Hydrologic pulsing promotes spatial connectivity and food web subsidies in a subtropical coastal ecosystem

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ABSTRACT: Resource pulsing is a widespread phenomenon, but its effects on ecosystem dynamics are often difficult to predict. Hydrological pulsing, in particular, is known to influence the structure and dynamics of fluvial and coastal ecosystems, but little information is available about its effects on trophic connectivity between wetlands and estuaries. We investigated the hypothesis that hydrologic pulsing drives 1-way trophic subsidies (e.g. suspended organic matter and freshwater fish) from wetland to estuary. Our study system is a coastal lagoon with an ephemeral mouth that, when closed, stores freshwater as a sustained flood pulse that is subsequently released when a connection with the sea is reestablished. We monitored isotopic composition of consumers and food sources over the course of an entire flood pulse to infer trophic linkages and spatial subsidies. Before the flood peak (April and May), freshwater and estuarine zones were largely dependent on local primary production sources (seston and C_3 plants vs. C_4 plants and microphytobenthos, respectively), essentially functioning as disconnected compartments. A sustained pulse of freshwater inflow (June to August) induced greater habitat connectivity and a net flow of biomass and energy from the freshwater zone into the estuarine zone. The opening of the lagoon outlet channel abruptly terminated the flood pulse and reduced freshwater subsidies to estuarine consumers, and both zones returned to dependence on autochthonous production. Our findings contribute to current concerns that artificial opening of sandbars in coastal lagoons alters natural ecological dynamics with significant effects on biodiversity and ecosystem processes.

KEY WORDS: Basal resource · Bayesian mixing model · Biomass assimilation · Estuary · Hydrologic connectivity · Production source · Salinity · Trophic ecology

INTRODUCTION

Food web dynamics are influenced not only by consumer–resource interactions, but also by environmental variation that directly affects ecosystem productivity, habitat suitability, and other factors affecting organism fitness and population growth (Winemiller & Layman 2005). Further complicating our ability to predict dynamics in response to environmental drivers is the fact that perceived patterns of variation are influenced by temporal and spatial scales of analysis (Petraitis & Latham 1999). Most, and probably all, ecosystems are subject to abiotic factors that pulse. Resource pulses, in particular, are defined as discrete, significant increases in resource availability over intervals of time and/or space (Yang et al. 2008). Envi-

ronmental seasonality affects local population dynamics and species interactions, but also can have regional effects when pulsing factors induce mass migrations or synchrony among regional populations (Liebhold et al. 2004). For example, Krenz et al. (2011) showed how coastal upwelling influenced by the El Niño Southern Oscillation affects food web subsidies within intertidal ecosystems over a large region. Resource subsidies associated with pulsing environmental factors strongly influence bottom-up (e.g. a fertilizer effect) and top-down (consumer control) effects in food webs of arid islands (Polis et al. 1997, Spiller et al. 2010).

Pulsing can induce responses that reverberate throughout the food web (Yang et al. 2008). Mass spawning by corals and reef fishes in response to lunar and oceanographic cues produces huge subsidies for planktivores (Pratchett et al. 2001), which can have long-term effects on fitness of the latter (McCormick 2003). Regional insect outbreaks in response to favorable climatic conditions can devastate plant biomass (Mattson & Addy 1975) while subsidizing insectivores (Yang 2004). Seasonal migrations can create food web subsidies at the landscape scale (Polis et al. 2004). For example, the decomposing carcasses of post-spawn anadromous salmon provide a pulse of marine-derived nutrients that stimulates primary production not only within oligotrophic streams, but also riparian terrestrial ecosystems (Naiman et al. 2002). Theoretical models indicate diverse responses to pulsing that range from transient population dynamics that may or may not influence species coexistence, to tipping the dynamics governing alternative stable states of ecosystems (Ostfeld & Keesing 2000, Holt 2008, Petersen et al. 2008).

Hydrologic changes, in particular, can trigger ecosystem pulsing that affects the structure and dynamics of fluvial and coastal ecosystems (Winemiller 1990, Poff et al. 1997, DeAngelis et al. 2005, Childers 2006, Warfe et al. 2011). Fish dynamics in hydrologically pulsed wetland ecosystems monitored over long time scales (e.g. Florida's Everglades) are strongly associated with seasonal cycles in precipitation and water depth that cascade to other components of the food web (DeAngelis et al. 1997, Childers 2006).

In southeastern Brazil, inter-annual and seasonal variation in precipitation drives changes in hydrology and salinity gradients that, in turn, affect the composition of local fish assemblages within coastal lagoons (Garcia et al. 2003, 2004). A suitable ecosystem model for evaluating hydrologic pulsing effects on spatial food web subsidies between freshwater and estuarine systems in this region is the Lagoa do

Peixe National Park (LPNP). Ecological conditions within LPNP are influenced not only by intra-annual variations in freshwater inflow, but also by ephemeral connections with the sea. Freshwater draining from the coastal plain is stored within the lagoon as a sustained flood pulse and then released over a relatively short period when a connection with the sea is established either (1) naturally by inflows or (2) artificially by mechanical excavation (Lanés et al. 2015). Thus, hydrology induces a freshwater flood pulse that causes aquatic ecosystem expansion with greater connectivity between the freshwater wetland and the estuarine zone and conditions promoting input of freshwater basal production sources and consumer taxa into the estuary.

We hypothesized that hydrologic pulsing influences food web dynamics along the longitudinal fluvial gradient via 1-way trophic subsidies (e.g. passive transport of suspended organic matter and dispersal of freshwater fish) from wetland to estuary. We used stable isotope methods to test this hypothesis based on extensive temporal and spatial sampling of production sources and consumers at locations along the longitudinal fluvial gradient, and used a Bayesian mixing model to estimate probability distributions for assimilation of alternative production sources. Stable isotope analysis (SIA) is commonly used to reconstruct trophic links connecting food web components across spatial (Hoeinghaus et al. 2011, Claudino et al. 2015) and temporal (Claudino et al. 2013, Garcia et al. 2017) scales, and several authors have used SIA to test effects of hydrologic changes on food web organization and spatial subsidies among ecosystems (Jardine et al. 2012, Abrantes et al. 2014, Kaymak et al. 2015, Ou & Winemiller 2016). In contrast with most previous studies that used seasonal or before-and-after sampling designs, our study analyzed a time series of samples taken over frequent, short intervals, which enabled us to view food web structure like a movie rather than a snapshot. This analysis provided a basis for estimating the timing and magnitude of a spatial food web subsidy to estuarine consumers.

