

Isotopic signature and the trophic interactions of *Aegla castro* Schmitt, 1942 (Crustacea: Anomura: Aeglidae)

Ana Clara Denadai¹  orcid.org/0000-0002-9821-4060

Vladimir Eliodoro Costa²  orcid.org/0000-0003-3889-7514

Milena Regina Wolf¹  orcid.org/0000-0003-1022-1360

Geslaine Rafaela Lemos Gonçalves³  orcid.org/0000-0001-7443-6913

Antonio Leão Castilho¹  orcid.org/0000-0003-0001-9054

¹ Zoology - Biological Science Graduate Course, Institute of Biosciences, São Paulo State University (UNESP). 18618-970 Botucatu, State of São Paulo, Brazil.

ACD E-mail: ana.denadai@unesp.br

MRW E-mail: milenarwolf@gmail.com

ALC E-mail: antonio.castilho@unesp.br

² Stable Isotopes Center “Prof. Carlos Ducatti”, São Paulo State University “Júlio de Mesquita Filho (UNESP)”. 18618-689 - Botucatu, SP – Brasil.

VEC E-mail: vladimir.costa@unesp.br

³ Scottish Association for Marine Science, Scottish Marine Institute, Oban, Argyll, PA37 1QA, Scotland, United Kingdom.

GRLG E-mail: geslainelemos@gmail.com

ZOOBANK: <http://zoobank.org/urn:lsid:zoobank.org:pub:C97FBFC0-B4B7-4228-9577-761FB16F523A>

ABSTRACT

Aeglids are endemic crustaceans from the Neotropical region of South America, with great importance in the freshwater food chain. We investigated the trophic relationships in different streams containing *Aegla castro* Schmitt, 1942 through the use of stable isotopes, testing the hypothesis that these animals occupy a lower position and that the environments are different and will modulate the isotopic ratios of carbon and nitrogen. *Aegla castro* showed a low trophic level in all locations, being an important link between producers and other trophic levels. They present differences in their isotopic signature for the sampling areas, indicating that environment can be a factor that modulates the trophic webs. We did not find intraspecific differences in isotopic signatures, probably due to the similar food items consumed by both juveniles and adults foraging in the same locations. Studies like this are becoming increasingly important due to the rapid degradation of freshwater environments and the lack of trophic knowledge about these endemic animals. It is increasingly important to understand how environmental changes (such as through anthropogenic action) is interfering in freshwater trophic relationships, and how this can affect the permanence of aeglids.

KEYWORDS

Carbon, environmental characteristics, food web, nitrogen, ontogenetic phases.

Corresponding Author
Ana Clara Denadai
ana.denadai@unesp.br

SUBMITTED 18 June 2021
ACCEPTED 10 February 2022
PUBLISHED 16 September 2022

DOI 10.1590/2358-2936e2022024



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INTRODUCTION

Aeglids are endemic crustaceans from the Neotropical region, restricted to South American freshwater ecosystems (Bond-Buckup and Buckup, 1994), and generally found in clean and well-oxygenated water. They have great ecological importance, due to their role in aquatic food chains, behaving as omnivores, generalists, and opportunists (Castro-Souza and Bond-Buckup, 2004; Bueno and Bond-Buckup, 2004; Santos *et al.*, 2008). They are a key species in nutrient cycling (Cogo and Santos, 2013) because they are able to feed on allochthonous plant matter, particulate organic matter and aquatic insect larvae (Magni and Py-Daniel, 1989; Bueno and Bond-Buckup, 2004). However, these animals are negatively affected by changes in habitat conditions (Bond-Buckup, 2003), mainly through human activities (Pérez-Losada *et al.*, 2002; Bond-Buckup *et al.*, 2010). So, it is necessary to intensify studies on aquatic fauna and their ecological interactions (Bond-Buckup and Buckup, 1994).

Aegla castro Schmitt, 1942 has a geographic distribution from the southern region of São Paulo state into northern Parana state (Bond-Buckup and Buckup, 1994). Even though this species has a wide geographic distribution it has not been extensively studied. Some previous studies are limited to those on population biology in Ponta Grossa, Paraná State (Swiech-Ayoub and Masunari, 2001); Itatinga, São Paulo State (Fransozo *et al.*, 2003); and Mauá da Serra, Paraná State (Marçal *et al.*, 2017).

Stable isotope analysis has been widely used in ecological studies (Peterson and Fry, 1987; Lajtha and Michener, 1994; Post, 2002). This method is based on the premise that predators enrich with heavy isotopes compared to their prey (Minagawa and Wada, 1984), integrating the isotopic signatures of different prey, over longer periods and incorporating this signature into their tissues. This type of signature is different from what is sampled in the analysis of stomach contents (Hyslop, 1980; Mao *et al.*, 2016). We chose the analysis of stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) in the present study.

These isotopic analyses provide a picture of diet integrated over a longer period of time, while conventional dietary analysis of stomach contents

only provides a short-term picture of diet. In addition, stable isotopes represent assimilated diet rather than what is ingested, which may or may not be digested and contribute to a consumer's nutrition (Bearhop *et al.*, 1999; Votier *et al.*, 2003). Conventional methods of dietary analysis may also be biased toward particular types of prey (Hobson *et al.*, 1994). Isotopic analyses also require small numbers of animals, which is of great value since this group is already at various levels of conservation threat (Ducatti *et al.*, 2011).

The carbon incorporated into plants undergoes isotopic discrimination throughout the photosynthesis process. In C_3 plants the value of $\delta^{13}\text{C}$ ranges from -22 to -34 ‰, and for C_4 plants it ranges from -9 to -16 ‰ (Vogel, 1993; Boutton, 1996; Ducatti *et al.*, 2011). Plants are the main food source for most animals, therefore, the $\delta^{13}\text{C}$ isotopic signature differentiates C_3 or C_4 producers in food chains (Peterson and Fry, 1987; Clapcott and Bunn, 2003). Nitrogen can be incorporated into algae/plants by association with nitrogen-fixing bacteria or by assimilation of nitrogen compounds from the air and soil (Caxito and Silva, 2015), while in animals it is assimilated solely by the incorporation of proteins (Gannes *et al.*, 1998). Nitrogen is used to determine trophic niches and trophic position because $\delta^{15}\text{N}$ of each trophic level is typically 3-5‰ greater than its nitrogen source (Minagawa and Wada, 1984; Peterson and Fry, 1987; Fry, 1991; Robinson, 2001), furthermore, enriched $\delta^{15}\text{N}$ may be associated with carnivorous behavior (Post, 2003) and may be used as an indication of anthropogenic inputs (Peterson, 1999).

This study aims to determine the isotopic signature and the trophic interactions of *A. castro* during both juvenile and adult stages. The hypotheses tested are: (1) *A. castro* occupy a lower position in the food chain, as a primary consumer, being an important link between producers and other trophic levels; (2) there is a difference between the isotopic ratios of *A. castro* juveniles and adults; and (3) the environments studied are different and modulate the isotopic ratios of carbon and nitrogen in *A. castro* and the other organisms in the trophic web. This knowledge about the ecosystem in which these animals occur, including the isotope ratios, is important to know if the environmental status modulates carbon and nitrogen ratios of wildlife and, consequently, the structure of food webs.

