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Isotopic signature of the caridean shrimp *Potimirim brasiliana* Villalobos, 1960 in different points of a pristine area in southeastern Brazil

Assinatura isotópica do camarão carídeo *Potimirim brasiliana* Villalobos, 1960 em diferentes pontos de uma área preservada no sudeste do Brasil

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Abstract: Aim: The aim of this study was to investigate the isotopic signature of *P. brasiliana* captured in three different points along the river flow toward the sea of the Prumirim River, northern coast of the state of São Paulo/Brazil in order to test the hypothesis of similarity between isotopic signature of individuals living in separated areas of the river. **Methods:** We used stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) at three points of the river and ANOVA and Bayesian Ellipses analyses were performed. **Results:** Our results showed that are a differential isotopic enrichment along the river course in ^{13}C, providing important results on the environmental condition and anthropogenic impacts in the region. In addition, the food biology of *P. brasiliana* was characterized as a primary consumer corroborating with the detritivores feeding habit observed in previous studies of stomach content analysis. **Conclusions:** Our research, limited to a single area along the northern coast of the state of São Paulo, uncovers intriguing findings that merit replication in other areas within the region. This is particularly crucial given the rising number of anthropogenic influences resulting from urban development, underscoring the need for improved monitoring of these areas.

Keywords: amphidromous species; Atyidae; freshwater shrimp; conservation; stable isotopes.

Resumo: Objetivo: O objetivo deste estudo foi investigar a assinatura isotópica de *P. brasiliana* capturada em três diferentes pontos ao longo do fluxo fluvial em direção ao mar do rio Prumirim com a finalidade de testar a hipótese de similaridade entre a assinatura isotópica de indivíduos que vivem em areas diferentes do rio. **Métodos:** Foram utilizadas análises de isótopos estáveis ($\delta^{13}C$ e $\delta^{15}N$) em três pontos do rio e foram realizadas análises de Stable Isotope Mixing Models e Bayesian Ellipses.

Resultados: Nossos resultados mostraram que há um enriquecimento isotópico diferencial ao longo do curso do rio em 13C, fornecendo resultados importantes sobre as condições ambientais e os impactos antropogênicos na região. Além disso, a biologia alimentar de *P. brasiliana* foi caracterizada como um consumidor primário, corroborando com o hábito alimentar de detritívoros observado em estudos anteriores de análise de conteúdo estomacal. **Conclusões:** Nossa pesquisa, limitada a uma única área ao longo da costa norte do estado de São Paulo, revela descobertas intrigantes que merecem ser replicadas em outras áreas dentro da região. Isso é especialmente crucial dada a crescente quantidade de influências antropogênicas resultantes do desenvolvimento urbano, ressaltando a necessidade de um monitoramento aprimorado dessas áreas.

Palavras-chave: espécies anfídromas; Atyidae; camarão de água doce; conservação; isótopos estáveis.

1. Introduction

Habitat use and trophic strategies are central topics in ecology, and modern tools can help us to improve the knowledge in these issues. For example, although stomach content analyses provide us important information about the diet habits of a species (such as taxonomy and composition of diet size, trophic chain, and predator-prey interactions) distinct tools have been widely used in the study of food communities (Layman et al., 2005). The stable isotopes are one of the appliances that complement these analyses, bringing information temporarily integrated (weeks to months) on diet habits, representing food sources that are in fact assimilated by the consumer (Davis et al., 2012).

Decapod crustaceans (Malacostraca, Decapoda) are a relatively very representative group with more than 13,800 extant species worldwide (WoRMS, 2023). However, there are few studies using isotopic tools focused on freshwater species inhabiting the Neotropical region: Viozzi et al. (2021) with the shrimp *Macrobrachium borellii* (Nobili, 1896) Burress et al. (2013) with the endemic aeglids from the southern, *Aegla uruguayana* Schmitt, 1942; Denadai et al. (2022) with *A. castro* Schmitt, 1942 and Burress et al. (2013) with the crab *Trichodactylus panoplus* (von Martens, 1869).