MATERIALS AND METHODS

Study area

LPNP has an area of 344 km^2 and is situated along the coastal plain of Rio Grande do Sul, the southernmost state in Brazil. This coastal plain is characterized by a flat topography, low altitude (<20 m a.s.l.) and low tidal range (~0.5 m; Fig. 1). Lagoa do Peixe is
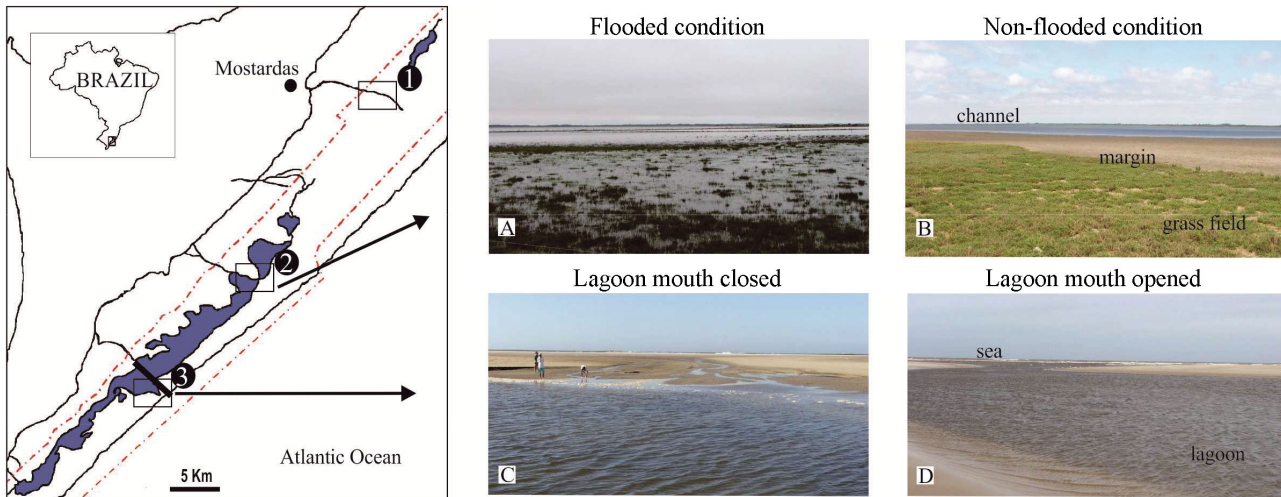
Fig. 1. Lagoa do Peixe National Park (LPNP) in the coastal plain of southern Brazil, with the location of the sampled areas (squares) at the freshwater wetland (1), at the estuarine zone (2), and at the lagoon mouth (3). Red dashed line denotes the boundaries of the national park. Photos taken at the mouth of the lagoon on 2 April and 11 November 2008 show the status of its connection with the sea (C,D) and the flooded (A) and non-flooded conditions (B) in the same location of the estuarine zone. Photos by A. M. Garcia

a shallow coastal lagoon (<50 cm, except its channel) surrounded by freshwater wetlands (Maltchik et al. 2010), except for its eastern border where sand dunes are prevalent (Fig. 1). The lagoon has a narrow outlet to the sea that is blocked from February/March to August/September each year by sand dunes until freshwater inflows, usually from winter rainfall, establish a connection. If the seasonal connection with the sea is not established naturally, earth-moving machinery is used to construct an outlet channel (200 m long, 40 m wide, 1.5 m deep) during late winter (August/September). The overall dimensions of both artificial and natural channels are similar. This periodic opening of the outlet is done to promote the entrance of marine shrimp larvae into the lagoon to favor commercial fisheries and also to drain water from the floodplain to increase pasture for livestock ranching (Lanés et al. 2015).

LPNP has a humid subtropical climate, with mean temperatures ranging from 14.6 to 22.2°C, and a mean annual temperature of 17.5°C; precipitation in the study area ranges from 1150 to 1450 mm yr^{-1}, with a mean of 1250 mm yr^{-1}. Prior studies on estuarine systems approximately 100 km south of LPNP have shown that rainfall anomalies cause hydrological changes that affect estuarine and freshwater fish assemblages (Garcia et al. 2004). To assess the potential influence of climatic variation on ecological dynamics, temperature and rainfall data were obtained from 2 meteorological stations (Rio Grande and Mostardas) near the study area (Fig. 1), and the status of the lagoon connection with the sea was moni-

tored throughout the study. Rainfall varied seasonally, with 2 periods of high rainfall: May to August 2008 and January to March 2009. The former wet period coincided with a closed lagoon mouth, which occurred from April to August 2008. At the estuarine site in particular, water flooded over the marginal areas during wet periods, increasing hydrological exchanges with freshwater wetlands. Average air temperature varied seasonally, with lowest values during the austral winter (14.1°C in June) and highest values in summer (23.7°C in February). Based on this climatic variation, we defined flooded and non-flooded phases of the hydrologic pulse in order to evaluate our initial hypothesis (Fig. 2).

Field collections and sample processing

Samples were obtained monthly from April 2008 to May 2009 (with the exception of September 2008 and March 2009) in 3 regions along the main longitudinal axis of the LPNP: (1) a freshwater wetland located near the northern limit of the park, (2) the upper–middle reach of the lagoon that encompasses an ecotone between estuarine and freshwater zones, and (3) the middle reach of the lagoon near the ephemeral connection with the sea (Fig. 1). These sites are subsequently referred to as freshwater wetland, estuarine zone, and lagoon mouth, respectively.