MATERIAL AND METHODS

The study locations are:

- Paranapanema sub-basin (São Paulo State, Fig. 1). (1) Itaúna stream (ITA, Itatinga city, 23°09'S 48°37'W), where the local vegetation is Atlantic forest and Cerrado biome (Montana semi-deciduous), with a lot of *Eucalyptus* reforestation. The sampling area is located on a private farm and the stream has preserved and continuous riparian forest with a good tree canopy and creeping plants (grasses and herbs).

- Tibagi sub-basin (Paraná State, Fig. 1). (2) Preto stream (PRE, Mauá da Serra city, 23°57'S 51°07'W), where the local vegetation is Atlantic forest located behind a restaurant and a road with small dirt roads and construction surrounding the area. Riparian forest is present as well as areas composed of creeping

plants (grasses). (3) Iapó stream (IAP, Castro city, 24°48'S 49°51'W), where the local vegetation is Atlantic forest (Dense Ombrophilous Forest with remnants of Mixed Ombrophilous Forest) and is surrounded by plantations on irregular or deforested land. Agriculture, livestock, pig farming, poultry farming, and the extraction of minerals constitute the main economic activities of the Castro region (Fasolo *et al.*, 2002). The stream is surrounded by discontinuous riparian Forest, and the sampling site specifically is composed of a deforested area with creeping plants (grasses), close to a road. (4) Quebra Perna stream (QPE, Ponta Grossa city, 25°10'S 49°58'W), where the local vegetation is Atlantic forest (Dense Ombrophilous Forest and natural fields). In this region there is the private park "Buraco do Padre" with tourist activities where the main attraction is a cave with a waterfall being accessed through an

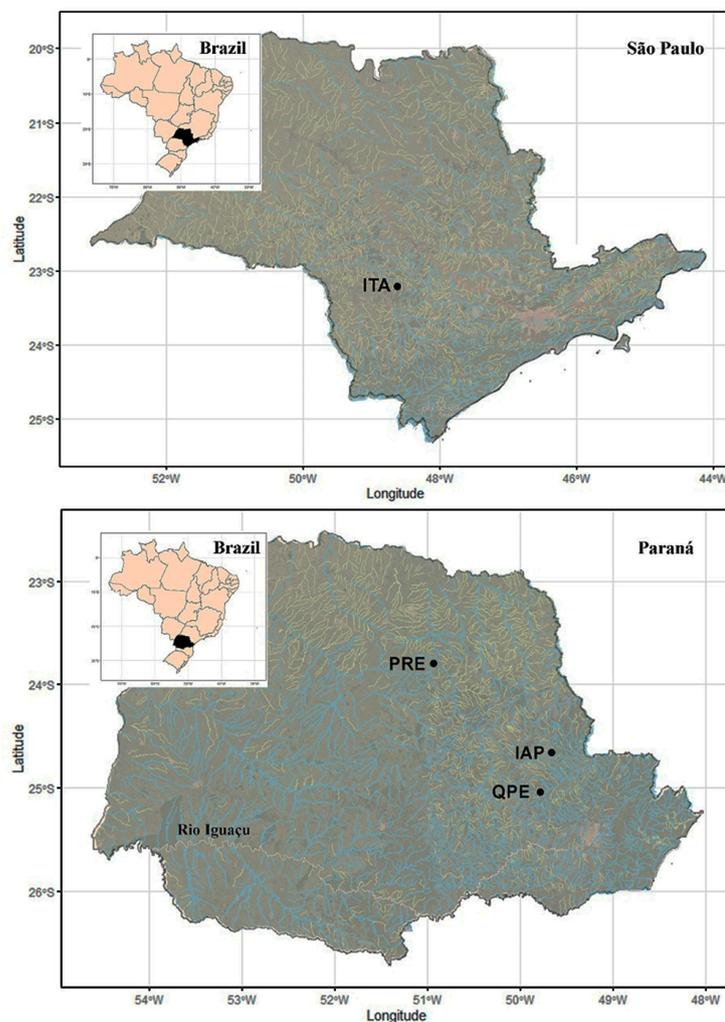


Figure 1. Study sites at Paranapanema sub-basin (São Paulo State): Itaúna stream (ITA) (Itatinga city); and Tibagi sub-basin (Paraná State): Preto stream (PRE) (Mauá da Serra city), Iapó stream (IAP) (Castro city) and Quebra Perna stream (QPE) (Ponta Grossa city).

approximately 730 m trail. Visitors usually go there for outdoor activities such as walking, bathing, rappelling, hiking and climbing. Around the park places with plantations and deforestation are observed.

Biological samples

The collections were made in August (PRE), September (IAP and QPE) and December (IAP) of 2018, using 6 traps measuring 24 cm × 19.5 cm × 8 cm, that were randomly distributed throughout each freshwater stream. A fish-based cat food was used as bait and was placed in a small, perforated plastic container attached inside the trap to attract the crabs (Bueno *et al.*, 2007). The trap opening was placed opposite to the water flow, allowing the bait odor to flow out the opening. All the traps were set after 17:00h, when aeglids are more active (Bueno and Shimizu, 2008) and were left in the field overnight and retrieved the next morning.

D-frame hand nets were used to actively collect smaller aeglids (mainly juvenile individuals), before the traps were set. Other organisms involved in the trophic interactions of aeglids, based on the food sources already described as a food item for aeglids, were collected manually, and they included leaves, algae and moss.

Aeglids were separated from other material, placed in individual plastic bags and sacrificed via cooling. All the material was analyzed after collection in the laboratory and morphometric maturity was determined based on the carapace length estimated previously (Marçal *et al.*, 2017). Macroinvertebrates were identified in the laboratory with the help of literature (Mugnai *et al.*, 2010). Fishes and shrimp were confirmed with researchers working in ecology and zoology of continental waters in Brazil.

Water quality and abiotic factors

The chemical and microbiological characteristics of freshwater were characterized according to the methods recommended by the Standard Methods for the Examination of Water and Wastewater. Microbiological analyzes were performed using the multi-tube technique, according to PRC N° 5, of 09/28/2017 (ANVISA, 2017), which establishes the absence of thermotolerant coliforms in 100 ml of water as a potability standard. In individual samples from wells, sources, springs and other forms of supply without channeled distribution, the presence of total coliforms is tolerated in the absence of thermotolerant coliforms. Abiotic factors, such as pH, electrical conductivity (Ec – $\mu\text{S}/\text{cm}$), turbidity (Turb), temperature (Temp – $^{\circ}\text{C}$), total dissolved solids (TDS – mg/L), chlorophyll (Chl – $\mu\text{g}/\text{L}$) and dissolved oxygen (DO – mg/L), were measured using a multi-parameter Eureka probe (Manta model 2–4.0).

Isotopic signature and trophic structure

The stable isotope analysis was conducted at the Stable Isotopes Center, Institute of Biosciences, UNESP, Botucatu Campus. The material used for the isotopic analyses for *A. castro* were from 32 individuals, of which 20 were juveniles and 12 were adults (Tab. 1). Males and females were analyzed together, because of the small size of the animals and the fact that we sacrificed as few as was possible. We analyzed the musculature of chelipeds together with the exoskeleton, due to the small amount of musculature removed from the chelipeds. Samples of bulk sediment were collected from the middle of river. The $\delta^{15}\text{N}$ values of sediment were examined for use as a basis for predicting the $\delta^{15}\text{N}$ values of aeglids. Sediment has no trophic position, but it can be a long-

Table 1. *Aegla castro*. Number of individuals (juveniles and adults) and means (\pm standard deviation) of carapace length (CL mm) (juvenile and adults), for aeglids at Itatinga from São Paulo State (ITA: Itaúna stream, Paranapanema sub-basin) and Mauá da Serra (PRE: Preto stream, Tibagi sub-basin), Castro (IAP: Iapó stream, Tibagi sub-basin) and Ponta Grossa (QPE: Quebra Perna stream, Tibagi sub-basin) in Paraná State.

