UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS

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ESTRUTURA TRÓFICA DE CRUSTÁCEOS DO GÊNERO *AEGLA* (PLEOCYEMATA: ANOMURA), ENDÊMICOS DAREGIÃO NEOTROPICAL DA AMÉRICA DO SUL: UMA ABORDAGEM UTILIZANDO A FERRAMENTA ISÓTOPOS ESTÁVEIS

DISSERTAÇÃO DE MESTRADO

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CONSIDERAÇÕES INICIAIS

Os eglídeos são crustáceos endêmicos da região Neotropical da América do Sul, com limite norte de distribuição no município de Claraval, no Estado de Minas Gerais (Brasil), e limite Sul na Ilha Madre de Dios, Província de Ultima Esperanza (Chile) (Bueno et al., 2007; Oyanedel et al., 2011). São restritos ao ambiente límnico, encontrados em lagos, rios de correnteza, e arroios (Feldmann, 1984; Feldmann et al., 1998; Francisco et al., 2007; Bueno et al., 2007), em ambientes com baixas temperaturas e altos teores de oxigênio (Feldmann et al., 1998; Bueno et al., 2007; Francisco et al., 2007; Oliveira et al., 2007).

Os eglídeos são considerados protagonistas na dinâmica de nutrientes e no fluxo de energia dos ecossistemas onde vivem, comportando-se como omnívoras generalistas e oportunistas (Castro-Souza and Bond-Buckup, 2004; Bueno and Bond-Buckup, 2004; Santos *et al*., 2008), portanto, um elo importante na teia trófica emáguas continentais. Podem se alimentar de matéria orgânica em decomposição, detritos vegetais, folhas e algas (Magni and Py-Daniel, 1989; Bueno and Bond-Buckup, 2004; Castro-Souza and Bond-Buckup, 2004), contribuindo com o processo de detritos vegetais, como demais crustáceos decápodos de ambientes límnicos, representando grande proporção na taxa de detritívoros, diminuindo as taxas de decomposição (Cogo and Santos, 2013; Cogo *et al*., 2014), além de se alimentaram de itens de origem animal, como macroinvertebrados (maioria Insecta) (Castro-Souza and Bond-Buckup, 2004; Colpo *et al*., 2012). Ademais, são fonte de alimento para outros organismos como jacarés, rãs, peixes, aves e mamíferos (Arenas, 1976; Pardini, 1998; Bueno and Bond-Buckup, 2004).

Essas características, os tornam de suma importância para se investigar o efeito sobre as populações locais de *Aegla* spp., diante das intensas práticas agrícolas, pastoris e urbanas, queprovocam a degradação de grandes extensões de mananciais hídricos. Essa degradação ambiental observada ao longo dos ecossistemas aquáticos continentais traz

consigo não somente prejuízos à qualidade de água, mas também, prejudica e altera consideravelmente as comunidades de invertebrados que habitam esses ambientes (Wetzel, 1993), desencadeando desequilíbrio de inúmeras espécies.

Atualmente, 89 espécies são conhecias do gênero *Aegla* (Bueno *et al*., 2017; Santos *et al*., 2017; Páez *et al*., 2018; Trombetta *et al*., 2019; Marçal *et al*., 2020; Marçal *et al*., 2021), sendo que um número considerável espécies vem sendo descobertas recentemente: *Aegla manuinflata* Bond-Buckup & Santos, 2009; *Aegla renana* Bond-Buckup & Santos, 2010,*Aegla georginae* Santos & Jara, 2013 e *Aegla ludwigi* Santos & Jara, 2013 (Santos *et al*.,2013), descritas no Rio Grande do sul; *Aegla pomerana* Bond-Buckup and Buckup, 2010; *Aegla muelleri* Bond-Buckup and Buckup, 2010; *Aegla brevipalma* Bond-Buckup & Santos, 2012; *Aegla leachi* Bond-Buckup Buckup, 2012 e *Aegla oblata* Bond-Buckup &Santos, 2012, descritas em Santa Catarina (Santos *et al*., 2012); *Aegla meloi* Bond- Buckup & Buckup, 2015; *Aegla loyolai* Bond-Buckup & Buckup, 2015; *Aegla lancinhas* Bond-Buckup & Buckup, 2015 (Santos *et al*., 2015), *Aegla marginata* Bond-Buckup & Buckup, 1994 (redescrita) (Moraes *et al*., 2017); *Aegla okora* Páez & Teixeira, 2018 (Páez*et al*., 2018), *Aegla charon* Bueno & Moraes (Bueno *et al*., 2017); *Aegla nebeccana* Trombetta e Teixeira (Trombetta *et al*., 2019) e *Aegla jacutinga* Marçal & Teixeira (Marçal *et al*., 2020), descritas no Paraná; *Aegla vanini* Moraes, Tavares & Bueno, 2016;*Aegla japi* Moraes, Tavares & Bueno, 2016; *Aelga jaragua* Moraes, Tavares & Bueno, 2016 e *Aegla jundiai* Moraes, Tavares & Bueno, 2016 (Moraes *et al*., 2016), descritas emSão Paulo e Paraná; *Aegla* quilombola Moraes, Tavares e Bueno 2017, descrita em São Paulo (Moraes, *et al*., 2017), e *Aegla chilota* Jara, Pérez-Losada e Crandall, na Ilha de Chiloé, na Patagônia ocidental (Jara *et al*., 2018).

Algumas espécies recentemente descritas já se encontram no Livro Vermelho dos Crustáceos do Brasil: Avaliação 2010-2014, publicado em 2016, como: *Aegla brevipalma*

Bond-Buckup & Santos, 2012 e *Aegla renana* Bond-Buckup & Santos, 2010, classificadas como criticamente em perigo; e *Aegla leachi* Bond-Buckup & Buckup, 2012; *Aegla manuinflata* Bond-Buckup & Santos, 2009, *Aegla oblata* Bond-Buckup & Santos, 2012 e *Aegla pomerana* Bond-Buckup & Buckup, 2010, classificadas como em perigo. Além de outras vinte espécies que também se encontram nesse livro, classificadas como criticamente em perigo, em perigo e vulnerável.

Desvendar claramente os hábitos alimentares e consequentemente o nível trófico das espécies é um processo demorado e trabalhoso, visto que são realizados através do conteúdo estomacal (Purcell and Sturdevant, 2001). Entretanto, esse método não analisa todos os itens alimentares, pelo fato de se encontrar itens em processo acelerado de digestão e pelos crustáceos dilacerarem suas presas, dificultando a identificação dos itens alimentares (Castro-Souza and Bond-Buckup, 2004). Dessa forma, nesta dissertação, optou-se por utilizar a técnica de isótopos estáveis, que são aqueles que ocorrem de forma comum na natureza,como o Carbono, Hidrogênio, Oxigênio, Nitrogênio e Enxofre (CHON'S) (Caxito and Silva, 2015). Em análises de estrutura trófica, posição trófica e identificação de itens alimentares, os isótopos mais utilizados são o carbono e o nitrogênio.

O carbono incorporado nas plantas é advindo do CO_2 atmosférico. Esse carbono sofre discriminação isotópica ao longo do processo de fotossíntese. Nas plantas C_3, o valor de $\delta^{13}C$ varia de -22 a -34‰ e nas plantas C_4 de -9 a -16‰ (Vogel, 1993; Boutton, 1996; Ducatti *et al*., 2011). A análise isotópica do carbono dos tecidos e o contraste existente na razão $^{13}C/^{12}C$, da ordem de 16‰ nos valores em $\delta^{13}C$ entre as plantas de ciclo fotossintético C_3 e C_4, possibilita a caracterização da dieta do animal (Ducatti *et al*., 2011). Em razão das plantas serem fonte primária de alimento aos animais, a assinatura isotópica dessas fontes é refletida nos tecidos específicos dos animais após certo tempo de

metabolismo (Gannes *et al*., 1998).