Fish were caught using 4 fishing gears (gillnets, beach seine, beam trawl, dip net) during each monthly survey. To catch larger fishes (>200 mm total length),
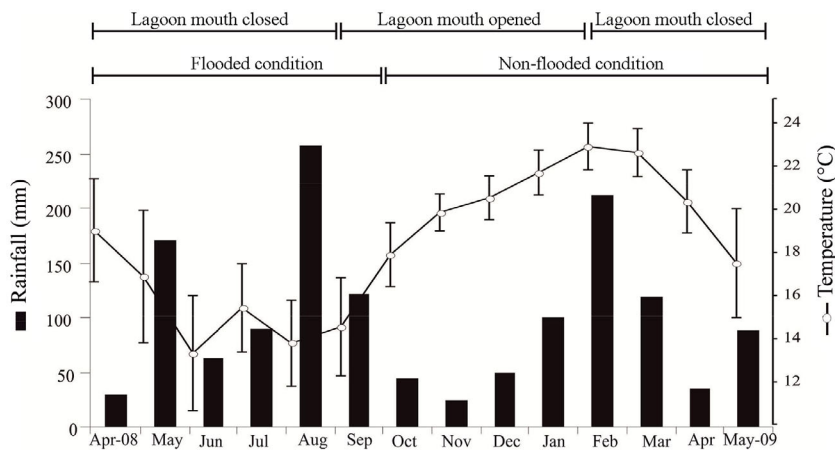
Fig. 2. Monthly variation in rainfall (mm, bars) and water temperature (°C, line) during the study period (April 2008 to May 2009)

2 gillnets (4 × 2 m, comprising panels with different mesh sizes of 15, 20, 30, and 35 mm) were deployed in the channel (2.0–2.5 m deep). One to 2 beach seine (9 m long, 2.4 m high, mesh size 13 mm wings and 5 mm center) hauls were made in unvegetated near-shore areas. Densely vegetated marginal habitats were sampled with 3 hauls of the beam trawl (mouth = 1 × 1 m, mesh = 5 mm) and dip netting for approximately 15 min. Beach seine and beam trawl hauls and dip netting were conducted in shallower waters (<1.5 m) and were effective in capturing smaller species (<50 mm), including those that take refuge in structurally complex habitats. Sampling with multiple gear types provides a more representative sample of the fish assemblage, especially when the ecosystem is composed of multiple habitat types with differing complexities and species of various sizes and behaviors (Rozas & Minello 1997, Chick et al. 2004, Garcia et al. 2006). Representative macroinvertebrates, such as adult and immature aquatic insects, gastropods, polychaete worms, shrimps, and crabs, were collected at each study site.

Samples of microphytobenthos, seston (phytoplankton and suspended fine particulate organic matter), and leaves from floating, emergent, and submerged macrophytes were collected at each site during each survey. This material was used for determination of the isotopic composition of major basal production sources. Macrophytes were collected by hand; samples of microphytobenthos were obtained by carefully removing the thin upper layer of flocculent or consolidated biofilm from substrates; and seston samples were obtained by filtering water through a pre-combusted (450°C, 4 h) Whatman glass fiber filter (GF/F) using a manual pump.

Immediately after collection, all specimens were placed on ice for transport to the laboratory where they were stored frozen. After thawing, fish were weighed (g), measured (mm total length, TL), and dissected to extract approximately 5 g of dorsal muscle tissue for isotopic analysis. For fish <50 mm TL, a composite sample was obtained by combining muscle tissue from 5 to 15 conspecifics from the same site. Gastropods, shrimps, and crabs were dissected, and a sample of muscle tissue was extracted for SIA. Due to their small sizes, polychaetes and aquatic insects were processed as whole specimens. Tissue samples from fish and macroinvertebrates were inspected, and any significant non-muscle material (e.g. bone, scales, exoskeleton) was removed before the samples were rinsed with distilled water, placed in sterile Petri dishes, and dried in an oven at 60°C until attainment of a constant weight (minimum of 48 h). Dried samples were ground to a fine powder with a mortar and pestle and stored in clean Eppendorf tubes. Sub-samples were weighed to 6–10 mg, pressed into Ultra-Pure tin capsules (Costech), and sent to the Centro de Isótopos Estáveis, Universidade Estadual Paulista (UNESP) for measurement of stable isotope ratios (^{13}C:^{12}C and ^{15}N:^{14}N). Results are reported as parts per thousand (‰) differences from a corresponding standard: $\delta^H X = [(R_{sample} / R_{standard}) - 1] \times 1000$, where X is a particular element (C, carbon; or N, nitrogen), the superscript H denotes the heavy isotope mass of the given element (^{13}C, ^{15}N), and R is the ratio of the heavy isotope to the light isotope for the element (^{13}C:^{12}C, ^{15}N:^{14}N). Standards were carbon in the PeeDee Belemnite and molecular nitrogen in air. Standard deviations of δ^{13}C and δ^{15}N replicate analyses were 0.14‰ and 0.13‰, respectively.

Data analysis

For some analyses, fishes were pooled into 5 functional guilds based on prior classification proposed by Garcia et al. (2001) and also considering Myers' (1938) model of primary- and secondary-division freshwater fishes, as follows: (1) estuarine resident species that typically occur and breed within the estuary; (2) estuarine dependents that are marine or freshwater spawning species found in large numbers within the estuary during certain periods of their life cycle; (3)

marine vagrants typically inhabiting marine habitats and rarely occurring within the estuary; (4) primary freshwater fishes with no tolerance for salinity that are confined to freshwater; and (5) secondary freshwater fishes with some salinity tolerance that may enter brackish water occasionally. Also, due to consistent differences in retention of ^{13}C by plants using the Calvin cycle (C_3) and Hatch-Slack cycle (C_4) photosynthetic pathways (Marshall et al. 2007), sampled macrophytes were pooled and analyzed as 2 groups, C_3 vs. C_4, according to the literature (Marshall et al. 2007), the range of $\delta^{13}C$ values observed in this study, and previous research in nearby coastal lagoon ecosystems (Garcia et al. 2007, Hoeinghaus et al. 2011). C_3 plants tend to have significantly lower average carbon stable isotope ratios (~ −27‰) than C_4 plants (~ −14‰), mainly due to differences in the enzymes involved in carbon fixation during photosynthesis (Marshall et al. 2007). Abundance patterns of these groups shift along the salinity gradient of the study site, with C_3 being the dominant aquatic macrophytes in the freshwater wetland and C_4 plants dominant in the estuarine/marine sites (Knak 2004, Rolon et al. 2011).