Freshwater shrimps are a very representative group of the crustacean fauna and a good model to apply an isotopic approach to ecological studies, especially amphidromous species that present a fresh and marine water dependency during the life cycle. The genus *Potimirim* Holthuis, 1954 is an excellent group that fits with this later characteristic. This genus has currently five species of tiny freshwater shrimps, all restricted to the American continent (Torati & Mantelatto, 2012; Mantelatto et al., 2021), among which two species occur in coastal rivers and streams of Brazil, *P. brasiliana* Villalobos, 1959 and *P. potimirim* (Müller, 1881) (Mantelatto et al., 2021). These shrimps have a fundamental role in the trophic web of limnic environments (Benzie, 1982) and in the sediment renewal process (Souza & Moulton, 2005; Lima et al., 2006). In addition, *Potimirim* species are only found in pristine areas with a high degree of environmental preservation in terms of quality of the water and marginal vegetation, which could be a good candidate for water quality indicator (F.L. Mantelatto Pers. Comm.).

The target species of this study, *P. brasiliana,* is endemic to Brazil and occur along the east coast, from the states of Bahia to Santa Catarina (Villalobos, 1959; Torati & Mantelatto, 2012; Rocha et al., 2013). This shrimp is usually found in clear flowing water, under stones, among roots of aquatic plants, and in the litter deposited at the bottom of rivers and streams that flow into the sea, in coastal regions (Barros & Fontoura, 1996; Rocha et al., 2013; Grilli et al., 2014), since it depends on brackish water to complete its larval development (Hoffmann & Negreiros-Fransozo, 2010; Grilli et al., 2014).

Potimirim brasiliana has an upstream migratory behavior in the river after the larval development, common to Atyid shrimps (Hoffmann & Negreiros-Fransozo, 2010; Bauer, 2011). This migration may drive to a shrimp population structure composed of several local populations or subpopulations inhabiting different rivers habitats (Bauer, 2011; Santos et al., 2022), which consequently can exhibit differential structures and sizes, as a result of the degree of isolation or the habitat characteristics, while the shrimps may migrate among them (Hanski, 1994; Terui et al., 2014; Santos et al., 2022).

Coastal streams may consist of shallow regions with turbulent hydrology, deep regions with slow water flow, and the formation of small and large waterfalls (Jönck & Aranha, 2010). Waterfalls can function as natural barriers that influence the distribution, abundance, and ecological relationships of some species, acting as biogeographical barriers within river basins (Reznick & Ghalambor, 2005; Greathouse & Pringle, 2006; Mazzoni et al., 2006).

Although certain waterfalls can promote isolation between subpopulations along the course of a river, some species of amphidromous shrimp are able to traverse upstream waterfalls in search of predator-free refuge environments, depending on several factors, such as habitat composition, elevation, predation, among others (Covich et al., 2009; Hein et al., 2011; Ebner et al., 2021).

The aim of this study was to investigate the isotopic signature of *Potimirim brasiliana* in three different points of the Prumirim River on the northern coast of the state of São Paulo/Brazil. Our hypothesis is that the isotopic signature of this shrimp is compatible with its previously described feeding habit (detritivores) and there are differences on the river sampled reflecting the different habitats exposition of shrimps.

2. Material and Methods

The sampling was carried out during the spring of September 2019 in the Prumirim River, a pristine coastal river from Ubatuba, São Paulo, Brazil. Three points were chosen, representing the layers of the river flow toward the sea: the first point (Upstream from a Waterfall = P1) was the most distant of the sea (~ 1.4 km along the course of the river); the second (Downstream from Waterfall = P2) was intermediary and the third point (Close to Sea = P3) was the closest to the sea (Figure 1).