Locality	Number of juveniles	Number of adults	CL juveniles (mm)	CL adults (mm)
ITA	3	2	4.56 (\pm 0.02)	20.48 (\pm 0.56)
PRE	6	4	9.16 (\pm 0.68)	17.05 (\pm 3.24)
IAP	6	2	10.01 (\pm 0.57)	19.99 (\pm 2.17)
QPE	5	4	13.83 (\pm 2.20)	23.4 (\pm 1.72)

term integrator of particles and dead organisms from the water column, and it is readily sampled (Lake *et al.*, 2001). Adults may experience sediment intake, which may occur occasionally or associated with the consumption of organic matter, where algae, bacteria and other microorganisms grow (Collins, 2019). The leaves, algae and moss collected were washed to eliminate debris and invertebrates from their surfaces (Burress *et al.*, 2013). For the animals, the material used for analyzes was muscle between the dorsal and ventral fin of fish and abdomen muscle of the shrimp.

All the macroinvertebrates were of small size, so they were processed whole. All samples were dehydrated at 50 °C, in a drying oven with air circulation for 48 hours (Nagata *et al.*, 2015). The dried samples were ground for six minutes in a cryogenic mill to complete homogenization. For $\delta^{13}\text{C}$ analyses, aeglids and macroinvertebrates were soaked in 1 M HCl for 3 hours to remove carbonates from exoskeletons, then they were re-dried for 24 hours (Carabel *et al.*, 2006; Nagata *et al.*, 2015; Bosley *et al.*, 2017).

Aliquots of the homogeneous material (50–70 \pm 1 μg for $\delta^{13}\text{C}$ and 500–600 \pm 1 μg for $\delta^{15}\text{N}$) were analyzed in duplicate and placed in tin capsules (5 mm, Sercon), except for material with HCL, which were placed in silver capsules inside tin capsules (Carabel *et al.*, 2006). The capsules were weighed and submitted to a continuous flow isotope ratio mass spectrometry system. Afterwards, the samples were placed in an elementary analyzer (Flash EA - Thermo Scientific, Bremen, Germany) coupled to an isotope ratio mass spectrometer (Delta V Advantage - Thermo Scientific, Bremen, Germany) with an interface (ConFlo IV - Thermo Scientific, Germany) that determines the isotopic ratio ($R_{\text{sample}} = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$) of each sample. The results are given in the relative difference of isotope ratio (δ) to a standard value (R_{standard}) expressed in ‰ in accordance with:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1$$

For $\delta^{13}\text{C}$, the R_{standard} is V-PDB (Vienna Pee Dee Belemnite) and for $\delta^{15}\text{N}$ it is atmospheric air. The standard uncertainty ($n = 10$) of analyzes was ± 0.20 ‰ for $\delta^{13}\text{C}$ and ± 0.15 ‰ for $\delta^{15}\text{N}$.

To provide a broad view of the trophic structure of the collected organisms, the trophic level of the organisms was estimated based on the nitrogen values in relation to samples from primary producers and then sequentially, primary and secondary consumers. The estimate of the trophic level (TL) was calculated according to the formula presented by Post (2002):

$$\text{TL} = \lambda + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{base}}) / \Delta^{15}\text{N}$$

Where: λ value 1 was used, since the organisms used in $\delta^{15}\text{N}_{\text{base}}$ were considered primary producers; $\delta^{15}\text{N}_{\text{consumer}}$ is the value of each consumer; and $\Delta^{15}\text{N}$ is the enrichment in $\delta^{15}\text{N}$ by trophic level, that is, the assumed trophic enrichment factor (TEF) was 3.4 ‰ as proposed by Minagawa and Wada (1984).

Statistical analyses

The predictors and response data were filtered by the variance inflation factor (function *vif*). The variance inflation factor excludes the highly correlated variables from the dataset through a stepwise procedure to deal with multicollinearity problems (Zuur *et al.*, 2010). A Principal Component Analysis (PCA) was used to detect the relationship between the sampling areas (ITA, PRE, IAP, and QPE) and the abiotic factors (pH, Ec, Turb, Temp, TDS, Chl, and DO) (Clarke and Ainsworth, 1993). After the PCA analysis, the permutational multivariate analysis of variance (PERMANOVA) was done to test the differences between the areas of sampling.

Since values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures were directly connected to each other, the multivariate analysis of variance (MANOVA) was performed to determine differences between age (juvenile or adult) and location (ITA, PRE, IAP, and QPE tributaries). Following this test, a residual analysis was performed in R program. Then, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data were analyzed separately with factorial analysis of variance (ANOVA) to identify differences for each isotope by age and location, followed by post-hoc Tukey's tests to show the differences.

To produce biplots with the signatures of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, in order to demonstrate the trophic web (*A. castro* and potential food sources), the mixture model for stable isotopes was used, through the SIMMR package

(Parnell and Inger, 2016). SIMMr adapts to a Bayesian mixture model to determine estimates of contributions from dietary sources in the aeglid diet, using isotopic data (Parnell and Inger, 2016).

The R program was used for statistical and graphical analyses (R Development Core Team, 2011).

RESULTS

Water quality and abiotic factors

Microbiological analyzes indicated that for all studied sites the total coliforms and thermotolerant coliforms are higher than the amount considered potable (Tab. 2), and therefore in all areas of sampling there is indication of human waste. Chemical analyzes of aluminum, iron and copper showed amounts outside of the maximum allowed value (Tab. 2). For aluminum all areas of sampling showed values above the maximum allowed value (0.2 mg.L^{-1}), between 2.59 mg.L^{-1} to 6.65 mg.L^{-1} . In IAP, iron showed values above the maximum allowed value (0.3 mg.L^{-1}) with a recording of 0.70 mg.L^{-1} , and copper was recorded at 1.71 mg.L^{-1} which is above the maximum allowed value (1.5 mg.L^{-1}) in QPE (Tab. 2).

The variance inflation factor excluded highly correlated variables (TDS and DO) from the dataset. The PCA Axis 1 explained 47.6 % while Axis 2 explained 31.3 % of the variation, and the scores plotted

(Axis 1 and 2) showed a separation between the areas of sampling according to the environmental factors (Fig. 2). In QPE the abiotic factors were predominant and were positively selected. PRE and ITA showed opposite relationships to Ec, Turb and Chl, and was opposite to Iapó stream (IAP). The Permanova showed differences between the areas of sampling and abiotic factors ($F = 2.46$, $df = 3$, $p = 1e-04$).