Bi-plots of $\delta^{15}N$ and $\delta^{13}C$ values of primary producers and organic sources, invertebrates, and fishes were used to evaluate patterns of isotopic variation across spatial and temporal scales. Sources of organic carbon assimilated by consumers are indicated by proximity between carbon isotope ratios ($\delta^{13}C$) of consumers and their food sources, whereas trophic position of each consumer is indicated by the relative position of their nitrogen isotope ratios ($\delta^{15}N$) in relation to an isotopic baseline (Peterson & Fry 1987). Analysis of variance (ANOVA) was used to compare average $\delta^{13}C$ and $\delta^{15}N$ values of sources and consumers among study sites and, for fishes only, among months. When a significant main effect was observed, pairwise comparisons of site means were performed using a Newman-Keuls post hoc procedure. Normality and homogeneity of variances were evaluated by the Kolmogorov-Smirnov and Cochran tests, respectively (Zar 1984).

To estimate relative contributions of basal production sources to fish assemblages of the freshwater wetland, estuarine zone, and lagoon mouth, we employed Bayesian mixing model with 4 basal production sources as end-members (seston, microphytobenthos, C_3 and C_4 plants) using data specific to each site. Mixing models were run using the Stable Isotope Analysis in R (SIAR) package in R statistical software (R Core Team 2012), which employs a Bayesian approach that incorporates uncertainties associated with sample variability and trophic enrichment on the assumption they are normally distributed (Parnell et al. 2010). Each mixing model was fit via a Markov chain Monte Carlo method that generated simulations of plausible values of dietary proportions consistent with the data using a Dirichlet prior distribution (Parnell et al. 2010). Each model was run based on 500 000 iterations, discarding the first 50 000, and considering a non-informative prior to guide the dietary proportion simulations. Upper and lower credibility intervals (95, 75, and 50%) were used to describe the range of feasible contributions for each food source to the consumer (Parnell et al. 2010).

In order to quantify food web subsidies occurring due to movement of carbon sources from the freshwater wetland into the estuarine zone, we ran 5-end-member SIAR mixing models to estimate, on a monthly basis, the relative contribution of freshwater- and estuarine-derived sources for estuarine resident fishes (silversides *Atherinella brasiliensis* and *Odonthestes argentinensis*) and individuals of primary freshwater fish species that were collected within the estuarine zone during the hydrologic pulse. Importantly, silversides are not piscivorous, and thus assimilation of freshwater-derived sources by these estuarine-resident taxa is not by consumption of freshwater fishes that moved into the estuarine zone. We used average values of carbon and nitrogen isotope ratios of seston and C_3 plants collected in the wetland, and microphytobenthos, C_4 plants, and seston collected in the estuary as representative freshwater- and estuarine-derived basal production sources. We chose freshwater seston and C_3 plants (mostly floating macrophytes) because these basal sources are transported from the freshwater wetland into the estuarine zone during floods, which create hydrologic connectivity between regions. Estuarine sources included in this model were based on the 4-end-member mixing model that identified the primary sources assimilated by the estuarine fish assemblage. Mixing models were computed assuming the silversides are 2 trophic levels above the basal production sources, and we used values of 0.54 ± 1.23‰ and 2.54 ± 0.11‰ to correct for trophic fractionation of isotopic ratios of carbon and nitrogen, respectively (Vanderklift & Ponsard 2003).

RESULTS

In total, 684 samples representing 52 fish species were obtained in the freshwater wetland (n = 352), estuarine zone (n = 187), and lagoon mouth (n = 145)

between April 2008 and May 2009 for analysis of carbon and nitrogen stable isotope ratios (Table S1 in the Supplement at www.int-res.com/articles/suppl/m567p017_supp.pdf). Local fish assemblages showed a gradual zonation in species composition across the freshwater–estuarine gradient. Primary and secondary freshwater fishes were restricted to freshwater wetland and estuarine zones, with the only exception being *Jenynsia multidentata* (secondary freshwater species), which occurred at all 3 sites. In contrast, estuarine residents, estuarine dependents, and marine vagrants were captured only within the estuarine zone and the lagoon mouth (Table S1).

At the assemblage scale, there was a significant trend in $\delta^{13}C$ and $\delta^{15}N$ along the freshwater to estuarine gradient, with lower values in freshwater (mean ± SD $\delta^{13}C$: −26.3 ± 2.86‰; $\delta^{15}N$: 7.5 ± 1.5‰) and much higher values in the estuarine ($\delta^{13}C$: −15.7 ± 3.4‰; $\delta^{15}N$: 10.4 ± 1.8‰) and lagoon mouth zones ($\delta^{13}C$: −13.8 ± 2.4‰; $\delta^{15}N$: 13.3 ± 2.1‰; Fig. 3, Table S1). Overall, invertebrates (e.g. crabs, shrimps, polychaete worms), macrophytes (C_3 and C_4), and other potential food resources (seston, microphytobenthos) showed similar trends. Specifically, both $\delta^{13}C$ and $\delta^{15}N$ values significantly increased from the freshwater wetland to the lagoon mouth (Fig. 3, Table S1).

For fish, quantitative estimates from Bayesian isotopic mixing models revealed that seston and C_3 plants (0.53 to 0.69 and 0.23 to 0.44, respectively) were the basal sources assimilated in the greatest proportions by fish inhabiting the freshwater wetland. Conversely, within the 2 mixohaline sites (estuarine zone and lagoon mouth), C_4 plants and microphytobenthos were the basal carbon sources assimilated in the greatest proportions. For the estuarine zone, in particular, C_4 plants had a much greater contribution to fish biomass (0.70 to 0.83) compared with C_3 plants (0.08 to 0.21), and C_4 plants and microphytobenthos were the basal carbon sources with

the highest contributions near the lagoon mouth (0.53 to 0.71 and 0.07 to 0.42, respectively; Fig. 3).