The Prumirim River covers an area of 21 km^2 and has an average flow of m^3/s (Andrade, 2008). It is predominantly composed of igneous and metamorphic rocks in the mountainous region. Mostly, it consists of small streams that originate from headwaters, flowing through steep valleys until they reach the coastal plain. Sometimes these streams transform into sinuous and meandering creeks or rivers, flowing into small beaches (Andrade, 2008). Between P1 and P2 there is a waterfall approximately eight meters high. The map of this study was made in the software QGIS 3.30.1 using the SIRGAS 2000 coordinate system, using Google's® satellite image.

The specimens were collected in all three points during the morning, by two-person using dip nets and sieves for approximately 30 minutes. Afterward, shrimp were stored in plastic bags with water from the sampling site and transported alive to the field laboratory of the Oceanographic Institute of the University of São Paulo (IO/USP), where they were cryo-anesthetized and identified using specific literature (Villalobos, 1959; Torati & Mantelatto, 2012). Thirty individuals were collected for each sampling point, totaling 90 individuals. To obtain the necessary weight of the material sufficient to perform the analysis of stable isotopes we grouped three individuals per sample. As a result, we obtained ten different isotopic values for each collection point. The samples were kept frozen until the time to run the analysis of stable isotopes. Both analyses used only adult specimens. We emphasize that the area P3 is an area with strong influence of the sea and anthropic, this can influence the enrichment of ^{13}C in our results.

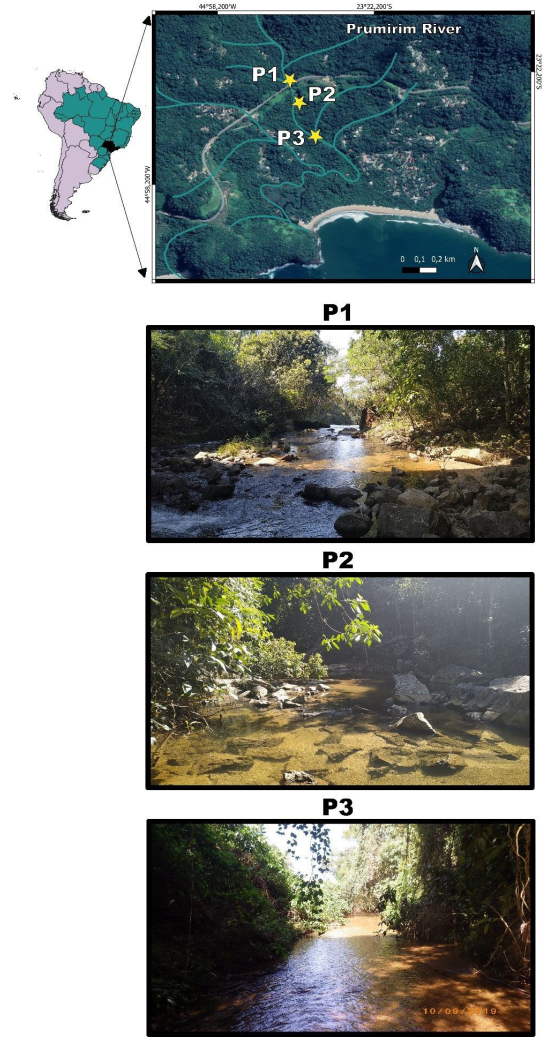

Figure 1. Schematic drawing and images of Ubatuba, São Paulo, Brazil, with the three sampling points in the Prumirim River: Upstream Waterfall Area (P1), Downstream Waterfall Area (P2), and Close to Sea Area (P3).

The analysis of stable isotopes (i.e., $^{13}C/^{12}C$ and $^{15}N/^{14}N$, respectively) has been frequently used as an informative and reliable tool to identify the main food source and the trophic position of consumers (Peterson & Fry, 1987; Post, 2002; Mao et al., 2016). Specifically, the isotopic values of $\delta^{15}N$ are indicators of the trophic level in species, given that $\delta^{15}N$ exhibits a slight enrichment of 2.5% – 5‰ in each trophic level (Minagawa & Wada, 1984; Bearhop et al., 2004). In the other hand, the values of $\delta^{13}C$ contributes better as indicators of habitat, since they allow the differentiation between the sources of primary production, that could change according to distinct habitat location (Fry, 2006). Thus, obtain the isotopic signature of different taxa from different sites across natural areas allows the estimation of their isotopic niche.