Isotopic signature and trophic structure

The Mean \pm standard deviation (SD) values of isotopic signatures for *A. castro* studied here were $-25.00 \text{ ‰} \pm 4.88$ for $\delta^{13}\text{C}$ (range $-33.04 \text{ ‰} - -13.53 \text{ ‰}$) and $7.64 \text{ ‰} \pm 3.04$ for $\delta^{15}\text{N}$ (range $3.54 \text{ ‰} - 13.96 \text{ ‰}$). We found different isotopic signatures in the sampled areas. For example, the highest values for $\delta^{15}\text{N}$ and the most negative for $\delta^{13}\text{C}$ were in IAP; the lowest values for $\delta^{15}\text{N}$ in QPE; the lowest negative for $\delta^{13}\text{C}$ in PRE; and intermediate values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were both in ITA (Fig. 3).

There was no difference between the juvenile and adult ontogenetic stages according to the combined $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (MANOVA, $F_{\text{ontogenetic}} = 1.15$, $Df = 2$, $p = 0.32$) values. Differences between locations were observed (MANOVA, $F_{\text{location}} = 32.60$, $Df = 6$, $p < 2e-16$). When $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were analyzed separately, differences were still not observed for the ontogenetic stages of juvenile and adult (ANOVA $\delta^{13}\text{C}$,

Table 2. Abiotic factors (mean \pm standard deviation), water quality, microbiological and chemical analysis in ITA (São Paulo State) and PRE, IAP and QPE (Paraná State), Brazil. Legend of abiotic factors: pH, Ec (Conductivity), Turb (Turbidity), Temp (Temperature), TDS (Total dissolved solids), Chl (Chlorophyll), DO (Dissolved oxygen). ITA: Itaúna stream, PRE: Preto stream, IAP: Iapó stream, QPE: Quebra Perna stream.

Abiotic factors	ITA	PRE	IAP	QPE
pH	8.55 ± 0.06	8.94 ± 0.23	8.96 ± 0.04	8.95 ± 0.24
Ec ($\mu\text{S/cm}$)	61.78 ± 0.15	39.26 ± 0.05	115.94 ± 0.40	90.34 ± 0.05
Turb	4.20 ± 0.52	2.04 ± 1.62	13.01 ± 0.96	5.03 ± 2.05
Temp ($^{\circ}\text{C}$)	22.91 ± 0.00	15.77 ± 0.10	17.13 ± 0.06	18.48 ± 0.02
TDS (mg/l)	39.51 ± 0.10	25.10 ± 0.02	74.18 ± 0.25	57.80 ± 0.14
Chl ($\mu\text{g/L}$)	1.43 ± 0.01	1.76 ± 0.08	3.57 ± 0.27	1.41 ± 0.01
DO (mg/l)	8.02 ± 0.01	9.15 ± 0.08	7.14 ± 0.06	8.73 ± 0.02
Microbiological analysis				
Total coliforms	7.3/100 mL	3.6/100 mL	>23/100 mL	>23/100 mL
Thermotolerant coliforms	7.3/100 mL	3.6/100 mL	9.2/100 mL	16.1/100 mL
Chemical analysis				
	Aluminum (6.65 mg.L^{-1})	Aluminum (3.28 mg.L^{-1})	Iron (0.70 mg.L^{-1}) Aluminum (2.59 mg.L^{-1})	Copper (1.71 mg.L^{-1}) Aluminum (4.36 mg.L^{-1})

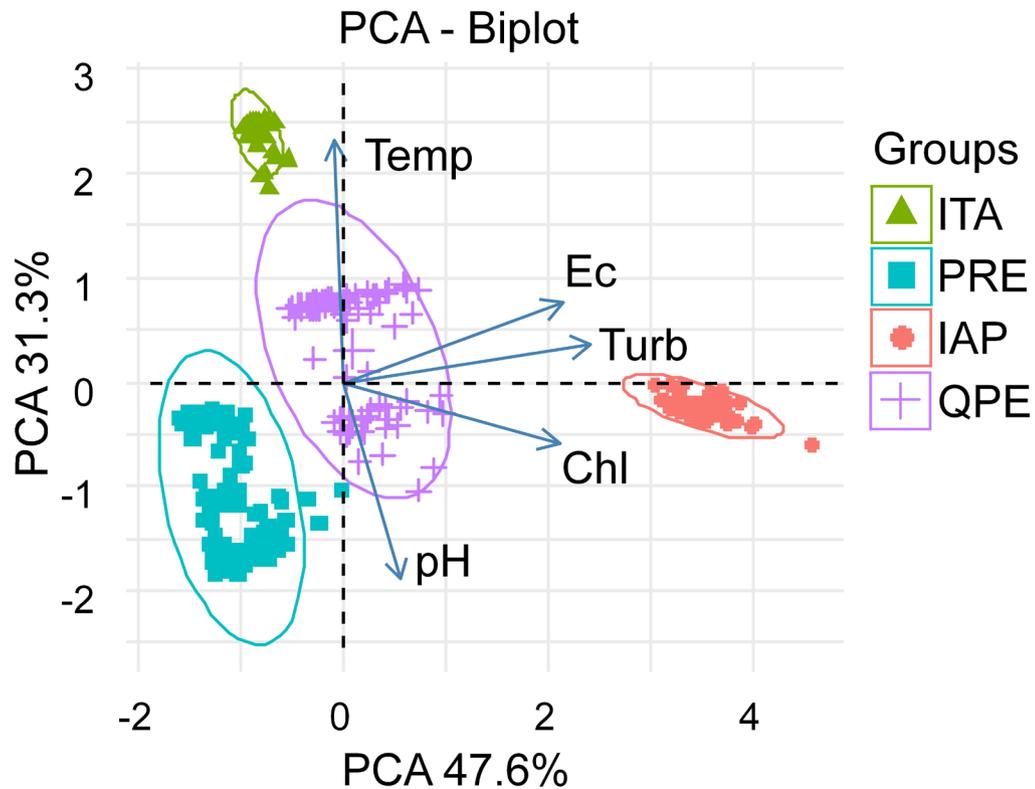


Figure 2. Results from the Principal Components Analysis (PCA) from the different sampling areas (ITA, PRE, IAP, and QPE) according to abiotic factors: pH, Ec (Conductivity), Turb (Turbidity), Temp (Temperature), Chl (Chlorophyll). The canonical axes explained 78.9% of the variation. ITA: Itaúna stream, PRE: Preto stream, IAP: Iapó stream, QPE: Quebra Perna stream.