O nitrogênio pode ser incorporado pelas plantas através da associação com bactérias fixadoras de nitrogênio ou a partir da assimilação de compostos nitrogenados do solo (Caxito and Silva, 2015). Já nos animais, sua assimilação ocorre pela incorporação das proteínas, visto que o fator metabólico pode ser mais importante nos carnívoros, porque o nitrogênio animal é mais homogêneo bioquimicamente e dominante nas proteínas (principal componente da dieta carnívora) (Gannes *et al*., 1998). Dessa forma, o nitrogênio permite estudos para determinação de nichos tróficos e posição trófica, além de poder ser utilizado como indicação de interferência antrópica. Cabana and Rasmussen (1996) descreveram que impactos antropogênicos, como descargas esgoto ou dejetos de práticas agrícolas transportados aos ambientes aquáticos, são responsáveis por 68% da modificação das assinaturas isotópicas do nitrogênio dos consumidores primários. Assim, os sinais isotópicos de nitrogênio antropogênico são transferidos para os consumidores através de relações tróficas (Cabana and Rasmussen, 1996). Portanto, é necessário conhecer os organismos residentes em determinados locais, e como estes estão envolvidos nas interações tróficas, além de, avaliar a qualidade dos ambientes onde vivem.

Dessa forma, a carência de estudos sobre ecologia e teia trófica trófica em eglídeos de em águas continentais, considerando seu endemismo e condições ambientais restritas (baixastemperaturas e altos teores de oxigênio), ressaltam a importância desse tipo de estudo, principalmente no que diz as modificações nos riachos, causados por atividade humana, levando a instabilidade, e até mesmo o desaparecimento de populações neste tipo de ambiente.

Para uma melhor compreensão e embasamento sobre o tema, essa dissertação foi dividida em três capítulos, que se encontram em formato de artigos. O capítulo inicial tem

como objetivo identificar a assinatura isotópica de *Aegla castro* Schmitt, 1942 comparando seus estágios de vida com a hipótese de que há diferença nos itens alimentares consumidos entre os jovens e os adultos. Também observamos os valores de nitrogênio entre as áreas de amostragem, testando a hipótese de a dieta dos animais de locais impactados teriam maiores teores de N. O segundo capítulo tem como objetivo identificar a assinatura isotópica de *Aegla parana* Schmitt, 1942 comparando seus estágios de vida com a hipótese de que há diferença nos itens alimentares consumidos entre os jovens e os adultos, e estudar sua teia trófica no afluente do rio Iguaçu (Rio Palmital), em União da Vitória, para compreender seu papel trófico na teia alimentar, com a hipótese de que serão encontrados em níveis tróficos inferiores sendo elos importantes entre os produtores e os demais consumidores. O terceiro capítulo tem como objetivo estudar a teia trófica de *A. castro* nas bacias sudestee sul do Brasil, para compreender: seu papel trófico na teia alimentar, se as diferenças entre as áreas irão apresentar diferentes razões isotópicas para os eglídeos devido a diferença de itens alimentares ofertados pelas distintas áreas de amostragem e se em diferentes áreas de amostragem esses animais podem se comportar de forma semelhante,mas dentro de diferentes teias tróficas moldadas pela ação humana, devido ao enriquecimento de nitrogênio.

REFERÊNCIAS

Arenas, R.L. 1976. La cordillera de la costa como refúgio de la fauna dulcícola preglacial. *Archivos de Biologia y Medicina Experimentales*, 10: 1–40

Boutton, T.W. 1996. Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change. p. 47–82. In: T.W. Boutton and S.I. Yamasaki (eds), Mass spectrometry of soils. Marcel Dekker, New York.

Bueno, A.A.P. and Bond-Buckup, G. 2004. Natural diet of *Aegla platensis* Schmitt and *Aegla ligulata* Bond-Buckup and Buckup (Crustacea, Decapoda, Aeglidae) from Brazil. *Acta Limnologica Brasiliensia*, 16: 115–127.

Bueno, S.L.S.; Shimizu, R.M. and Rocha, S.S. 2007. Estimating the population size of *Aegla franca* (Decapoda: Anomura: Aeglidae) by mark-recapture technique from an isolated section of Barro Preto stream, county of Claraval, state of Minas Gerais, southeastern Brazil. *Journal of Crustacean Biology*, 27: 553–559.

Bueno, S.L.D.S.; Camargo, A.L. and Moraes, J.C.B. 2017. Uma nova espécie de eglídeo estigobítico de águas subterrâneas lênticas no sudeste do Brasil, com um traço morfológico incomum: pleópodes curtos em machos adultos. *Nauplius*, 25: e201700021.

Cabana, G. and Rasmussen, J.B. 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences*, 93: 10844–10847.

Castro-Souza, T. and Bond-Buckup, G. 2004. O nicho trófico de duas espécies simpátricas de *Aegla* Leach (Crustacea, Aeglidae) no tributário da bacia hidrográficado Rio Pelotas, Rio Grande do Sul, Brasil. *Revista Brasileira de Zoologia*, 21: 805– 813.

Caxito, F.A. and Silva A.V. 2015. Isótopos estáveis: fundamentos e técnicas aplicadas à Caracterização proveniência geográfica de Produtos alimentícios. *Geonomos*, 23: 10–17.

Cogo, G.B. and Santos, S. 2013. The role of aeglids in shredding organic matter in Neotropical streams. *Journal of Crustacean Biology*, 33: 519–526.

Cogo, G.B.; Biasi, C. and Santos, S. 2014. The effect of the macroconsumer *Aegla longirostri* (Crustacea, Decapoda) on the invertebrate community in a subtropical stream. *Acta Limnologica Brasiliensia*, 26: 143–153.

Colpo, K.D.; Ribeiro, L.C.; Wesz, B. and Ribeiro, L.O. 2012. Feeding preference of the South American endemic anomuran *Aegla platensis* (Decapoda, Anomura, Aeglidae). *Naturwissenschaften*, 99: 333–336.

Ducatti, C.; Martins, C.L.; Arrigoni, M.B.; Martins, M.B.; Vieira Jr., L.C. and Denadai, J.C. 2011. Utilização de isótopos estáveis em ruminantes. *Revista Brasileira de Zootecnia*, 40: 68–75.

Esteves, F.A. 1988. Fundamentos de limnologia. Rio de Janeiro, Ed. Interciência/Finep, 575p.

Feldmann, R.M. 1984. *Haumuriaegla glaessneri* n. gen. and sp. (Decapoda; Anomura; Aeglidae) from Haumurian (late Cretaceous) rocks near Cheviot, New Zealand. *Journal of Geology and Geophysics*, 27: 379–385.

Feldmann, R.M.; Veja, F.J.; Applegate, S.P. and Bishop, G.A. 1998. Early Cretaceous arthropods from the Tlayúa formation at Tepexi de Rodríguez, Puebla, México. *Journal of Paleontology*, 72: 79–90.

Francisco, D.A.; Bueno, S.L.S. and Kihara, T.C. 2007. Description of the first juvenile of *Aegla franca* Schmitt, 1942 (Crustacea, Decapoda, Aeglidae). *Zootaxa*, 1509:17–30. Gannes, L.Z.; Del-Rio, C.M. and Koch, P. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology.