Stable isotopes analysis was carried out in the Center for Stable Isotopes of the Institute of Biosciences of UNESP, Botucatu, São Paulo, Brazil. After thawing, the abdominal muscle tissue was extracted from each shrimp and dried at 60 ºC for 48 h, and the samples were ground in a cryogenic grinder for 5 min at 990 rpm. Subsequently, the samples were weighed from each sampling point, samples weighing approximately 50-80μg for ^{13}C and 400-500 μg for ^{15}N were used to measure, whose CO_2 and N_2 were obtained by combustion and measured in a mass spectrometer, which determines the obtained $\delta^{13}C$ and $\delta^{15}N$ enrichment values relative to their respective international standards. The isotopic composition values were obtained relative to the international standard V-PDB (Vienna Pee Dee Belemnite) for $\delta^{13}C$ and atmospheric air N_2 for $\delta^{15}N$, with an analysis error of 0.2 ‰ and calculated by Equation 1:

$$\delta X_{(sample,\ standard)} = \left[\left(R_{sample} / R_{standard}\right) - 1\right] \times 10^3 \qquad (1)$$

Where: δX represents the isotope enrichment of the chemical element X (^{13}C or ^{15}N) of the sample relative to the respective international standard, and R represents the ratio between the least abundant and the most abundant isotope. Although our samples did not undergo a lipid removal chemical process, they resulted a C:N ratio < 3.5, indicating that it was not necessary to perform a lipid correction for $\delta^{13}C$ values [see Post et al. (2007)].

The Stable Isotope Bayesian Ellipses in R "SIBER" package (Jackson et al., 2011) was used to analyze and determine isotopic niches and their overlapping among shrimps from samples collected in the different points across the river using 40% of data size. Two niche metrics were utilized to describe niche width: SEAc (size-corrected standard ellipse area) and SEAb (Bayesian standard ellipse area). We obtained posterior modes of SEAb, when the 95% credibility intervals were non-overlapping. Finally, we test the differences of ellipse sizes by calculating the probability of posterior distributions (smaller or larger).

The data were considered normal and homoscedastic by the multivariate analyses then an Analysis of Variance (ANOVA) one-way was used to test niche differences between sampling points, considering isotopic values as response variable and sampling points as categorical variable. Subsequently, Tukey's post hoc test was performed to verify the differences between niches. These analyses and figures were performed in the software R using the packages "multcompView" (Graves et al., 2019), "dplyr" (Wickham et al., 2023) and "ggplot2" (Wickham, 2016).

3. Results

The isotopic values obtained in the three points were: $\delta^{13}C$ and $\delta^{15}N$ of P1 ranged from –26.69 to –23.42‰ (mean ± SD = –25.70 ± 1.02‰), and 5.30 to 6.82‰ (5.91 ± 0.48h), respectively. $\delta^{13}C$ of P2 ranged from –26.15 to –24.08‰ (mean ± SD = –24.7 ± 0.58‰) and $\delta^{15}N$ ranged from 5.50 to 7.18‰ (mean ± SD = 6.41 ± 0.48‰). In P3 $\delta^{13}C$ ranged from –25.55 to –24.59‰ (mean ± SD = –24.96 ± 0.32‰) and $\delta^{15}N$ ranged from 5.39 to 6.78‰ (mean ± SD = 6.33 ± 0.45‰).

There was a significant difference in the food sources of *P. brasiliana* in the three points sampled (Figure 2). However, the point with the greatest difference was Upstream from Waterfall (P1) containing higher values of $\delta^{13}C$ and lower values of $\delta^{15}N$ (Figure 2).