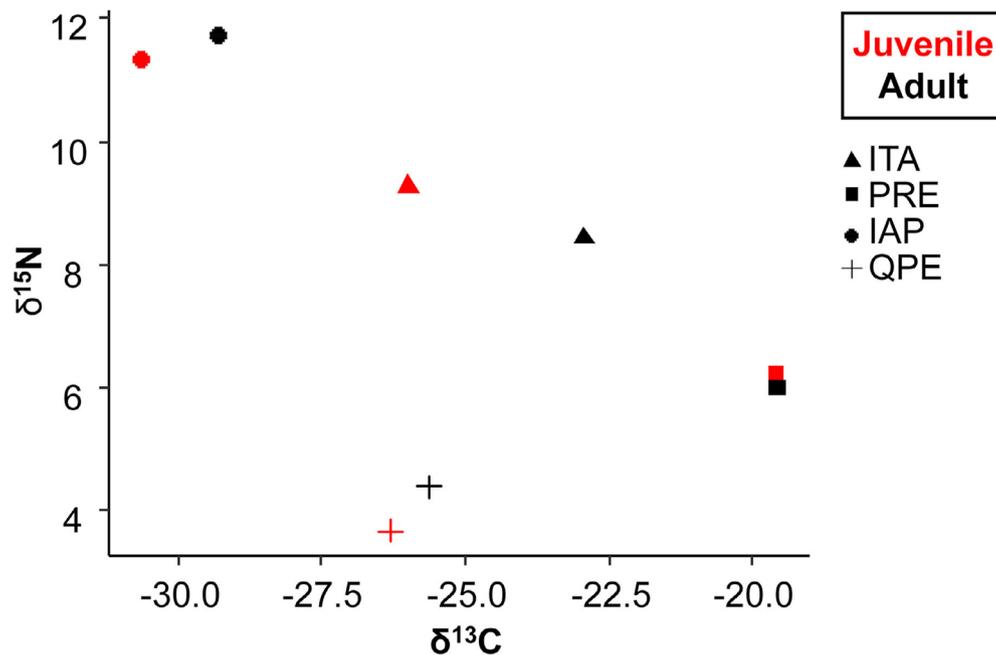


Figure 3. Isotopic biplot of variation in mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) values calculated for juveniles and adults of *Aegla castro* at Itatinga from São Paulo State (ITA: Itaúna stream, Paranapanema sub-basin) and Mauá da Serra (PRE: Preto stream, Tibagi sub-basin), Castro (IAP: Iapó stream, Tibagi sub-basin) and Ponta Grossa (QPE: Quebra Perna stream, Tibagi sub-basin) in Paraná State.

$F_{\text{ontogenetic}} = 1.55$, $Df = 1$, $p = 0.22$; ANOVA $\delta^{15}\text{N}$, $F_{\text{ontogenetic}} = 0.01$, $Df = 1$, $p = 0.98$), respectively. For the different sampling areas, the differences were evident for C and N analyzed separately, *i.e.*, $\delta^{13}\text{C}$ (ANOVA, $F_{\text{location}} = 17.79$, $Df = 3$, $p = 3.48\text{e-}07$) and $\delta^{15}\text{N}$ (ANOVA, $F_{\text{location}} = 92.43$, $Df = 3$, $p = 2\text{e-}16$). These differences and similarities of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between sampling areas according to the Tukey test are shown in Fig. 4.

Nineteen distinct isotopic signatures were analyzed for the representative organisms that co-habit with *A. castro*. These include fish, macroinvertebrates, leaves, moss, shrimp, algae and sediment (Tab. 3). As we found no difference in the diet of juveniles and adults, we studied them in the trophic web as *A. castro*, without making any distinction between these stages.

Leaves (ITA), sediment (PRE and IAP), and algae (QPE) were identified as the first level of the food web and *A. castro* was a primary consumer together with other organisms such as macroinvertebrates, *Astyanax bockmanni* Vari and Castro, 2007 and *Macrobrachium*

sp. The secondary consumers were characiform and siluriform fishes (PRE), and the cyprinodontiform fishes *Phalloceros harpagos* Lucinda, 2008 in IAP and *Phalloceros pellos* Lucinda, 2008 in QPE. We did not find any top predators (above TL 4) in our study (Tab. 3, Fig. 5).

It is possible to propose that *A. castro* feeds on items such as leaves, algae, moss and sediment and that this might be the same niche as Megaloptera in ITA and Coleoptera in PRE (Fig. 5). However, the values of the base of the food chain are different between the areas of sampling. These differences could be caused by the environments or by human actions that enrich the environment with higher nitrogen values. This can be seen when comparing the leaf values of $\delta^{15}\text{N}$ 5.68 ‰ in ITA, $\delta^{15}\text{N}$ 3.28 ‰ in PRE, $\delta^{15}\text{N}$ 10.03 ‰ in IAP, and $\delta^{15}\text{N}$ 0.81 ‰ in QPE. We emphasize that the base value at IAP (starts with $\delta^{15}\text{N}$ 8.35 ‰) is a high value compared to the other areas of sampling with leaves $\delta^{15}\text{N}$ 5.68 ‰ in ITA, sediment $\delta^{15}\text{N}$ 2.63 ‰ in PRE, and algae $\delta^{15}\text{N}$ 0.44 ‰ in QPE (Tab. 3, Fig. 5).

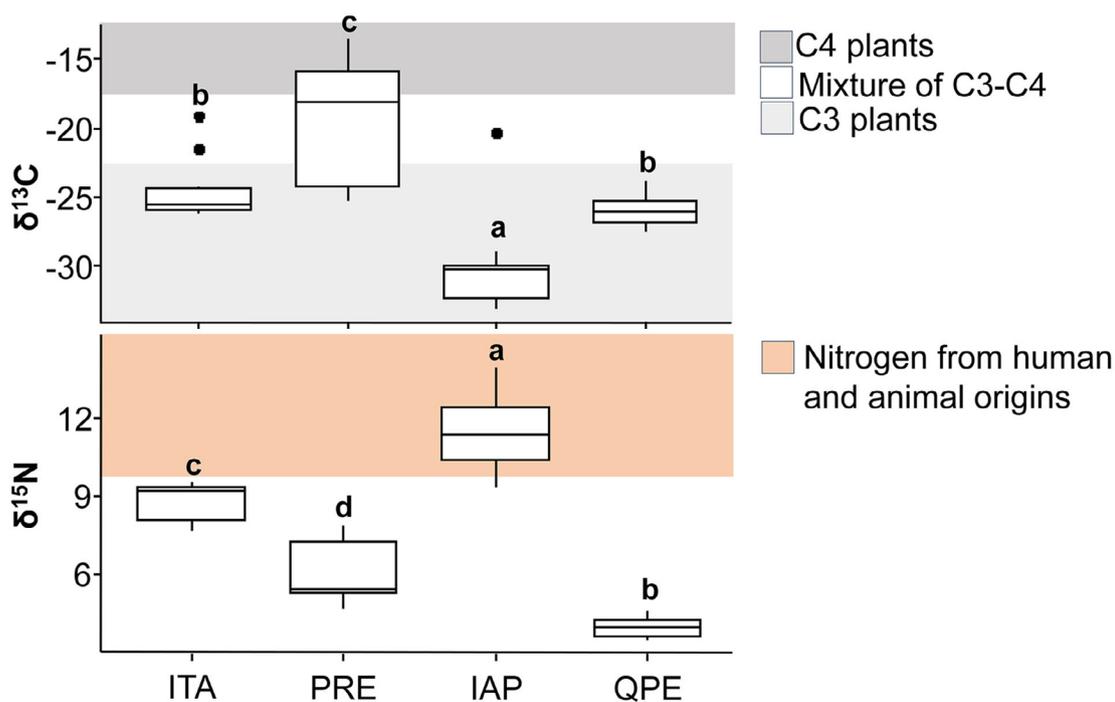


Figure 4. Variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for *Aegla castro* at Itatinga from São Paulo State (ITA: Itaúna stream, Paranapanema sub-basin) and Mauá da Serra (PRE: Preto stream, Tibagi sub-basin), Castro (IAP: Iapó stream, Tibagi sub-basin) and Ponta Grossa (QPE: Quebra Perna stream, Tibagi sub-basin) in Paraná State. Median values are represented by horizontal lines inside boxes (first and third quartiles); vertical lines represent minimum and maximum values. Differences between samples are indicated by different letters (Tukey test, $p < 0.05$). In C_3 plants, the value of $\delta^{13}\text{C}$ ranges from -22 ‰ to -34 ‰ (light gray), in C_4 plants from -9 ‰ to -16 ‰ (gray), and intermediate values between C_3 and C_4 (white) (Vogel, 1993; Boutton, 1996; Ducatti *et al.*, 2011). $\delta^{15}\text{N}$ values considered to come from human and animal origins are indicated by the orange bar (Heaton, 1986; McClelland and Valiela, 1998; Hoffman *et al.*, 2012).