Comparative Biochemistry Physiology, 119: 725–737.
Jara, C.G.; Pérez-Losada, M. and Crandall, K.A. 2018. *Aegla chilota*, new species of anomuran freshwater crab from Chiloé Island, western Patagonia. *Nauplius*, 26: 1– 11.
Livro Vermelho dos Crustáceos do Brasil: Avaliação 2010-2014. Organização: Marcelo Pinheiro & Harry Boos. Porto Alegre, RS: Sociedade Brasileira de Carcinologia - SBC, 2016.
Magni, S.T. and Py-Daniel, V. 1989. *Aegla platensis* Schmitt, 1942 (Decapoda: Anomura) a predator of Simuliidae immature stages (Diptera: Culicomorpha). *Revistade Saúde Pública*, 23: 258–259.
Marçal, I.C.; Páez, F.P.; Souza-Shibatta, L.; Sofia, S.H. and Teixeira, G.M. 2021. *Aegla buenoi*. sp. (Decapoda: Anomura): first record of aeglid crab from Cinzas River basin, Brazil. *Zootaxa*, 5005: 291–303.
Marçal, I.C.; Páez, F.P.; Souza-Shibatta, L.; Sofia, S.H. and Teixeira, G.M. 2020. Taxonomic revision of *Aegla lata* Bond-Buckup & Buckup, 1994 (Decapoda: Anomura: Aeglidae), with the description of a new species of *Aegla Leach*, 1820 fromthe Upper Paraná Ecoregion, Brazil. *Journal of Crustacean Biology*, 40: 425–434.
Moraes, J.C.B.; Terossi, M.; Buranelli, R.C.; Tavares, M.; Mantelatto, F.L. and Bueno, S.L.S. 2016. Morphological and molecular data reveal the cryptic diversity among populations of *Aegla paulensis* (Decapoda, Anomura, Aeglidae), with descriptions offour new species and comments on dispersal routes and conservation status. *Zootaxa*4193: 1–48.
Moraes, J.C.B.; Tavares, M. and Bueno, S.L.D.S. 2017. Taxonomic review of *Aegla marginata* Bond-Buckup & Buckup, 1994 (Decapoda, Anomura, Aeglidae) with description of a new species. *Zootaxa*, 4323: 519–533.
Oliveira, G.T.; Fernandes, F.A.; Bueno, A.A. and Bond-Buckup, G. 2007. Seasonal variations in the intermediate metabolism of *Aegla platensis* (Crustacea, Aeglidae). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 147: 600–606.
Oyanedel, A.; Valdovinos, C.; Sandoval, N.; Moya, C.; Kiessling, G.; Salvo, J. and Olmos, V. 2011. The southernmost freshwater anomurans of the world: Geographic distribution and new records of Patagonian aeglids (Decapoda: Aeglidae). *Journal of Crustacean Biology*, 31: 396–400.
Páez, F.P.; Marçal, I.C.; Souza-Shibatta, L.E.N.I.C.E.; Gregati, R.A.; Sofia, S.H. and Teixeira, G.M. 2018. A new species of *Aegla* Leach, 1820 (Crustacea, Anomura) fromthe Iguaçu River basin, Brazil. *Zootaxa*, 4527: 335–346.
Pardini, R. 1998. Feeding ecology of the Neotropical river otter *Lontra longicaudis* in an Atlantic Forest stream, southeastern Brazil. *Journal of Zoology*, 245: 385–391.
Purcell, J.E. and Sturdevant, M.V. 2001. Prey selection and dietary overlap among zooplanktivorous jellyfish and juvenile fishes in Prince William Sound, Alaska. *Marine Ecology Progress Series*, 210: 67–83.
Santos, S.; Ayres-Peres, L.; Cardoso, R.C. and Sokolowicz, C.C. 2008. Natural diet of the freshwater anomuran *Aegla longirostri* (Crustacea, Anomura, Aeglidae). *Journalof Natural History*, 42: 1027–1037.
Santos, S.; Bond-Buckup, G.; Buckup, L.; Pérez-Losada, M.; Finley, M. and Crandall, K.A. 2012. Three new species of *Aegla* (Anomura) freshwater crabs from the Upper Uruguay River hydrographic basin in Brazil. *Journal of Crustacean Biology*, 32: 529–540.
Santos, S.; Jara, C.G.; Bartholomei-Santos, M.L.; Pérez-Losada, M. and Crandall, K.A.

2013. New species and records of the genus *Aegla* Leach, 1820 (Crustacea, Anomura,Aeglidae) from the west-central region of Rio Grande do Sul, Brazil. *Nauplius*, 21: 211–223.

Santos, S.; Bond-Buckup, G.; Buckup, L.; Bartholomei-Santos, M.L.; Pérez-Losada, M.; Jara, C.G. and Crandall, K.A. 2015. Three new species of aeglidae (*Aegla* Leach, 1820) from Paraná state, Brazil. *Journal of Crustacean Biology*, 35: 839–849.

Santos, S.; Bond-Buckup, G.; Gonçalves, A.S.; Bartholomei-Santos, M.L.; Buckup, L. and Jara, C.G. 2017. Diversity and conservation status of *Aegla* spp. (Anomura, Aeglidae): an update. *Nauplius*, 25: 1–14.

Trombetta, A.S.; Páez, F.P.; Santos, S. and Teixeira, G.M. 2019. *Aegla nececcana* n. sp. (Crustacea, Aeglidae) from Ivaí Basin, Paraná, Brazil. *Zootaxa*, 4712: 138–150.

Vogel, J.C. 1993. Variability of carbon isotope fractionation during photosynthesis. p. 29–46. In: J.R. Ehleringer; A.E. Hall and G.D. Farquhar (eds), Stable isotopes and plant carbon: water relations. Academic Press, San Diego.

Wetzel, R.G. 1993. Limnologia. Lisboa, Portugal, Ed. Fundação Calouste Gulbenkian.

CHAPTER I

Isotopic signature of *Aegla castro* Schmitt, 1942 (Crustacea: Anomura) in different environments in southeastern and southern basins from Brazil

Abstract

Aeglids are endemic to the Neotropical region of South America associated to clean and well-oxygenated water, and are negatively affected by alterations in these habitat conditions. In this study, we investigated the stable isotope signature ($\delta^{13}C$ and $\delta^{15}N$) in juvenile-adult stages of anomuran crabs *Aegla castro* Schmitt, 1942 in Paranapanema sub-basin and in Tibagi sub-basin, from Brazil. The aeglids did not present differences in their isotopic signature for the life stages, probably due to the similar food itemsconsumed by juvenile-adults once they probably forage in the same places However, the natural resources and the resources of the sampling areas with anthropogenic disturbance, promoted different isotopic signatures. These results were an important indication of a greater amount of organic waste in the tributary of the Iapó River, observed through the lower value of δ13C and the higher value of δ15N when compared to other tributaries with better conservation status. As several species of aeglides are threatened by recent human disturbances in limnic environments, our results may be evidence of this.

Keywords: Carbon, endemic crab, environmental characteristics, nitrogen,ontogenetic phases.

INTRODUCTION

Aeglidae is an endemic crustacean to the Neotropical region, restricted to South-American freshwater ecosystem (Bond-Buckup and Buckup, 1994). This anomuran crabs have been frequently associated to clean and well-oxygenated water and are negatively affected by human changes to their habitat (Bond-Buckup, 2003).

Studies about aeglids have been intensified in recent years due to the fast habitat degradation and consequently disappearance of certain species in these aquatic ecosystems (Pérez-Losada *et al*., 2002; Bond-Buckup *et al*., 2010). Since in the last evaluation of the species at risk of extinction, 57 species of *Aegla* were found to be under threat or almost 70% of the 82 species evaluated (Santos *et al*., 2017). Introduction of exotic species, water pollution and dam construction without appropriate natural resource management planning are some of the several human interferences in freshwater environments (Pérez-Losada *et al*., 2002; Bond-Buckup *et al*., 2010).