The Bayesian estimations of the standard ellipse areas (SEAb) of *P. brasiliana* showed differences in the niche width of the Upstream Waterfall Area (P1) and the other two sampling points, and a relationship between the points Downstream Waterfall (P2) and Close to Sea (P3) (Figure 2; Table 1). There was a niche overlap in all points,

Table 1. Samples of *Potimirim brasiliana* from Prumirim river, São Paulo, Brazil, analyzed (n) and SIBER analysis including the ellipse area corrected for small size (SEAc) and the estimated posterior mode of its isotopic niche width as the standard ellipse area of bayesian standard ellipse area (SEAb) in ‰^{2} together with the 95% credibility interval (in brackets).

Sites	SEAc	SEAb
P1 = Upstream from Waterfall	1.175	1.07 (0.56—2.19)
P2 = Downstream from Waterfall	0.995	0.80 (0.42—1.66)
P3 = Close to Sea	0.164	0.22 (0.11—0.46)

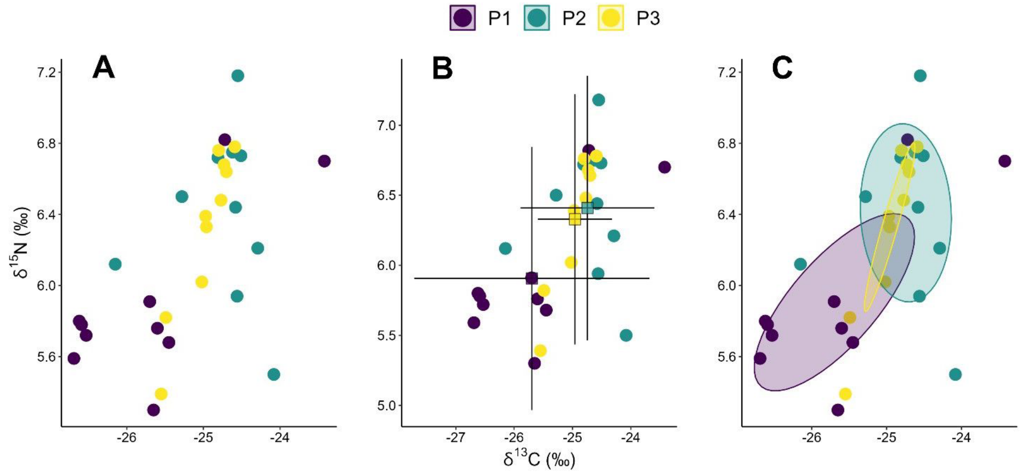

Figure 2. A) Dispersion point **B)** dual isotope plot (mean value) and **C)** SEAb (Bayesian standard ellipse area) of *Potimirim brasiliana* at the three sampling points in the Prumirim River (data pattern in 40% of size samples), Ubatuba, São Paulo, Brazil. P1 = Upstream Waterfall Area, P2 = Downstream Waterfall Area and P3 = Close to Sea Area.