Table 3. Stable isotope signatures for carbon and nitrogen (mean \pm SD) and the estimated trophic level of the organisms, in the freshwater community trophic web with *Aegla castro* at ITA (São Paulo State) and PRE, IAP and QPE (Paraná State), Brazil. ITA: Itaúna stream, PRE: Preto stream, IAP: Iapó stream, QPE: Quebra Perna stream.

Localities	Sources	$\delta^{13}\text{C}\text{‰}$	$\delta^{15}\text{N}\text{‰}$	Estimated Trophic Level
ITA - Itatinga	Leaves	-33.01 (\pm 0.23)	5.68 (\pm 0.13)	1.0
	Algae	-29.96 (\pm 0.30)	7.67 (\pm 0.85)	1.58
	Megaloptera	-25.54 (\pm 0.08)	8.73 (\pm 0.00)	1.89
	<i>Aegla castro</i>	-24.46 (\pm 2.33)	8.85 (\pm 0.77)	1.93
	Sediment	-27.83 (\pm 0.07)	10.61 (\pm 0.31)	2.45
	<i>Astyanax bockmanni</i>	-26.75 (\pm 1.46)	11.27 (\pm 0.04)	2.64
	Oligochaeta	-25.20 (\pm 0.04)	11.69 (\pm 0.01)	2.76
	<i>Macrobrachium</i> sp.	-25.79 (\pm 0.08)	11.70 (\pm 0.10)	2.77
PRE - Mauá da Serra	Sediment	-27.97 (\pm 0.13)	2.63 (\pm 0.37)	1.0
	Leaves	-31.11 (\pm 0.26)	3.28 (\pm 0.01)	1.19
	Moss	-26.53 (\pm 0.32)	4.08 (\pm 0.08)	1.43
	Coleoptera	-21.54 (\pm 0.11)	5.80 (\pm 0.16)	1.93
	<i>Aegla castro</i>	-19.59 (\pm 4.65)	6.12 (\pm 1.17)	2.03
	Trichoptera	-25.46 (\pm 0.01)	6.13 (\pm 0.13)	2.03
	Algae	-25.68 (\pm 0.06)	6.30 (\pm 0.60)	2.08
	Ephemeroptera	-24.06 (\pm 0.11)	6.51 (\pm 0.11)	2.14
	Megaloptera	-24.28 (\pm 0.04)	6.99 (\pm 0.39)	2.28
	Plecoptera	-23.41 (\pm 0.14)	7.93 (\pm 0.25)	2.56
	Odonata	-22.50 (\pm 0.21)	8.26 (\pm 0.03)	2.66
	Diptera (Chironomidae)	-24.61 (\pm 0.24)	8.29 (\pm 0.22)	2.66
Characiformes	-22.93 (\pm 0.07)	10.20 (\pm 0.36)	3.23	
Siluriformes	-24.49 (\pm 0.06)	11.07 (\pm 0.09)	3.48	
IAP - Castro	Sediment	-26.75 \pm 0.14	8.35 \pm 0.13	1.00
	Leaves	-29.48 \pm 0.54	10.03 \pm 0.03	1.49
	Algae	-35.57 \pm 3.01	11.29 \pm 0.91	1.86
	<i>Aegla castro</i>	-29.99 \pm 3.70	11.54 \pm 1.54	1.93
	Odonata	-34.81 \pm 0.05	13.03 \pm 0.01	2.37
	<i>Phalloceros harpagos</i>	-35.04 \pm 0.13	15.17 \pm 0.01	3.00
QPE - Ponta Grossa	Algae	-32.39 \pm 0.54	0.44 \pm 0.13	1.00
	Leaves	-30.89 \pm 0.46	0.81 \pm 0.13	1.11
	Sediment	-26.07 \pm 0.66	3.96 \pm 0.65	2.04
	<i>Aegla castro</i>	-25.96 \pm 1.14	4.03 \pm 0.40	2.06
	<i>Phalloceros pellos</i>	-24.00 \pm 0.42	8.94 \pm 0.25	3.50

