisms often have higher $\delta^{15}N$ values than freshwater ecosystems (Bilby et al. 1996, Hicks et al. 2005). Anadromous fish are effective transporters of nutrients from the ocean to adjacent freshwater bodies, where they may provide energy subsidies to the freshwater food web both directly (compounding the diet of piscivores) or indirectly (via excretions and carcasses) (Browder & Garman 1994, Walters et al. 2009). For instance, experimental work conducted by Post & Walters (2009) revealed that the anadromous alewife *Alosa pseudoharengus* excreted an average of 24.71 µg N g^{-1} of wet fish mass per hour during their spring spawning. The authors concluded that migrating fish that aggregate in high densities within small streams can have substantial effects on local food webs. A similar role seems to be played by juvenile mullets, which actively move in high numbers from the surf zone into estuaries and coastal streams. Our findings suggest that juvenile mullets at the lower stream reach partially conserve their marine carbon and nitrogen stable isotope signatures, but the fish captured further upstream switched their stable isotope composition to a more freshwater profile. This shift from marine-derived to freshwater-derived material incorporated into muscle tissues of juvenile mullets strongly suggests that this species might act as a biotic vector, carrying marine-derived nutrients into freshwater areas of coastal streams.

This hypothesis is supported by the fact that these marine estuarine-dependent mullets are abundant and frequent year-round in the adjacent marine area (Rodrigues & Vieira 2012) and are the only marine-related fish species entering these coastal streams at high abundances (Bastos et al. in press). Other evidence supporting this biotic vector hypothesis was the result of our mixing model showing a steadily decreasing trend in the contribution of marine production sources to juvenile mullets from the sea to the coastal stream, and the opposite trend in the contribution of freshwater production sources, which had a marked increase from the lower stream reach to the headwater reach. These trends strongly suggest that marine-born juvenile mullets enter and stay inside the coastal stream for a sufficiently long time to reflect a new diet based on freshwater production sources. This suggests that juvenile mullets had enough time to feed and grow inside the coastal stream in order to effectively transfer the marine-derived material in their tissues into the freshwater food web of the coastal stream, either by direct predation or indirectly by carcass deposition or excretion. This hypothesis seems to be corroborated by 2 factors—the isotope turnover time in fish muscle tissues needed in order to reflect a new diet varies from a few weeks to several months (Hesslein et al. 1993, Herzka & Holt 2000), and juvenile mullets are significantly larger (average TL: 33.3 mm) inside these coastal streams than in the adjacent surf zone (28.7 mm) (Bastos et al. in press).

The stomach contents analyses demonstrated the direct predation on juvenile mullets with average size of 26.3 mm by large body-size (TL > 150 mm) freshwater predatory fish like thraira, pike characin and pike cichlid, especially in the stream site closest to the sea. This finding seems to corroborate the mixing model results showing higher contribution of marine production sources to the predatory fish in the lower stream reach. The mixing model also showed that marine-derived material was also incorporated into muscle tissues of small body-size (TL < 50 mm) non-migratory freshwater fish like characins, cichlids and cyprinodontiforms. Considering these small prey fish are not big enough to feed upon juvenile mullets like the larger freshwater predatory fish, we hypothesized these non-migratory freshwater fish assimilated marine sources through an indirect transport of marine-derived nutrients via carcass deposition or excretion by juvenile mullets that entered the coastal stream. Therefore, marine-derived nutrients transported by juvenile mullets could be incorporated both into intermediate (small prey fish) and high trophic levels (large predatory fish) of the freshwater food web of the coastal stream.

Alternatively, it could be argued that such transport of marine-derived nutrients into the coastal stream could result not from animal movements, but rather from salinity intrusions into the coastal stream during storm surge events, which could carry marine nutrients and phytoplankton into this system. Previous work in a large nearby estuary (Patos Lagoon) has shown, for instance, that salinity intrusions can

carry nutrient-rich waters that influence estuarine phytoplankton (Abreu et al. 1995). However, we believe this mechanism plays a minor role in small coastal streams, because saltwater inflow into these streams during storm surge events produces ephemeral salinity gradients. For instance, Bastos et al. (in press) conducted monthly samplings for 1 yr at lower stream reach and headwater reach sites of 3 coastal streams (including the stream studied herein) but did not find salinity levels higher than 2. Moreover, an investigation of one storm surge event at the coastal stream studied here revealed that the salinity gradient formed due to the salinity intrusion disappeared soon after the event, and the stream rapidly reverted to freshwater conditions (M. C. L. M. Oliveira unpubl. data).

In summary, despite the lower number of samples and lack of spatial replication of some food web components, our study reveals that the marine surf zone and coastal streams are not independent ecosystems along the coastline of southern Brazil. Food webs of these habitats are connected through the movement of abundant marine-born juvenile mullets, which enter these coastal streams from the adjacent surf zone. Our study provides evidence that juvenile mullets may transport marine-derived nutrients that incorporate into intermediate and high trophic levels of the freshwater food web. Further studies are needed to (1) produce a more detailed description of the freshwater food webs of coastal streams, including analyses of stomach contents and stable isotope ratios of major components; (2) investigate movement patterns and residence time of mullets along this marine–freshwater gradient using, for instance, otolith microchemical analysis and/or mark-recapture techniques; (3) investigate additional coastal streams, including some with ephemeral and intermittent connections to the sea (Figueiredo & Calliari 2006); (4) to evaluate experimentally how excretions and carcasses of mullets affect the POM (e.g. in the sediment) within the coastal stream; and (5) experimental studies including stable isotopic field surveys in several coastal streams where the passage of marine juvenile mullets would be allowed in some streams and blocked in others, coupled with a concomitant monitoring of limnological parameters (e.g. water quality and nutrient profiles).

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