In general, aeglids are considered omnivores, generalists and opportunists (Bueno and Bond-Buckup, 2004), feeding on decomposing organic matter, plant debris and alga, as well as aquatic insect larvae, such as Ephemeroptera, Neuroptera and Diptera (Burns, 1972; Magni and Py-Daniel, 1989; Bueno and Bond-Buckup, 2004).

Some published works on the diet of juvenile-adults show that some Aegla spp. do not have a different diet during their ontogenetic phases (Bueno and Bond-Buckup, 2004; Santos et al., 2008). These studies report that the non-difference between juvenile-adult diets for *Aegla platensis* Schmitt, 1942 and *Aegla ligulata* Bond-Buckup and Buckup, 1994 could be due to differences in species size, which could influence dietary variation (Bueno and Bond-Buckup, 2004) and also because these species have the same range of distribution in the stream (Santos et al., 2008).

However, other published works show that feeding habits of aeglids can change

throughout their development, especially when they are different species (Bueno and Bond-Buckup, 2004; Burress et al., 2013; Collins, 2019). Therefore, different diets between the ontogenetic phases can occur due to different factors, such as competition and cannibalism. However, the difference in the functional morphology of the mouthparts, locomotion system and sensory capacities can also lead to this difference in the diet between the phases. In addition, the differences in life cycles, which produce distinct size classes at different times of the year when different food items are available (Laughlin, 1982), or are needed for the processes of growth and reproduction.

Ontogenetic shifts in niche are important for organisms that present strong relationships between resource use, growth rate and predation risk, especially in relation to body size (Miller *et al*., 1988). These ontogenetic changes are ecologically important because they reduce competition (Werner and Gilliam, 1984), minimize predation risks caused by habitat changes (Werner *et al*., 1983) and maximize growth through dietary changes (Olson, 1996), leading to complex interactions and dynamics in communities (Persson, 1999).

The stomach content analysis is the most traditional method used to describe the feeding habit and the possible connection in the trophic web of several animal groups (Hyslop, 1980; Williams, 1981; Mantelatto and Christofoletti, 2001; Purcell and Sturdevant, 2001). However, this method has requirements and some limitations that should be considered: 1) sampling size, *i.e.*, a large number of individuals are needed; 2) advanced stages of digestion or fragmentation caused by the mouthpieces of crustaceans affect the accurate identification (Castro-Souza and Bond-Buckup, 2004); and 3) focuses only on recently consumed food items lacking information about long-term feeding or foraging location (Carmichael *et al*., 2004).

As aeglids usually have a small population size, the use of techniques that do not

require a large number of specimens is recommended. Therefore, we propose the use of stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) that have been used extensively by animal ecologists to elucidate patterns in food webs (Bearhop *et al*., 2004; Burress *et al*., 2013; Mao *et al*., 2016). Their utility lies in the fact that stable isotope ratios in the proteins of consumers reflect those of the proteins in their diet in a predictable manner (Hobson and Clark, 1992; Hobson, 1999). The carbon and nitrogen isotopic composition of consumer tissues are thus a function of: $\delta^{15}N$ and $\delta^{13}C$ of each prey species; the relative proportions of each prey species assimilated and in certain instances, foraging location. Moreover, the stable isotope signatures of tissues generally reflect the diet over the period during which the tissue was synthesized (Bearhop *et al*., 2002).

The $\delta^{13}C$ is produced by primary producers, one of the most important groups for the functioning of ecosystems, because of their energy-providing role (Daufresne and Loreau, 2001), for the base of trophic web. For example, the autotrophic protists are the dominant primary producers in aquatic ecosystems and contribute roughly 50% to global primary production, a similar importance of the land plants (Boenigk *et al*., 2018). As well, aquatic plants are important; as they interact and influence hydrological environments, interact with a wide range of other organisms, from microbes to vertebrates, for example, providing habitat and food (Wood *et al*., 2017).

The carbon incorporated in plants suffers isotopic discrimination throughout the process of photosynthesis. In C_3 plants (about 85% of plant species - a large part of dicots), the $\delta^{13}C$ value ranges from -22 to -34‰, and for C_4 plants (dicots and a large part of monocots, *e.g.* grasses, corn, rice), ranges from -9 to -16‰ (Vogel, 1993; Boutton, 1996; Ducatti *et al*., 2011), and makes it possible to characterize an animal's diet through isotopic analysis of the carbon in tissues (Ducatti, 2011). Plants are the primary source of food for animals, so the isotopic signature of $\delta^{13}C$ differentiates C_3 or C_4 producers in the food

chains (Peterson and Fry, 1987; Clapcott and Bunn, 2003).

Nitrogen can be incorporated by algae/plants through association with nitrogen-fixing bacteria or from the assimilation of nitrogenous compounds in the air and in the soil (Caxito and Silva, 2015), whereas in animals it is assimilated by the incorporation of proteins (Gannes *et al*., 1998). The nitrogen is used to determine trophic niches and trophic position, because $\delta^{15}N$ of each trophic level is typically 3-5‰ higher than its source of nitrogen (Minagawa and Wada, 1984; Peterson and Fry, 1987; Fry, 1991; Robinson, 2001). Furthermore, $\delta^{15}N$ can be associated with increased carnivorous feeding (Post, 2003) or can be used as an indication of anthropogenic interference in environments (Peterson, 1999). For example, Tanu *et al*. (2020) studied anthropic interference in mangrove and reported that plants from polluted sites had $\delta^{15}N$ values of 5-14‰, while the ones from unaffected sites the values were around 3‰, suggesting that anthropogenic N may be regulating $\delta^{15}N$.

Studies which aim to unveil the food web or food habit for *Aegla* spp. using isotope stable are scarce in the literature, because most have used the stomach content analysis (as mentioned above). Biasi *et al*. (2019) investigated the feeding preferences of shredders including *Aegla longirostri* Bond-Buckup and Buckup 1994 and their isotope values when fed on the leaf litter of trees and grass species in a feeding-choice experiment. The carbon isotopic values for *A. longirostri* from experiment was $\delta^{13}C$ - 23.71± 0.25‰, and $\delta^{13}C$ - 24.38 ± 0.14 in natural environments. The isotopic values revealed an enrichment of grass carbon in the tissues, although the tree carbon was also tracked in substantial rates.

The isotopic signature in studies for the aeglids are scarce, since the majority sought to work with population structure, reproductive biology and abundance (Swiech-Ayoub and Masunari, 2001; Fransozo *et al*., 2003). *Aegla* spp. stand out as important

links in the food chains, consumers of algae, vegetable remains, macrocrustaceans (Bueno and Bond-Buckup, 2004) and aquatic insect larvae (Magni and Py-Daniel, 1989). In addition, they are an important source of food for vertebrates such as fish, amphibians, birds (Arenas, 1976) and mammals (Medina, 1998; Cassini *et al*., 2009). Due the high endemism of *Aegla* spp., restrict distribution and the number of species already considered threatened, studies focusing studies focusing on populations that have not yet been investigated, especially with the recent loss of species due to anthropic impacts, are extremely important to increase knowledge about these populations. Therefore, the use ofisotope stables besides the basic information on feed ecology that still is not completely understood for aeglids would make possible the verification of human is interference in the environment, through the increase of nitrogen in the stream.