Monthly analysis of the isotopic composition of fish assemblages revealed important changes in the spatial trend in average $\delta^{13}C$ across study sites (Fig. 4), which coincided with the effect that the closure of the lagoon's connection with the sea has on the flood dynamics of this system. During the flooding episode recorded between April and October 2008, mean fish $\delta^{13}C$ values across all sites were significantly lower (−23.5‰) compared with those recorded during the non-flooded period (−20.0‰; $F_{1,687} = 32.28$, p < 0.00). When functional guilds were taken into account, we observed that this difference was primarily due to the occurrence of salinity intolerant, primary freshwater fishes, especially small characids (*Astyanax eigen-*
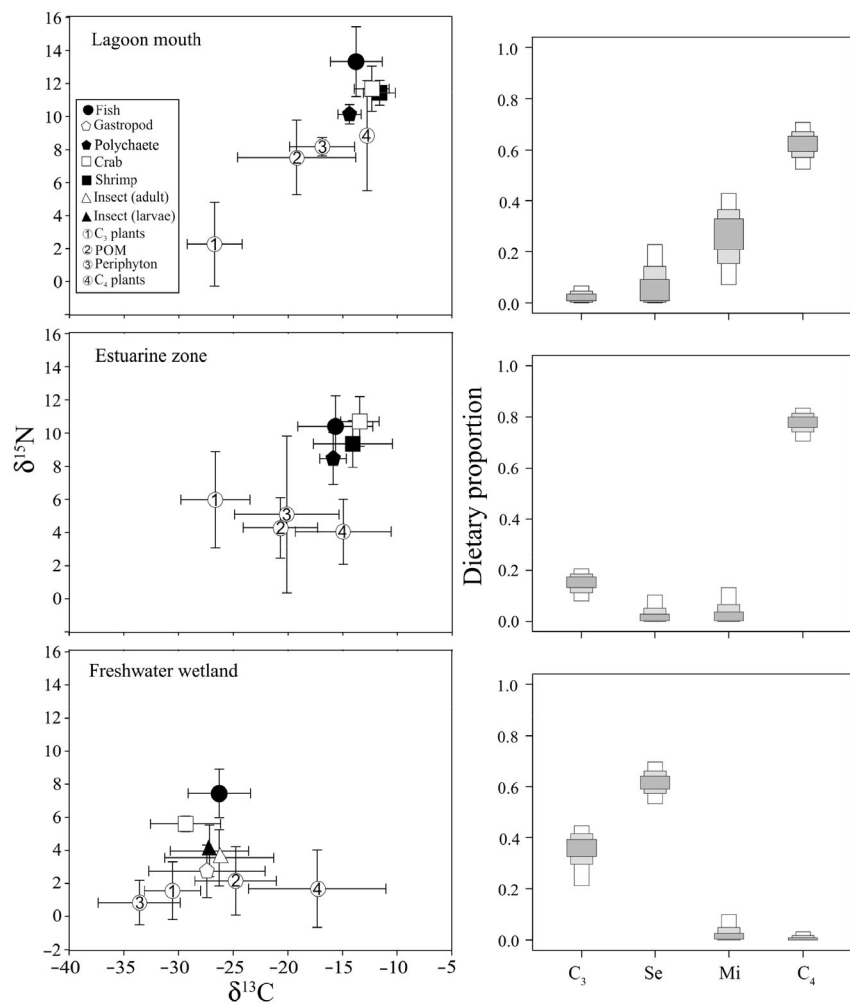

Fig. 3. Mean (+SD) of $\delta^{15}N$ and $\delta^{13}C$ values for primary producers and organic sources (open circles numbered 1 to 4), prey, and fishes (left column) and relative contributions of basal food sources (C_3 and C_4 plants, seston [Se], and microphytobenthos [Mi]) to fishes at the freshwater wetland, estuarine zone, and lagoon mouth sites (right column). Each 'Dietary proportion' plot shows 50 (gray), 75 (light gray) and 95% (white) Bayesian credibility intervals

manniorum, 59.7 ± 11.8 mm TL; *A. fasciatus*, 35.7 ± 8.8 mm TL; *Cheirodon interruptus*, 32.4 ± 6.0 mm TL) and poeciliids (*Phalloceros caudimaculatus*, 28.6 ± 8.7 mm TL), that occurred in the estuarine zone from July to November (Fig. 4). Fish collected from the estuary in July had lower $\delta^{13}C$ values (−21.6‰), but the isotopic composition of these primarily freshwater species increased ($F_{4,38} = 7.51$, p < 0.00) nearer to the estuarine site until approaching values (−17.7‰) similar to those observed for estuarine dependent fishes. Estuarine resident fishes also showed significant differ-