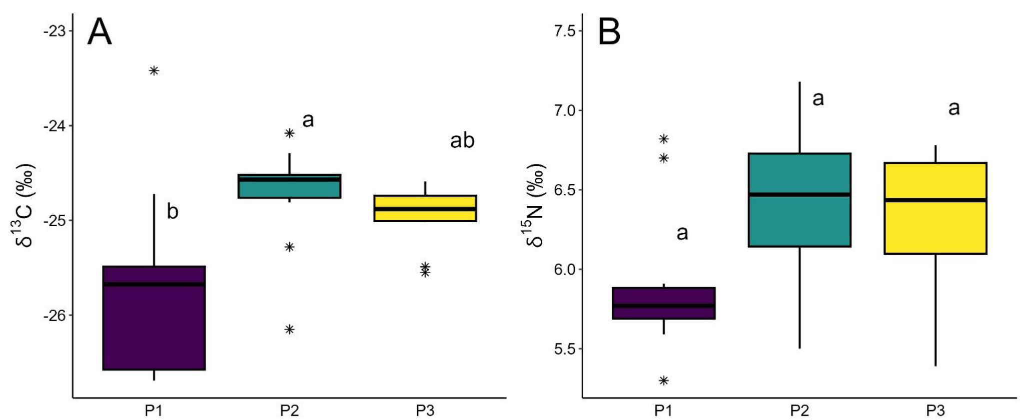

Figure 3. Box plot of *Potimirim brasiliana* isotopic values **A)** $\delta^{13}C$ and **B)** $\delta^{15}N$ by sampling points (P1 = Upstream Waterfall Area, P2 = Downstream Waterfall Area and P3 = Close to Sea Area), in Ubatuba, São Paulo, Brazil. Letters above the box plots indicate the differences in Tukey test, which followed the ANOVA (p < 0.05).

the point Close to Sea is completely overlapped by both the point Downstream Waterfall and Upstream Waterfall, while these two are slightly overlapping between them (Figure 2; Table 1). The width of the niches (Figure 2; Table 1) in the Upstream Waterfall area was higher than the other two points (Figure 3).

The ANOVA test of niche differences between sampling points was significant for both isotopic values of $\delta^{13}C$: $F = 4.98$, $p = 0.01$ and $\delta^{15}N$: $F = 3.27$, $p = 0.05$ (Figure 3). This result shows that differences were identified between sampling points for $\delta^{13}C$ but not for $\delta^{15}N$ (Figure 3).

4. Discussion

The results showed an evident and significant change in the carbon source, showing the importance of the marine influence on carbon assimilation. The isotopic nitrogen signature did not show an evident gradient, but there was a high variation in the isotopic nitrogen values upstream, which may suggest a diversification of food items in these habitats. Also, the different isotopic results of different ^{13}C between the sampling points initially indicate a high enriched carbon values indicate use of detritus as main source of carbon, corroborating other studies on stomach content (Abele & Blum, 1977).

Previous studies of stomach content analyses indicate that shrimps of the genus *Potimirim* are detritivores, feeding on particles scraping the substrate (Felgenhauer & Abele, 1985). In addition, Abele & Blum (1977) showed that 18% of the stomach contents analyzed were composed of plant material and 88% of unidentified material due to the degree of crushing found. Although neither of the studies cited above used the stable isotopes, our results corroborate them since the isotopic signature found ($\delta^{13}C$ between -28 and -23) indicates values composed of C3 plants (Ducatti et al., 2011), classifying this species as the basis of the food web of that environment as a primary consumer and confirming by the first time, via isotopic data, its important ecological role in this environment.

In the area above the waterfall (P1), we observed higher values of $\delta^{13}C$, characteristic of more preserved areas and less degraded by anthropic actions. Despite the lack of data from other species that make up the ecological niche of the Prumirim River, our results, using a caridean shrimp as a study model demonstrated that small stretches of a river considered a pristine area, can present different environmental quality, and consequently influence the life cycle and fauna existing of this environment.

The Prumirim region (including beach, river, and Atlantic Forest) is mainly affected by landfill and human occupation (this causes concern mainly regarding the degradation of mangroves), but also has tourism activities such as diving and hiking (Vasconcellos & Sanches, 2009; CTBio, 2020). Regarding fishing, the conservation status is considered stable, and the region is part of a marine protected area (MPA), however, landed fishing is allowed in the region (Vasconcellos & Sanches, 2009; CTBio, 2020).

The results of stable isotope values also indicated that there was a general overlapping of niches among all sampled points. This means that individuals captured in the upper part of the waterfall did not use exclusively the habitat in which they were collected, probably due to amphidromous migratory behavior. After larval development in brackish water, amphidromous shrimp settle to the bottom as post-larvae and must find a freshwater habitat to migrate upstream to the habitat of adults (Bauer, 2013; Santos et al., 2022). This migration passing through the different sampling points during the life cycle probably influenced the pattern obtained in the analysis of stable isotopes. Therefore, the overlapping of the isotopic niche was strong evidence that shrimps do not reside for a long time in only one section of the river, but they displace along the river, even when adults, that reinforces the hypothesis that there are a few differences in the isotopic signature among the distinct points for *P. brasiliana* at the Prumirim River.