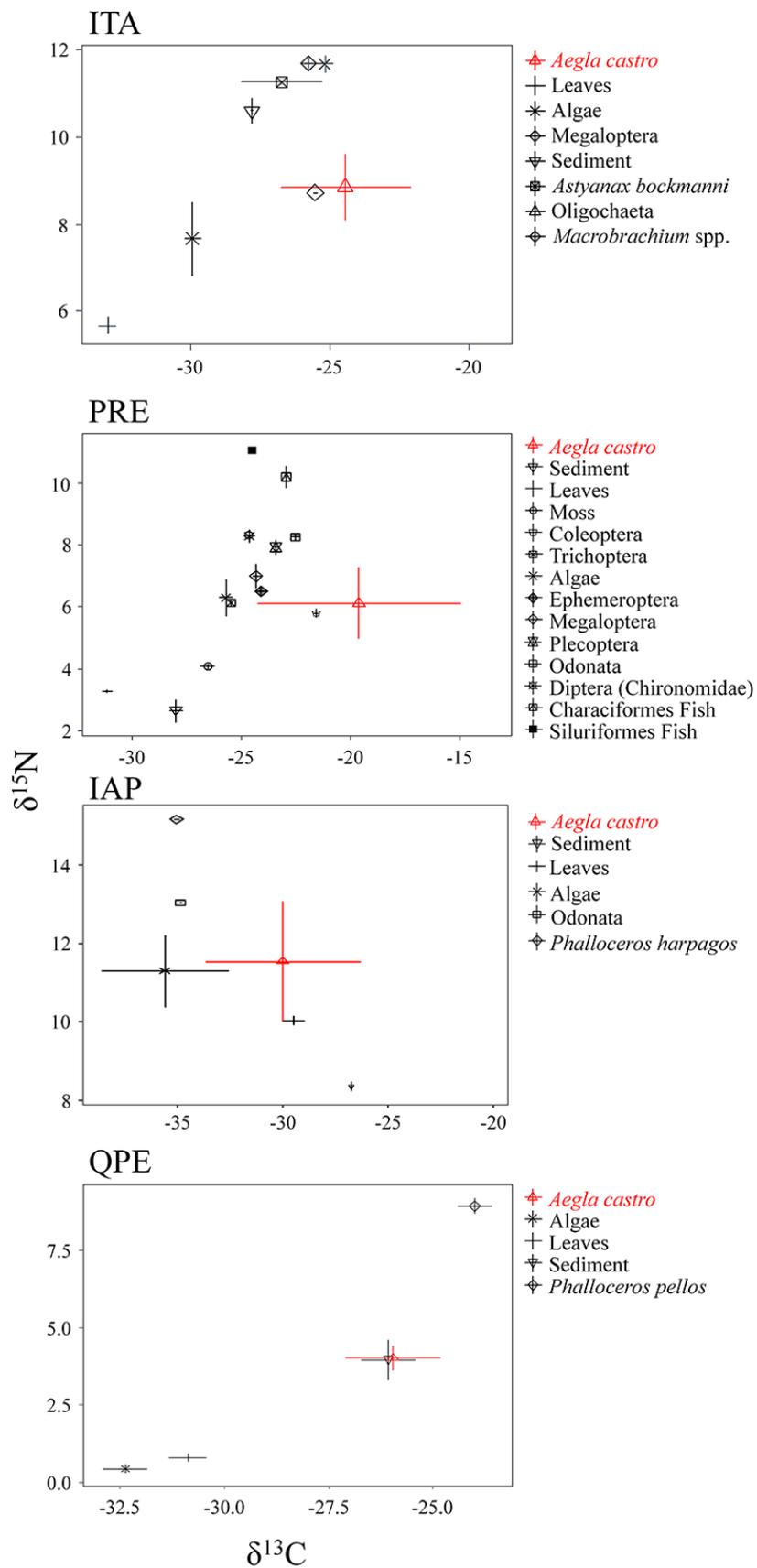


Figure 5. Biplots of stable isotopes using the mean for $\delta^{13}C$ and $\delta^{15}N$ (\pm SD). Calculated values for *Aegla castro* and potential food sources from ITA (Itaúna stream), PRE (Preto stream), IAP (Iapó stream), and QPE (Quebra Perna stream).

DISCUSSION

Aegla castro is an important link (primary consumer) between producers and other trophic levels in all locations and we reject the hypothesis that there are differences between the juvenile and adult diets, indicating that their food sources are independent of life stage, due to their direct development or because they use similar food resources during their ontogenetic development, and probably because they forage in the same locations, having the same food items available for both phases. So, they usually eat the same resource available to the adults in the habitat (Bond-Buckup *et al.*, 1996). Santos *et al.* (2008) found that adult and juvenile females of *Aegla longirostri* Bond-Buckup and Buckup, 1994 also did not show differences in their diets, which may be due to having the same distribution range in the water flow.

Otherwise, Bueno and Bond-Buckup (2004) showed differences between the juvenile and the adult diets of *Aegla platensis* Schmitt, 1942 and *Aegla ligulata* Bond-Buckup and Buckup, 1994 proposing that the difference in growth pattern, including differences in size, influenced the dietary variation. Burress *et al.* (2013) showed a difference for $\delta^{13}\text{C}$ between the ontogenetic phases of *Aegla uruguayana* Schmitt, 1942, suggesting a shift in the relative importance of carbon sources, but this difference was not significant for $\delta^{15}\text{N}$. So, the authors suggested that *A. uruguayana* feeds within the same trophic level in both life stages.

Water quality and abiotic factors

The presence of coliforms, aluminum (in all locations), iron (in IAP) and copper (in QPE) can be explained by both natural and anthropogenic actions. Natural actions, could be the presence of wild animal fecal material or even the type of soil found in all regions studied, which is an alic soil type, with aluminum saturation above 50 % (EMBRAPA, 2006), for example. Anthropogenic actions, on the other hand, can come from waste from farms (ITA), restaurants (PRE), agricultural practices and animal production (IAP), and waste deposited around streams or that reach them improperly (Nriagu, 1990; Brown and Peake, 2006; Jordão *et al.*, 2007; Kinsella and Crowe, 2016) via water treatment plants

(Gobena *et al.*, 2017; Sillanpää *et al.*, 2018), animal waste (Green and Kramer, 1979), or metallurgical industries (Douay *et al.*, 2008), among other sources. In addition, organic matter, as well as chemical elements, can be carried by rain and running water, coming from other locations, and be influencing the results at these collection points (Pereira *et al.*, 2020). Our objective with these analyzes was not to demonstrate exactly the origins of these alterations, but to show that these alterations are indicative of modifications in the natural environment and, consequently, this group of animals, sensitive to alterations, are also affected.

The areas of study were different according to the variable environmental factors. For example, pH did not show great variation between locations, remaining within the range of normal aquatic life with values between 6–9, according to CONAMA Resolution 357/05, and the temperature found in all locations were within the range already described for *A. castro*, from 15 °C to 22 °C (Swiech-Ayoub and Masunari, 2001). These factors though, would be less influencing the presence of *A. castro* at the localities, since they are within a tolerable range for the species. Other factors, such as Ec, Turb, and Chl indicate good conditions at QPE, but the opposite was demonstrated at IAP, PRE, and ITA showed intermediate values for water quality and abiotic factors.

IAP shows signs of being the most impacted region, for example, electrical conductivity values greater than 100 $\mu\text{S}\cdot\text{cm}$ is an indication of an impacted environment (CETESB, 2007). This sampling site also demonstrates an increase in turbidity that is most-likely related to the presence of animal and plant organic matter, in addition to suspended sediments from rocks and soil (Perdigão *et al.*, 2018).

Isotopic signature and trophic structure

We found differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and their trophic structure was characterized by distinct isotopic signatures. These differences are due to environmental differences, which alter the rates of these components between the sampling areas, a factor that generates different trophic interactions. Furthermore, we observed significant anthropogenic influence through high nitrogen values. Thus, in different streams these aeglids can behave similarly, in a low trophic position, but within different trophic webs shaped by the environment.

Primary producers are important groups for the functioning of ecosystems, due to their role as energy suppliers for the base of the trophic web (Daufresne and Loreau, 2001). Sediment, on the other hand, has no trophic position, but it can be a long-term integrator of particles and dead organisms from the water column (Lake *et al.*, 2001). So, these organic or inorganic elements supplying energy to primary consumers significantly contribute to the diet of *A. castro*.

Our sampled rivers ranged in width and degree of overhanging riparian vegetation. In our results, most of the $\delta^{13}\text{C}$ values are found within the range of C_3 plants, except in PRE, where Coleoptera ($-21.54 (\pm 0.11)$) and *A. castro* $-19.59 (\pm 4.65)$ showed values just above the -22 ‰. These slightly elevated values may indicate consumption of some C_4 plants. Consequently, the diversity of carbon inputs into each river probably plays a large role in influencing the resulting $\delta^{13}\text{C}$ signatures, and, the most negative carbon values may indicate some feeding from terrestrial carbon sources (Rounick and Winterbourn, 1986), which are more typical of shredders (Vannote *et al.*, 1980). We did not find aeglids out of the water, but there are reports in populations along some stretches of the Paraná state streams of these animals being seen out of the water, making it possible for them to feed on carbon sources from outside the stream.

In our study, the $\delta^{15}\text{N}$ values at the base of the food chain (carbon sources and sediment) were different between sampling areas, indicating that there is a different enrichment of nitrogen between the locations, supplying different trophic webs. The $\delta^{15}\text{N}$ values of primary producers increase with anthropogenic waste discharge (McClelland and Valiela, 1998) and are transferred to consumers through trophic relationships (Cabana and Rasmussen, 1996; Hansson *et al.*, 1997). For example, when we observe the sediment values in IAP ($8.35 \text{ ‰} \pm 0.13$) and ITA ($10.61 \text{ ‰} \pm 0.31$) compared with PRE ($2.63 \text{ ‰} \pm 0.37$) and QPE ($3.96 \text{ ‰} \pm 0.65$), as well as the highest leaf ($10.03 \text{ ‰} \pm 0.03$) and algae ($11.29 \text{ ‰} \pm 0.91$) values from IAP compared to the other sampling areas, it is evident that the base of the chain in IAP is enriched with nitrogen. The sediment was therefore enriched around ~ 7 ‰ between the different sampling areas. Tanu *et al.* (2020) reported that plants in areas facing human disturbances had $\delta^{15}\text{N}$ values of 5–14 ‰, while unaffected areas had

values only up to 3 ‰, suggesting that anthropogenic nitrogen may be regulating $\delta^{15}\text{N}$. Nitrate (NO_3) from human and animal waste is enriched in $\delta^{15}\text{N}$, with isotopic compositions of ~ 10 ‰ to ~ 22 ‰, due to denitrification and ammonia volatilization (Heaton, 1986; McClelland and Valiela, 1998; Hoffman *et al.*, 2012). Thus, we can consider that IAP is the location with the greatest anthropogenic interference through the enrichment of nitrogen in several items, and that ITA shows the beginning of an increase in nitrogen, through what we observed in the sediment.