ences in their average $\delta^{13}C$ values during the flood period ($F_{1,106} = 108.37$, p < 0.00), and had lower values (−18.7‰) when compared with the non-flooded period (−13.5‰; Fig. 4). Temporal variation in $\delta^{13}C$ during flooded vs. non-flooded conditions (Fig. S1 in the Supplement) also occurred for some basal sources, such as microphytobenthos ($F_{1,18} = 6.47$, p < 0.02) and seston ($F_{1,17} = 11.44$, p < 0.00), which had lower $\delta^{13}C$ during flooded (−21.8 and −22.6‰, respectively) vs. non-flooded (−17.2 and −18.4‰, respectively) conditions. Average $\delta^{13}C$ values of other sources (C_3 and C_4
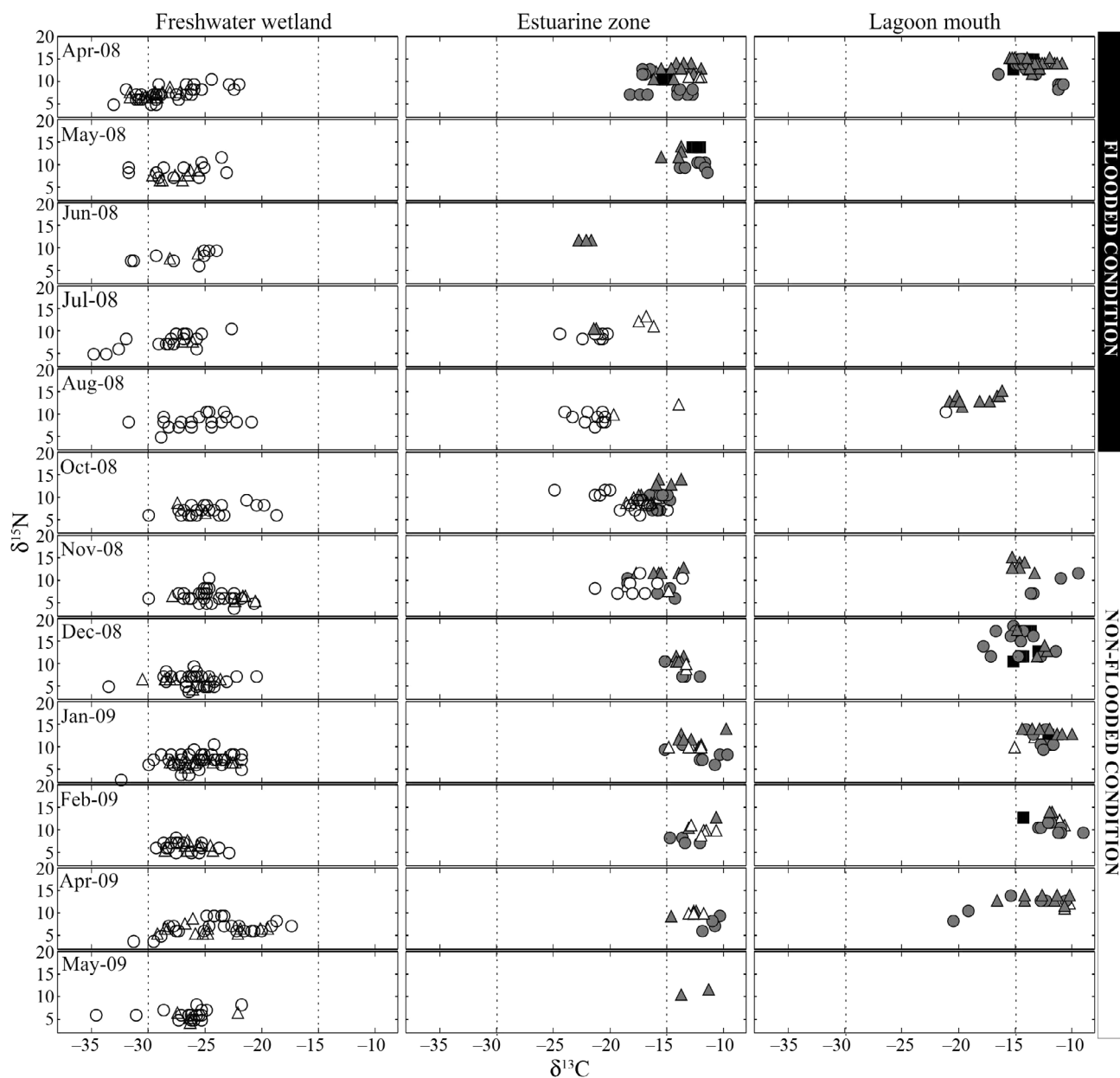

Fig. 4. Monthly variation in the $\delta^{15}N$ and $\delta^{13}C$ values for fish guilds (primary freshwater: open circles; secondary freshwater: open triangles; estuarine dependent: grey circles; estuarine resident: grey triangles; marine vagrants: dark squares) from the freshwater wetland, estuarine zone, and lagoon mouth. Vertical bar on the right denotes flood status within the estuarine zone
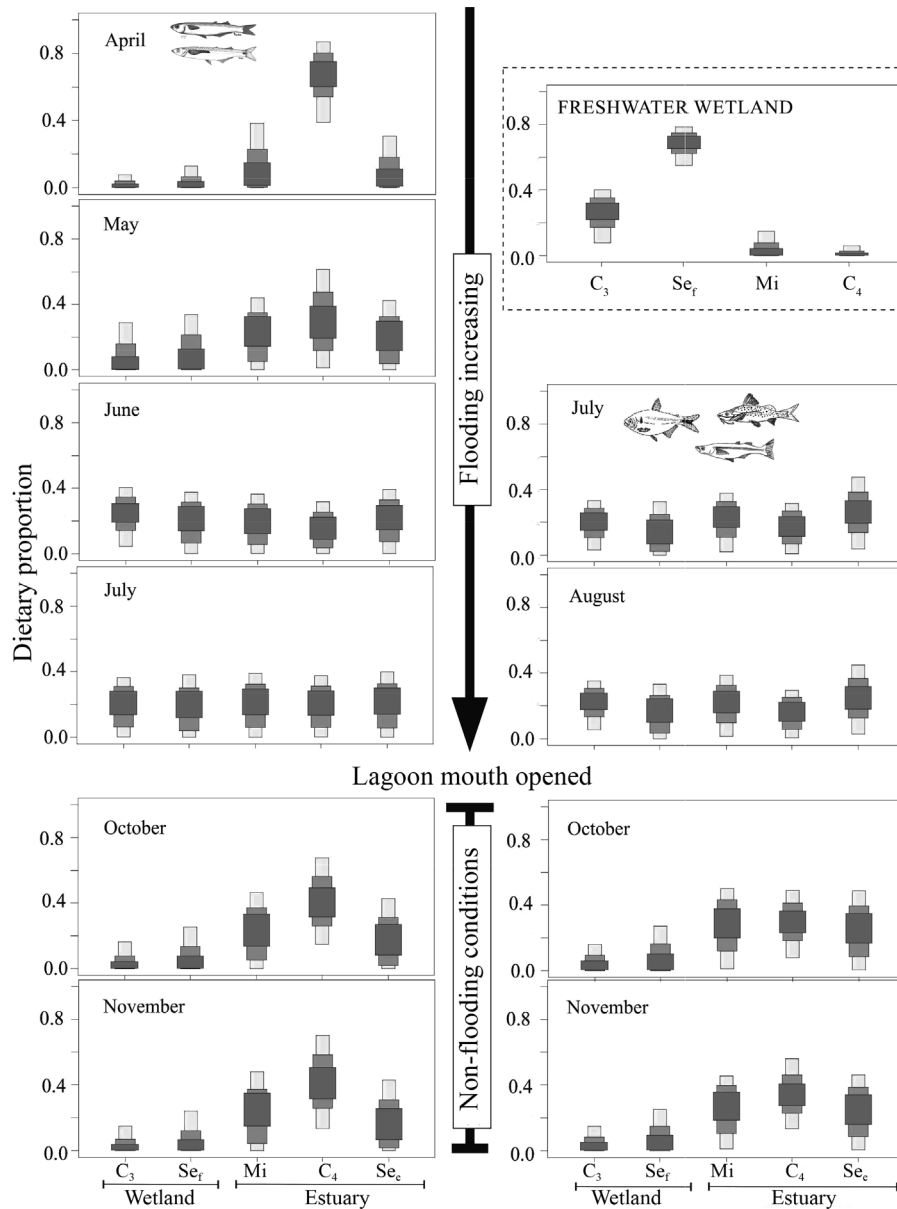
Fig. 5. Monthly relative contributions of basal food sources (C_3 and C_4 plants, seston [Se_f and Se_e], and microphytobenthos [Mi]) to biomass of estuarine-resident fish (*Atherinella brasiliensis*, *Odonthestes argentinensis*) (left column) and primary freshwater fish (*Astyanax eigenmanniorum*, *A. fasciatus*, *Cheirodon interruptus*, *Phalloceros caudimaculatus*) (right column) from the estuarine zone during flooding and non-flooding conditions. Each plot shows the 50 (black), 75 (gray), and 95% (lighter gray) Bayesian credibility intervals of the feasible contributions