Our objective is (a) the identification of the isotopic signature of A. castro comparing its life stages with the hypothesis of a difference between young-adults, since the lack of habitat overlap, probably due to the influence of size, results in a source consequently distinct food; (b) the identification of the isotopic signature of A. castro comparing its sampling areas, with the hypothesis that the environments are different between the sampling areas and will modulate the isotopic signature of the eglids, since the animals eat what is available in nature. We also look at the nitrogen values between the sampling areas, testing the hypothesis that nitrogen will have a high value at an impacted site.

MATERIAL AND METHODS

Study area

We chose four different locations to collect *A. castro* based on itsgeographic distribution and the state of preservation, *i.e.*: Paranapanema sub-basin (São Paulo state), (1) Itaúna stream (ITA) (Itatinga city); and Tibagi sub-basin (Paraná state), (2) Preto

stream (PRE) (Mauá da Serra city), (3) Iapó stream (IAP) (Castro city) and (4) Quebra Perna stream (QPE) (Ponta Grossa city). The locations were described through personal observations and using google maps to view the surrounding area of thesampling areas:

_ ITA tributary (23°9’38.21”S 48°37’55.56”W): the local vegetation is Atlantic forest and Cerrado biome (Montana semi-deciduous), with a lot of eucalyptus reforestation. The sampling area is located at a private farm and the stream has preserved and continuous riparian forest with a good tree canopy and creeping plants (grass and herbaceous).

_ PRE tributary (23°57'00"S 51°07'00"W): the local vegetation is Atlantic forest (Dense Ombrophilous Forest with remnants of Mixed Ombrophilous Forest). It is located behind a restaurant (with visible effluent release) and a road with small dirt roads and constructions surrounding the area. Riparian forest is present as well areas composed of creeping plants (grass).

_ IAP tributary (24º48'37"S 49º51’30"W): the local vegetation is Atlantic forest (Dense Ombrophilous Forest with remnants of Mixed Ombrophilous Forest). The location is surrounded by plantations (e.g. soybeans, beans and potatoes) 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 bydiscontinuous Riparian Forest, and the sampling site specifically is composed by a deforested area with creeping plants (grass), close to a road.

_ QPE tributary (25°10'19"S 49°58'08"W): the local vegetation is Atlantic forest (Dense Ombrophilous Forest and natural field), and the sampling site is inside the private park “Buraco do Padre”. It is a touristic park where the main attraction is a cave with a waterfall being accessed through an approximately 730 meters trail. Visitors usually go there for outdoor activities as walking, bathing, rappelling, hiking and climbing. Around the park places with plantations and deforestation are observed.

Biological samples

Aeglids were collected during August and September (winter) and December (spring) 2018, using 6traps measuring 24cm × 19.5cm × 8cm, 6 traps measuring 24cm × 14.5cm × 7cm and 6 traps measuring 17.5cm × 12.5cm × 5cm, that were randomly distributed throughout eachfreshwater 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 toflow 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 juvenileindividuals), before the traps are set. Morphometric maturity was determined based on the carapace length estimated previously (Marçal *et al*., 2017). The collected aeglids werestored in coolers containing ice for further analysis.

Isotopic analyses of $\delta^{13}C$ $\delta^{15}N$

The material used for the isotopic analyses were the two chelipeds musculature, obtained from 32 animals, from which 20 were juveniles and 12 were adults (See Tab. 1). All samples were dehydrated at 50°C, in a drying oven with air circulation for 48hours (Nagata *et al*., 2015). The dried samples were ground for six minutes in a cryogenicmill to complete homogenization. For $\delta^{13}C$ analyses, the samples were soaked in 1M HClfor 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).

The stable isotope analysis was conducted at the Stable Isotopes Center, Institute of Biosciences, UNESP, Botucatu Campus. Aliquots of the homogeneous material (50-70 ± 1 μg for $\delta^{13}C$ and 500-600 ± 1 μg for $\delta^{15}N$) 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 2000 - 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 (Rsample = $^{12}C/^{13}C$ or $^{15}N/^{14}N$) of each sample. The results are given in the relative difference of isotope ratio (δ) of a standard value (Rstandard) expressed in ‰ accordance with:

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

For $\delta^{13}C$, the $Rs_{standard}$ is V-PDB (Vienna Pee dee Belemnite) and for $\delta^{15}N$ is atmospheric air. The standard uncertainty (n = 10) of analyzes was ± 0.2‰ for $\delta^{13}C$ and ± 0.15‰ for $\delta^{15}N$.

Table 1. Number of individual (juvenile-adults) and means (± standard deviation) of carapace length (CL mm) (juvenile-adults), for aeglids in 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	CL adults
ITA	3	2	4.56mm (± 0.02)	20.48mm (± 0.56)
PRE	6	4	9.16mm (± 0.68)	17.05mm (± 3.24)
IAP	6	2	10.01mm (± 0.57)	19.99mm (± 2.17)
QPE	5	4	13.83mm (± 2.20)	23.4mm (± 1.72)

Statistical analyses

Since values of $\delta^{13}C$ and $\delta^{15}N$ signatures were directly connected to each other, the multivariate analysis of variance (MANOVA) was performed to determine differences between age (juvenile-adult) and location (ITA, PRE, IAP, and QPE tributaries). Following this test, a residual analysis was performed in the R program. Then, $\delta^{13}C$ and $\delta^{15}N$ data were analyzed separately with factorial analysis of variance (ANOVA) to identify differences for each isotope by age (juvenile-adult) and location (ITA, PRE, IAP, and QPE tributaries), followed by post-hoc Tukey's tests to show the differences. The R program was used for statistical and graphical analyses (R Development Core Team, 2011; available at: www.R-project.org). The factorial ANOVA was used Incorporation, StatSoft: STATISTICA (data analysis software system, version 12).

RESULTS

Mean ± standard deviation (SD) values of isotopic signatures for the *A. castro* studied here were -25.00‰ ± -4.88 for $\delta^{13}C$ (range -33.04‰ − -13.53‰) and 7.64‰ ± 3.04 for $\delta^{15}N$ (range 3.54‰ − 13.96‰). In the IAP we obtained the highest values for δ15N and the most negative for δ13C; in QPE the lowest values for δ15N; in PRE, the least negative for δ13C and ITA presented intermediate values for δ13C and δ15N (Fig. 1).

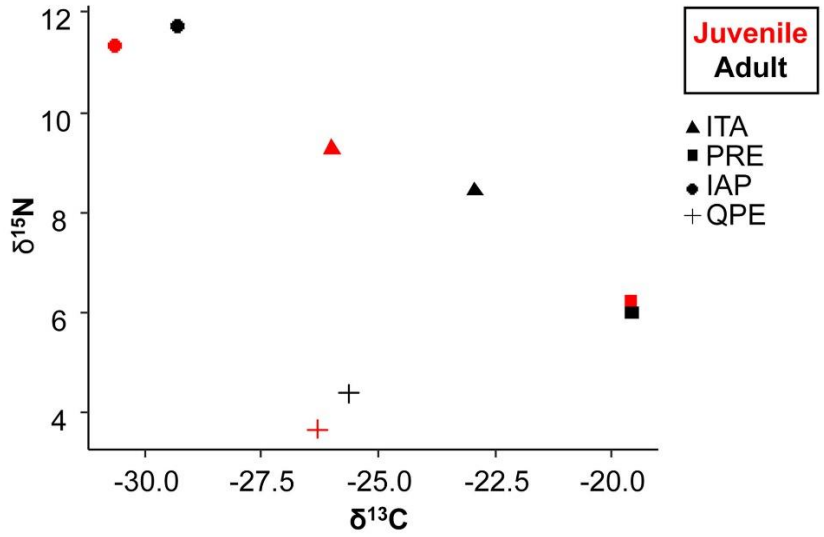

Figure 1. Isotopic biplot of variation in mean $\delta^{13}C$ and $\delta^{15}N$ (‰) values calculated for juvenile-adults to *A. castro* in Itatinga from São Paulo state (ITA: Itaúna stream, Paranapanema sub-basin) and Mauá da Serra (PRE: Preto stream, Tibagi sub-basin), Castro (IAP: Iapó stream, Tibagi sub-basin) and Ponta Grossa (QPE: Quebra Perna stream, Tibagi sub-basin) in Paraná state.