The factors that could alter $\delta^{15}\text{N}$ values in this environment are sewage and fertilizer discharge (Tanu *et al.*, 2020). Variation in $\delta^{15}\text{N}$ values of primary consumers may be due to anthropogenic impacts (Cabana and Rasmussen, 1996) via isotopic fractionation during ammonification (Heaton, 1986). In IAP, where animal farming and agriculture are the main economic activities, the high $\delta^{15}\text{N}$ values could be influenced by discharge from these activities.

Aeglids, together with other organisms (macroinvertebrates, *A. bockmanni* and *Macrobrachium* sp.) were found to be primary consumers. There are a few studies that attempt to build the trophic web for, or at least indicate the trophic position of *Aegla* spp., but we know that as a primary consumer they are an important link between producers and higher level consumers, and as detritivores, they directly facilitate the reduction of organic particles, simplifying their transport to other environments (Castro-Souza and Bond-Buckup, 2004; Santos *et al.*, 2008; Cogo and Santos, 2013). Burrell *et al.* (2013) showed that *A. uruguayana* occupies a low trophic position with an isotopic signature around 9 ‰. Biasi *et al.* (2019) demonstrated that shredders, like *Aegla* spp., might have feeding preferences for grass species that do not contribute significantly to higher trophic levels, due to their low enrichment in tissues. Other studies that did not have *Aegla* spp. as a focus, but studied them as part of the trophic web, described their isotopic ratios: in Lago Moreno Oeste, 7 ‰ (Bonato *et al.*, 2018); in Lago Moreno Leste, 8.4 ‰; and in Lago Nahuel Huapi, 5.9 ‰ and 5 ‰ (Argentina) (Arcagni *et al.*, 2013; Arcagni *et al.*, 2015). These values found for *Aegla* spp. are similar to those found here for *A. castro*, except in IAP that presented higher values, as already reported.

We noted that *A. castro* may be feeding on items low in the trophic web, such as leaves, algae, moss and sediment. Ingestion of plant debris as the main food item in aeglids has been previously demonstrated. Plants debris was found represented between 45–90 % in the stomachs of aeglids (Bueno and Bond-Buckup, 2004; Castro-Souza and Bond-Buckup, 2004; Santos *et al.*, 2008). Other studies have also found sediment as a part of aeglid diets (Bueno and Bond-Buckup, 2004; Castro-Souza and Bond-Buckup, 2004; Santos *et al.*, 2008; Burrell *et al.*, 2013). Its ingestion can occur accidentally during feeding (D’Incao *et al.*, 1990), due to the deliberate ingestion of organic matter (an easy source of energy), or by ingesting colonies of microorganisms, algae and bacteria (Graça, 2001; Bueno and Bond-Buckup, 2004; Castro-Souza and Bond-Buckup, 2004).

The large standard deviation for carbon for the aeglids in our study indicate a wider range of food sources. Furthermore, assuming aeglids are omnivores, *A. castro* probably overlaps for food resources with Megaloptera in ITA and Coleoptera in PRE, as they are close to each other in the trophic web. Megaloptera are known predators, feeding on parts of or whole animals (Merritt *et al.*, 2017) and aquatic Coleoptera are generally scrapers, feeding on periphyton (attached algae and associated material) or herbivores (grazing mineral and organic surfaces) (Merritt *et al.*, 2017). Thus, food items may be available in a similar way for these different groups.

At ITA, *A. bockmanni* and *Macrobrachium* sp. are primary consumers, while Characiformes and Siluriformes (PRE), *P. harpagos* in IAP and *P. pellos* in QPE are secondary consumers. The fish and shrimp do not present themselves as possible prey for aeglids, but aeglids could serve as a food source for these animals, particularly as juveniles, and especially for fish, which occupy higher trophic levels. Fish show plasticity in the food they consume and have different classifications in terms of their eating habits (Schneider *et al.*, 2011; Rocha *et al.*, 2009; Bonato and Fialho, 2014). *Macrobrachium* sp. is considered omnivorous (Collins *et al.*, 2007), but with an important carnivorous component (Lima *et al.*, 2014). *Macrobrachium nipponense* (De Haan, 1849) was shown to have a high value for $\delta^{15}\text{N}$ and its trophic position was also high (TL = 3.38), along with

several fish (Mirzajani *et al.*, 2020). As demonstrated in our study, *Macrobrachium* sp. and fish showed great versatility in their diets, ingesting many items that were below them in the food web.

In addition, the large standard deviation found for aeglids from all locations in our study, could indicate a range of other food sources that we did not sample. Furthermore, it is known that anomurans can shift their diets. The migration of *Aegla* was noted for *Aegla paulensis* Schmitt, 1942 (see López, 1965), where this species could move a distance of approximately 300 m along a stream. We, therefore, consider that the variable isotopic values for *A. castro* may reflect food sources from other regions, accessed both by the displacement of individuals or brought in by flood water. The omnivorous benthic behavior of aeglids regulates the flow of energy and the recycling of nutrients (Evans-White *et al.*, 2001; Buck *et al.*, 2003), as their food is a combination of resources with different conversion efficiency (Krivan and Diehl, 2005).

Our study demonstrates that aeglids are important components of the trophic web, occurring at low trophic levels and acting as important links between producers and other levels. We also found differences in the carbon and nitrogen isotopic ratios for *A. castro* and for the other organisms in the food web between the sampling areas. Differences in the $\delta^{15}\text{N}$ values at the base of the food web between the sampled areas, indicate the possible influence of anthropogenic activity that can modify local enrichment of streams and interfere in trophic relationships. Studies like this one are becoming increasingly important due to the rapid degradation of freshwater environments and the lack of trophic knowledge about their endemic animals. Similarly, it is important to understand how anthropogenic action is interfering with trophic relationships, and how it can affect the permanence of critical species such as aeglids.

ACKNOWLEDGEMENTS

The authors are indebted to the “Fundação de Amparo à Pesquisa do Estado de São Paulo” (2016/20177-0, 2019/00587-8), “Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq” (PQ 311034/2018-7 to ALC), and “Coordenação de Aperfeiçoamento de Nível Superior - CAPES”

(Ciências do Mar II 23038.004310/2014-85, PROEX 23038.000802/2018-25, and CAPES Print 88881.310767/2018-01) for funding. We also appreciate the “Instituto Chico Mendes de Conservação da Biodiversidade” (ICMBio), and the “Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis” (IBAMA) for granting permission to collect the samples.

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