plants) did not change significantly between flooding conditions ($F_{1,16}$ = 0.0656, p < 0.80 and $F_{1,5}$ = 0.0057, p < 0.94, respectively; Fig. S1).

Mixing models revealed that freshwater-derived basal sources (C_3 plants and seston) contributed to estuarine residents (silversides *Atherinella brasiliensis* and *Odonthestes argentinensis*) during flood conditions (April through July; Fig. 5). Contributions of these sources were nearly 0 in April, but gradually increased to up to 40% in June and July. Over the

same period, contributions of estuarine-derived basal sources to estuarine fish biomass declined. However, 2 mo after the opening of the lagoon mouth and cessation of flood conditions and hydrologic connectivity with the freshwater wetland, the contribution of freshwater-derived basal sources to estuarine resident fish decreased, and C_4 plants and microphytobenthos again were the main sources supporting estuarine resident fishes (Fig. 5). Primary freshwater fishes, such as characids and poeciliids, assimilated

carbon derived mostly from seston and C_3 plants in the freshwater wetland. Conspecifics caught within the estuarine zone during the late flooding period (July to August) assimilated freshwater- and estuarine-derived basal sources in similar amounts. Similar to estuarine resident fishes, the percent contribution of estuarine-derived sources (microphytobenthos and C_4 plants) to these freshwater species inhabiting the estuarine zone increased to up to 69% following cessation of flood conditions (Fig. 5).

DISCUSSION

Pulsing hydrology in Lagoa do Peixe affected both habitat productivity and habitat connectivity, which together resulted in trophic subsidies from the freshwater wetland to estuarine consumers. This subsidy occurred via passive transport of basal sources (e.g. terrestrial detritus in the form of fine particulate organic matter) as well as dispersal by freshwater fishes and probably other freshwater taxa along the fluvial gradient. Seston (phytoplankton plus suspended particulate organic matter of undetermined origin) from the freshwater wetland was apparently transported in significant quantities into the estuary during the flood pulse. The assimilation of this isotopically distinct seston (approximately 4 and 6‰ lower $\delta^{13}C$ than seston from the estuarine zone and lagoon mouth, respectively) by estuarine primary consumers (e.g. zooplankton) and their subsequent consumption by fish could explain why we observed an average reduction of approximately 5‰ in carbon isotope ratios of estuarine fishes (*Odonthestes argentinesis* and *Atherinella brasiliensis*) during the flood period. Carbon isotope ratios of estuarine fish gradually shifted back to typical estuarine isotopic values observed during pre-flooding conditions, suggesting their prey transitioned back to assimilating locally produced estuarine seston.

In addition to passive transport of seston during flooding conditions, food web subsidies also occurred via animal dispersal from the wetland into the estuary. Animal dispersal in fluvial systems is often directional, either by downstream drift or upstream swimming against the prevailing current (Flecker et al. 2010, Oliveira et al. 2014). For example, anadromous fish that ascend rivers to spawn deliver large amounts of nutrients from excretion and carcass decomposition that subsidize freshwater and riparian ecosystems by enhancing primary production (Naiman et al. 2002, Koshino et al. 2013). Juvenile marine fish that migrate into small coastal streams in Brazil excrete

marine-derived nutrients and are consumed by resident freshwater piscivores (Oliveira et al. 2014), thereby subsidizing the base and top of the food web simultaneously. During the Lagoa do Peixe flood pulse, salinity-intolerant primary freshwater fishes (e.g. small characins such as *Astyanax eigenmanniorum*, *A. fasciatus*, and *Cheirodon interruptus*) moved into the estuarine region and began to assimilate material derived from local basal production sources.

In this system, these species generally consume cladocerans, other microcrustaceans, algae, and vascular plant fragments (F. Correa unpubl. data). Consumption of locally produced microcrustaceans and vegetation would explain the spatial and temporal dynamics in isotopic values for these species, especially for those specimens sampled after the opening of the lagoon mouth. In addition to assimilating significant amounts of estuarine resources during their brief periods of residence in the estuary, these freshwater fishes contribute to nutrient dynamics via excretion and also delivered a pulse of biomass and energy that can be exploited by avian (Bugoni et al. 2005) and mammalian (Colares & Waldemarin 2000) predators. Studies of other coastal lagoons have demonstrated that a great diversity of freshwater fishes from different trophic guilds (e.g. detritivores, zoobenthivores, piscivores) can colonize estuarine zones during high freshwater discharges (Garcia et al. 2003). However, their potential effects on fitness, feeding habits, and survival of estuarine competitors and predators as well as overall effects to estuarine food web organization remain largely unknown.