There was not difference between the life stages according to the combined $\delta^{13}C$ and $\delta^{15}N$ (MANOVA, $F_{ontogenetic} = 1.15$, Df = 2, p = 0.32). Differences among regions were observed showed difference between the locations (MANOVA, $F_{location} = 32.60$, Df = 6, $p < 0.05$). When $\delta^{13}C$ and $\delta^{15}N$ were analyzed separately, still thedifferences were not observed for the ontogenetic phases (juvenile-adult) (ANOVA $\delta^{13}C$,$F_{ontogenetic} = 1.55$, Df = 1, p = 0.22; ANOVA $\delta^{15}N$, $F_{ontogenetic} = 0.01$, Df = 1, p = 0.98), respectively. For the sampling areas, the differences were evident for C and N analyzed separately, $\delta^{13}C$ was different (ANOVA, $F_{location} = 17.79$, Df = 3, $p < 0.05$), as well as $\delta^{15}N$(ANOVA, $F_{location} = 92.43$, Df = 3, $p < 0.05$). The differences and similarities of $\delta^{13}C$ and$\delta^{15}N$ by sampling areas according to the Tukey test are shown in Fig. 2.

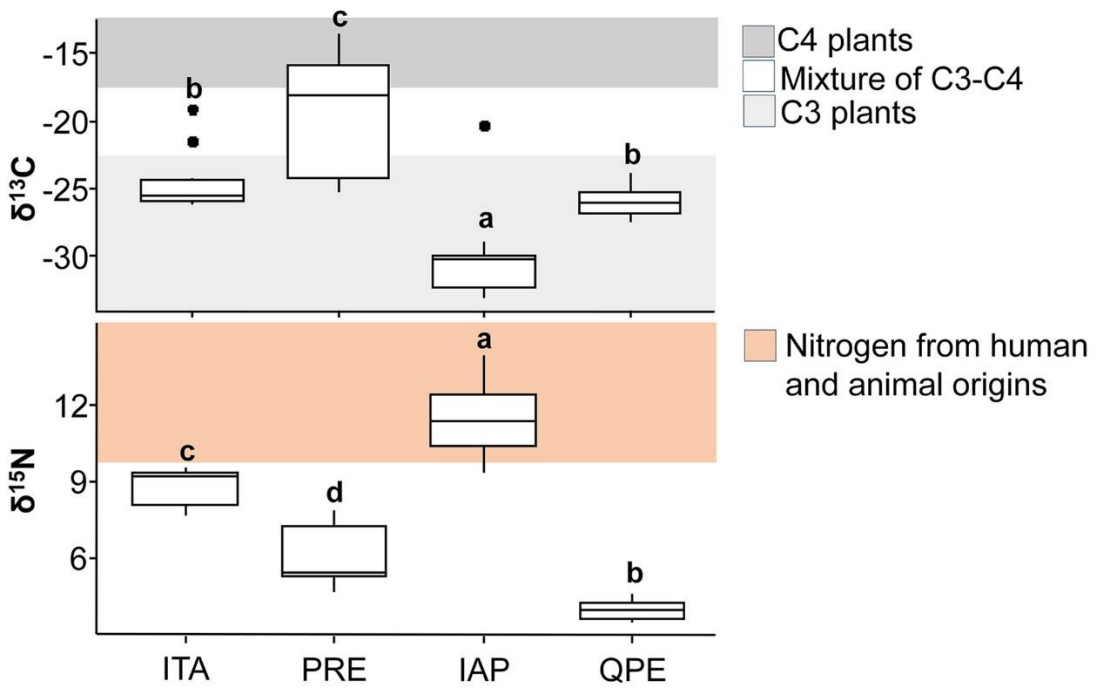

Figure 2. Variation in $\delta^{13}C$ and $\delta^{15}N$ of *A. castro* in Itatinga from São Paulo state (ITA: Itaúna stream, Paranapanema sub-basin) and Mauá da Serra (PRE: Preto stream, Tibagi sub-basin), Castro (IAP: Iapó stream, Tibagi sub-basin) and Ponta Grossa (QPE: Quebra Perna stream, Tibagi sub-basin) in Paraná state. Horizontal lines inside boxes (first and third quartiles) represent median values; vertical lines represent minimum and maximum values. Differences between samples are indicated by different letters (Tukey test, $p<0.05$). C_3 plants, the value of $\delta^{13}C$ 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). In the orange bar $\delta^{15}N$ values are considered to come from human and animal origins (Heaton, 1986; McClelland e Valiela 1998; Hoffman *et al*., 2012).

DISCUSSION

Aegla castro did not have significant variation in the isotopic signature between juvenile-adult, indicating that the food source aspect is independent of life stage or they use similar food resources during their ontogenetic development; so, we refuted our first hypothesis. Burress *et al*. (2013) showed a difference for $\delta^{13}C$ between the ontogeny phases of *Aegla uruguayana* Schmitt, 1942, suggesting a shift in the relative importance of C sources, but this difference was not significant for $\delta^{15}N$. So, the authors suggested that *A. uruguayana* feeds within the same trophic level in both life stages.

The absence of difference between the isotopic signatures by *A. castro*'s juvenile-adult can be because these animals have direct development and have the mouth parts and locomotion apparatus already developed in the early juvenile stages (Bueno and Bond-Buckup, 1996; Bond-Buckup *et al*., 1996) and because the juveniles could reflect the female isotopic signature, and possibly have the same distribution in the rivers, which would not show adifference between signatures. Santos et al. (2008) also found in *Aegla longirostri* Bond-Buckup & Buckup, 1994 that there are no differences in diet of juvenile-adult, justifying through the same range of distribution in the water flow.

This behavior probably leads to the sharing of food items, with contributions from each different source. Resource sharing can be due to several factors, despite overlap in prey choices, for example, the same resource can be shared according to the selection of prey size. In any case, if the resources are sufficiently abundant and not limiting, then competition between individuals does not occur and the selection of prey is done only according to the morphological and physiological characteristics of each species (Bocher et al., 2014).

Our study brings the results that *Aegla* spp. isotopic signatures were different among studied tributaries. This prove our second hypothesis that the environment was responsible to modulate the isotopic signature, since animals eat what is available in the

wild.

$\delta^{13}C$ *signature*

The carbon varied from -33.04‰ – -13.53‰. Our results showed difference between the locations, this shows that in some regions there is more consumption of C_3 carbon than C_4. Material collected in ITA, IAP and QPE tributaries show that animals consumed only C_3 plants, and PRE is the only location that presented intermediate values between C_3 and C_4.

The higher consumption of C_3 plants by aeglids may be associated to physiological and anatomical differences between C_3 and C_4 plants (Hatch and Slack, 1970). The higher consumption of C_3 plants by aeglids may be associated to physiological and anatomical differences between C_3 and C_4 plants (Hatch and Slack, 1970). Those shredders may have a limited capacity to process grass leaves (C_4) and may lack digestive enzymes or symbionts (Clapcott and Bunn, 2003). Furthemore, Caswell *et al.* (1973) hypothesized that C_4 plants are a poor food resource for herbivorous species when compared to C_3 plants. The nutrient availability in C_4 plants is lower because most nutrients are concentrated in the cells of the vascular bundle which are hard or even impossible to digest (Caswell *et al.*, 1973).