An additional explanation for the overlapping of niches among all sampled points could be the sharing of food such as leaf litter which is considered as a significant food resource for atyids (Cross et al., 2008). As the leaves and other types of organic matter move from one point of the river to another, following the current towards the sea, they can be shared by shrimps living in these different stretches. Despite P1 being connected with the other two points, it was noted that there is a difference in the size and distance of the ellipses compared to the others. This indicates that there is a variation in the food content at that sampling point, probably due to a greater variety of food at the point Upstream waterfall, or also, to greater availability of food due to a possible lower abundance of competing species in the area (Antonio & Richoux, 2014).

During sampling, one of us (FLM), observed by snorkeling that some individuals showed the behavior of climbing of rocks that were in the course of the river and close to the margins, walking on them in rapids areas. Lima & Oshiro (2002) and Lima et al. (2006) reported a greater preference of *P. brasiliana* (as *P. glabra*) adults to environments with rocks, slopes, and points with greater hydrodynamism. This preference is also observed in some freshwater prawns (*Macrobrachium* spp.) that show upstream migration (Kikkert et al., 2009; Hongjamrassilp et al., 2021). According to Covich et al. (2009) and Ebner et al. (2021) these shrimps crawl through very high and steep waterfalls seeking refuge environments from predators and most resource availability (Bauer, 2023).

In addition, we cannot discharge that rainfall frequency and river discharge may also influence the migration process of these shrimps, especially in a subtropical region with frequent storm during the rainy season (spring and summer). Unfortunately, we have not checked volume of water during the sampling, but according to Andrade et al. (2011), the discharge of the Prumirim River in September (considered as dry season, with low average rainfall) was 6 m^3/s which is considered one of the lowest, compared to the other rivers in the region. Even though in a low rainfall volume, the river flow among the sampled areas remains continuous, favoring the necessary orientation (positive rheotaxis) for the shrimp migration (Bauer, 2023). Such climbing behavior enables the upstream migratory movement carried out by *P. brasiliana* and, consequently, maintains connectivity with the different points of the river.

The majority of the studies that used stable isotopes of decapods as a model to test habitat connectivity, as far as we know, are restricted to penaeid shrimp and marine environments.

Fry (1981) evaluated the migration from inshore seagrass meadows to offshore areas of *Farfantepenaeus aztecus* (Ives, 1891) by indicating the presence of $\delta^{13}C$ values of seagrass based food (common of inshore areas) in subadult individuals at offshore areas; Fry et al. (1999) used populations of *F. duorarum* (Burkenroad, 1939) and traced higher connectivity of juvenile migration from inshore seagrass meadows areas to offshore region, in comparison to inshore mangrove-lined bays; and Taylor et al. (2017) studied *Melicertus plebejus* (Hess, 1865) highlighting the important role that connectivity plays in the recruitment of migratory species. Besides its accuracy in studies with decapods, this tool is also widely used to investigate migrations and connectivity of fish populations at different habitats (sea, estuaries, river, lakes, etc.) (Hesslein et al., 1991; Lugendo et al., 2006; Selleslagh et al., 2015).

Potimirim brasiliana completes its life cycle with its larval stages going to brackish waters, as one of the typical amphidromous species behaviors (Bauer, 2013; Grilli et al., 2014), and returning to upstream areas as juveniles, resisting to hydrodynamism and climbing the waterfall. Considering that the damming of rivers is one of the potential threats to populations of this species (Mantelatto et al., 2016), we glimpse here some measurable indicators that can be used to monitor populations and use as a tool to mitigate impacts in populations that depend on movement between watercourse areas.

Our research, restricted to a single area along the northern coast of state of São Paulo, reveals interesting findings that should be replicated in other areas along the region, especially due to the increasing number of anthropogenic influences caused by urbanist development, justifying the importance of better monitoring of these areas.

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