Estimates of spatial food web subsidies generally have involved asymmetric productivity across aquatic–terrestrial or marine–terrestrial ecotones, with the more productive habitat being a net donor (e.g. coastal marine systems, riparian forests) and less productive habitat being a net recipient (e.g. arid islands, forest streams) (Polis et al. 2004). We are unaware of prior evidence for reciprocal subsidies between aquatic habitats with similar productivity, such as freshwater wetlands and estuaries. Whether inputs of wetland-derived allochthonous organic matter and freshwater fish into estuaries induce trophic cascades probably depends on the relative productivity of the 2 systems and the degree to which there are reciprocal subsidies. Potential bottom-up and top-down effects could be further elucidated by field experiments. For example, Nakano et al. (1999) and Nakano & Murakami (2001) conducted field experiments and dietary analyses to estimate reciprocal subsidies across aquatic–terrestrial ecotones and their influence on trophic cascades. Baxter et al. (2004) demonstrated how habi-

tat alteration and introduction of alien predators suppressed flows of resources across ecotones and associated reciprocal subsidies.

Fish guilds responded differently to the pulse of freshwater inflow and degree of connectivity with the sea. Estuarine dependent (e.g. *Brevoortia pectinata*, *Micropogonias furnieri*, *Paralichthys orbignyanus*) and marine vagrant (e.g. *Diapterus rhombeus*, *Eucinostomus melanopterus*, *Menticirrhus littoralis*, *Trachinotus marginatus*, *Ulaema lefroyi*) fishes were absent in mixohaline zones (estuary, lagoon mouth) during the latter stages of flooding when characids, poeciliids, and other freshwater fishes were prevalent. Marine fishes responded to the freshwater pulse by moving southward toward the sea, but they could not exit the lagoon because sand dunes blocked the outlet. Several species from the marine guild (e.g. croakers, mullets, silversides) were observed in high densities within shallow pools along the margins of the outlet zone. Mass mortality of marine species sometimes occurs during periods of drought as well as periods of freshwater expansion into mixohaline zones (A. M. Garcia pers. obs.) and would explain the absence of estuarine dependent and marine vagrant fish when the lagoon mouth was closed. Similar to other coastal lagoons in the region (Garcia et al. 2012), marine fishes returned to the mixohaline zones soon after the connection to the sea was opened with machinery. Because we did not sample marine habitat outside the lagoon outlet, we can only speculate about effects of estuarine discharge of resources on the nearshore marine food web. Using SIA, Savage et al. (2012) showed that freshwater primary production subsidizes suspension-feeding bivalves in coastal waters of New Zealand when high flow pulses transport material from the estuary to the adjacent coast.

High-resolution temporal sampling of food web components was crucial to reveal how pulsed freshwater inflows affect trophic subsidies between zones of a coastal lagoon ecosystem. All estuarine systems experience hydrologic pulsing, with patterns varying from seasonal (e.g. wet/dry tropics, temperate regions with spring snowmelt) to highly variable and relatively unpredictable regimes (dryland rivers). Appropriate sampling designs are required to reveal spatiotemporal patterns of food web dynamics caused by hydrologic pulsing (Yang et al. 2008). Resource pulses occur at multiple scales, and a local subsidy for one species (e.g. short-lived, sedentary organism) may be insignificant for another (e.g. long-lived, migratory organism) (Zackrisson et al. 1999, Yang et al. 2008). Therefore, in order to quantify resource pulses, it is crucial to define relevant spatial and temporal scales matching re-

source–consumer interactions. Because we sampled food web components with greater frequency (monthly) compared with most prior studies (e.g. pre- vs. post-flood or seasonal sampling), our analysis was able to reveal the timing, magnitude, and duration of trophic subsidies along the longitudinal fluvial gradient. Analysis of fish guilds also allowed us to identify pulses at ecologically relevant scales in space and time.

Our findings provide evidence that human interference with the connectivity of coastal wetlands, lagoons, and the sea can greatly affect food web dynamics, including trophic subsidies among their compartments. Mechanical opening of the lagoon outlet terminated the flood pulse and reduced delivery of freshwater subsidies to the estuarine zone, but it also allowed estuarine dependent species to access critical habitat for early life stages. Excavation of the Lagoa do Peixe outlet channel has been done multiple times over the past 150 yr to allow entrance of shrimp *Farfantepenaeus paulensis* from the sea into the lagoon and to drain water from the floodplain to increase pasture for cattle (Lanés et al. 2015). Ecological responses to artificial connections between coastal lagoons and marine systems are difficult to predict, and fish species diversity may decline (Saad et al. 2002), increase (Griffiths 1999), or change little (Lanés et al. 2015). There is general concern that artificial opening of sandbars in coastal lagoons alters natural ecological dynamics with potential negative effects on biodiversity and ecosystem services (Griffiths 1999, Saad et al. 2002). Our study contributes to this discourse by demonstrating how the timing of freshwater inflows and opening of the outlet affect food web dynamics in different regions of the lagoon system.

Future studies of coastal ecotones could examine the influence of pulsing allochthonous inputs on the distribution of trophic interactions of varying strength within the regional food web. Prior theoretical and empirical work suggests that greater proportions of weak trophic interactions enhance stability in species-rich food webs, and strong trophic interactions promote destabilizing oscillations (Kokkoris et al. 2002, Cross et al. 2013). Landscape mosaics, including fluvial ecosystems and other ecotones, might have greater proportions of weak links within the regional food web when there is periodic exchange of organisms among habitat compartments (Bellmore et al. 2015). Research that combines comparative and experimental approaches to estimate interaction strength could test this hypothesis and further elucidate ecological responses to pulsing hydrology and food web subsidies.

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