Fhurthemore, this preference can be by the association of plants with the microbiota. For example, *A. platensis* showed preference for leaves with higher levels of microorganism conditioning (Colpo *et al.*, 2012). For shredders, microorganisms colonizing the surface of leaves have nutritional importance, since they enhance the food quality of the plant litter (Ward and Cummins, 1979; Vannote *et al*., 1980; Graça, 2001).

Based on these evidences, we suggest that aeglids showed a preference for C_3 species. Then, we emphasize the importance of the carbon item in the aeglids feed.

Castro- Souza and Bond-Buckup (2004) demonstrated for *Aegla leptodactyla* Buckup and Rossi,1977 and *Aegla camargoi* Buckup and Rossi, 1977, that macrophytes are one of the most important food components. Bueno and Bond-Buckup (2004) found algae and vascular plants present in the food of *A. platensis* and *A. ligulata* and Colpo *et al.* (2012) demonstrated the food preference of *A. platensis* for leaves when they had microorganisms.

δ^{15}N *signature*

The isotopic nitrogen signature can indicate the trophic levels, but also the carnivorous feeding and indication of anthropogenic interference, because of the increase of the N in the enviroments (Fry, 1991; Peterson, 1999; Robinson, 2001; Post, 2003).

In the locations that presented the lowest values for nitrogen, *e.g.* QPE, the isotopic signature can only be indicative of carnivorous-omnivorous feeding. According to Bueno and Bond-Buckup (2004), there is a tendency for *Aegla* spp. become carnivorous in adult phase and also assimilate a greater proportion of invertebrates in the adult phases. This pattern is found in *A. uruguayana*, *A. platensis* and *A. ligulata* (Bueno and Bond- Buckup, 2004; Burress *et al*., 2013; Collins, 2019). Diets consisting of protein-rich (composed mainly by invertebrates) also result in faster growth rates (Bondar *et al*., 2005) demonstrating a benefit of animal consumption rather than plant, indicating why these animals end up having a mixed diet, between sources of carbon and nitrogen. The isotopic signature in these locations can be indicate of the organic matter intake, which may occur through the ingestion of sediments associated with organic matter, where algae, bacteria and other microorganisms grow (Collins, 2019).

The N is high in this region probably due to the anthropic action. In IAP is located close to the main economical pole of animal and agriculture farming in Castro city.

Therefore, the high $\delta^{15}N$ value is clearly influenced bydischarge of water enriched with wastes of the nearby areas of pig, dairy cattle and poultryproduction into the stream, as well as water flow enriched with organic matter rich in ^{15}N,leading to ^{15}N enrichment of the animals at this location. So, the highest values observed for IAP are an indicative of environments in which nitrogen from human and animal origins predominate (Heaton, 1986; McClelland and Valiela, 1998; Hoffman *et al*., 2012).

The $\delta^{15}N$ values in the environment can also be altered by sewage and fertilizer discharge (Tanu *et al*., 2020). Cabana and Rasmussen (1996) state that 68% of the variation in $\delta^{15}N$ values of primary consumers is due to anthropogenic impacts, such as sewage discharge or waste from agricultural practices transported to aquatic environments, as a result of isotope fractionation during ammonification (Heaton, 1986). The $\delta\ ^{15}N$ values of primary producers increase with the anthropic waste discharge (McClelland and Valiela, 1998) are transferred to consumers through trophicrelationships (Cabana and Rasmussen, 1996; Hansson *et al*., 1997). Anthropic interference has also been found for other animal and plant groups. It has been reported that plants in sites facing human disturbances had $\delta^{15}N$ values of 5–14‰, while unaffected sites had values up to 3‰, suggesting that anthropogenic N may be regulating$\delta^{15}N$ (Tanu *et al*., 2020).

According to our results, *A. castro* from QPE apparently had the most equilibrated value for $\delta^{15}N$ (4.02‰). The juvenile-adults of *A. uruguayana* had the $\delta^{15}N$ value around 9‰ (Burress *et al*., 2013), showed an ontogenetic shift from herbivore– detritivore to omnivore, and occupied a much lower trophic position than is typical for crayfishes. In the localities of ITA and PRE is possible to see that the $\delta^{15}N$ values start increase in comparison to the QPE's $\delta^{15}N$ isotopic signal. It is possible that these tributaries, in special ITA. Further studies at these locations should be carried out to better understand the rise in nitrogen.

Our study showed no difference in ontogeny diets, therefore, being able to feed on the same food items and foraging in the same places, indicating a low competition between resources. We also demonstrated that the environment is very important for the aeglids, being able to changethe isotopic signature of the species. Furthermore, IAP had the influence of anthropogenicnutrient inputs affecting the sensitivity of natural isotopic ratios observed in the present study. In general, *Aegla* spp. occurs in habitats with good environmental conditions and therefore, they are likely to be extremely affected by changes or environmental disturbances. Therefore, this study is an evidence of how the high levels of nitrogen probably by human interference (*e.g*., agriculture, sewage) were assimilated in the aeglids diet. In addition, it is an important base of information for future comparisons to check the changes in the environment according to isotopic levels. Investigations aiminganthropic impact analyses and conservation are essential for this group.

REFERENCES

Arenas, R.L. 1976. La cordillera de la costa como refúgio de la fauna dulcícola preglacial. *Archivos de Biologia y Medicina Experimentales*, 10: 1–40.

Bearhop, S.; Adams, C.E.; Waldron, S.; Fuller, R.A. and MacLeod, H. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of AnimalEcology*, 73: 1007–1012.

Bearhop, S.; Waldron, S.; Votier, S.C. and Furness, R.W. 2002. Factors that influence assimilation rates, and fractionation of nitrogen and carbon isotopes in avian blood and feathers. *Physiological and Biochemical Zoology*, 75: 451–458.

Biasi, C.; Cogo, G.B.; Hepp, L.U. and Santos, S. 2019. Grass species as a source of allochthonous energy for shredders and fungal decomposers in a subtropical stream. *Fundamental and Applied Limnology*, 192: 331-341.

Bocher, P.; Robin, F.; Kojadinovic, J.; Delaporte, P.; Rousseau, P.; Dupuy, C. and Bustamante, P. 2014. Trophic resource partitioning within a shorebird community feeding on intertidal mudflat habitats. *Journal of Sea Research*, 92: 115–124.

Boenigk, J.; Wodniok, S.; Bock, C.; Beisser, D.; Hempel, C.; Grossmann, L.; Lange, A. and Jensen, M. 2018. Geographic distance and mountain ranges structure freshwater protist communities on a European scale. *Metabarcoding and Metagenomics*, 2:e21519.

Bondar, C.A.; Bottriell, K.; Zeron, K. and Richardson, J.S. 2005. Does trophic position of the omnivorous signal crayfish (*Pacifastacus leniusculus*) in a stream food web

vary with life history stage or density? *Canadian Journal of Fisheries and Aquatic Sciences*, 62: 2632–2639.

Bond-Buckup, G. 2003. Família Aeglidae. p. 21–116. In: G.A.S Melo (ed), Manual de identificação dos Crustacea Decapoda de água doce do Brasil. Editora Loyola, São Paulo.

Bond-Buckup, G.; Bueno, A.A.P. and Keunecke, K. 1996. O primeiro estágio juvenil de *Aegla prado*, Schmitt, 1942 (Crustacea, Anomura, Aeglidae). *Revista Brasileira de Zoologia*, 13: 1049–1061.

Bond-Buckup, G. and Buckup, L. 1994. A família Aeglidae (Crustacea, Decapoda, Anomura). *Arquivos de Zoologia*, 32: 159–346.

Bond-Buckup, G.; Jara, C.G.; Buckup, L.; Bueno, A.A.; Perez-Losada, M. and Crandall, K.A. 2010. Description of a new species of Aeglidae, and new records of relatedspecies from river basins in Argentina (Crustacea, Anomura). *Zootaxa*, 2343: 18–30.

Bosley, K.M.; Copeman, L.A.; Dumbauld, B.R. and Bosley, K.L. 2017. Identification of burrowing shrimp food sources along an estuarine gradient using fatty acid analysis and stable isotope ratios. *Estuaries and Coasts*, 40: 1113–1130.

Boutton, T.W. 1996. Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change. p. 47–82. In: T.W. Boutton and S.I. Yamasaki (eds), Mass spectrometry of soils. Marcel Dekker, New York.

Bueno, A.A.P. and Bond-Buckup, G. 2004. Natural diet of *Aegla platensis* Schmitt and *Aegla ligulata* Bond-Buckup and Buckup (Crustacea, Decapoda, Aeglidae) from Brazil. *Acta Limnologica Brasiliensia*, 16: 115–127.

Bueno, A.A.P. and Bond-Buckup, G. 1996. Os estágios iniciais de *Aegla violacea* Bond-Buckup and Buckup, 1994 (Crustacea, Decapoda, Anomura). *Nauplius*, 4: 39–47.

Bueno, S.L.S. and Shimizu, R.M. 2008. Reproductive biology and functional maturity in females of *Aegla franca* (Decapoda: Anomura: Aeglidae). *Journal of Crustacean Biology*, 28: 652–662.

Bueno, S.L.S.; Shimizu, R.M. and Rocha, S.S. 2007. Estimating the population size of *Aegla franca* (Decapoda: Anomura: Aeglidae) by mark-recapture technique from an isolated section of Barro Preto stream, county of Claraval, state of Minas Gerais, southeastern Brazil. *Journal of Crustacean Biology*, 27: 553–559.

Burns, J.W. 1972. The distribution and life history of South American freshwater crabs (*Aegla*) and their role in trout streams and lakes. *Transactions of the American Fisheries Society*, 101: 595–607.

Burress, E.D.; Gangloff, M.M. and Siefferman, L. 2013. Trophic analysis of two subtropical South American freshwater crabs using stable isotope ratios. *Hydrobiologia*, 702: 5–13.

Cabana, G. and Rasmussen, J.B. 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences*, 93: 10844–10847.

Carabel, S.; Godínez-Domínguez, E.; Verísimo, P.; Fernández, L. and Freire, J. 2006. An assessment of sample processing methods for stable isotope analyses of marine foodwebs. *Journal of Experimental Marine Biology and Ecology*, 336: 254–261.

Carmichael, R.H.; Rutecki, D.; Annett, B.; Gaines, E. and Valiela, I. 2004. Position of horseshoe crabs in estuarine food webs: N and C stable isotopic study of foraging ranges and diet composition. *Journal of Experimental Marine Biology and Ecology*, 299: 231–253.

Cassini, M.H.; Fasola, L.; Chehébar, C. and Macdonald, D.W. 2009. Scale-dependent analysis of an otter-crustacean system in Argentinean Patagonia. *Naturwissenschaften*, 96: 593–599.

Castro-Souza, T. and Bond-Buckup, G. 2004. O nicho trófico de duas espécies simpátricas de *Aegla* Leach (Crustacea, Aeglidae) no tributário da bacia hidrográficado Rio Pelotas, Rio Grande do Sul, Brasil. *Revista Brasileira de Zoologia*, 21: 805– 813.

Caswell, H.; Reed, F.; Stephenson, S.F. and Werner, P. 1973. Photosynthetic pathways and selective herbivory: a hypothesis. *The American Naturalist*, 107: 465–480.

Caxito, F.A. and Silva A.V. 2015. Isótopos estáveis: fundamentos e técnicas aplicadas à Caracterização proveniência geográfica de Produtos alimentícios. *Geonomos*, 23: 10–17.

Clapcott, J.E. and Bunn, S.E. 2003. Can C_4 plants contribute to aquatic food webs of subtropical streams? *Freshwater Biology*, 48: 1105–1116.

Collins, P. 2019. Trophic ecology. p. 97–132. In: S. Santos and S.L.S. Bueno (eds), Aeglidae: Life History and Conservation Status of Unique Freshwater Anomuran Decapods, CRC Press, Taylor and Francis Group, New York.

Colpo, K.D.; Ribeiro, L.C.; Wesz, B. and Ribeiro, L.O. 2012. Feeding preference of the South American endemic anomuran *Aegla platensis* (Decapoda, Anomura, Aeglidae). *Naturwissenschaften*, 99: 333–336.

Daufresne, T. and Loreau, M. 2001. Ecological stoichiometry, primary producer–decomposer interactions, and ecosystem persistence. *Ecology*, 82: 3069–3082.

Ducatti, C.; Martins, C.L.; Arrigoni, M.B.; Martins, M.B.; Vieira Jr., L.C. and Denadai, J.C. 2011. Utilização de isótopos estáveis em ruminantes. *Revista Brasileira de Zootecnia*, 40: 68–75.

Fasolo, P.J.; Carvalho, A.P.; Potter, R.O.; Bognola, I.A.; Bhering, S.B. and Martorano, L.G. 2002. Caracterização dos solos do Município de Castro, PR. Embrapa Solos-Boletim de Pesquisa e Desenvolvimento 09 (INFOTECA-E). https://www.researchgate.net/publication/344546805_Boletim_de_Pesquisa_e_Desenvolvimento_09_Caracterizacao_dos_Solos_do_Municipio_de_Castro_PR - ISSN 1678–0884.

Fransozo, A.; Costa, R.C.; Reigada, A.L.D. and Nakagaki, J.M. 2003. Population structure of *Aegla castro* Schmitt, 1942 (Crustacea: Anomura: Aeglidae) from Itatinga (SP), Brazil. *Acta Limnologica Brasiliensia*, 15: 13–20.

Fry, B. 1991. Stable isotope diagrams of freshwater food webs. *Ecology*, 72: 2293–2297.

Gannes, L.Z.; Del-Rio, C.M. and Koch, P. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comparative Biochemistry Physiology*, 119: 725–737.

Graça. M.A.S. 2001. The role of invertebrates on leaf litter decomposition in streams - a review. *International Review of Hydrobiology*, 86: 383–393.

Hansson, S.; Hobbie, J.E.; Elmgren, R.; Larsson, U.; Fry, B. and Johansson, S. 1997. The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration.*Ecology*, 78: 2249–2257.

Hatch, M.D. and Slack, C.R. 1970. Photosynthetic CO_2-pathways. *Annual Review of Plant Physiology*, 21: 141–162.

Heaton, T.H. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. *Chemical Geology*, 59: 87–102.

Hobson, K.A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, 120: 314–326.

Hobson, K.A. and Clark, R.G. 1992. Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. *Condor*, 94: 181–188.

Hoffman, J.C.; Kelly, J.R.; Peterson, G.S.; Cotter, A.M.; Starry, M.A. and Sierszen, M.E. 2012. Using $\delta^{15}N$ in fish larvae as an indicator of watershed sources of

anthropogenicnitrogen: Response at multiple spatial scales. *Estuaries and Coasts*, 35: 1453–1467.

Hyslop, E.J. 1980. Stomach contents analysis - a review of methods and their application. *Journal of Fish Biology*, 17: 411–429.

Laughlin, R.A. 1982. Feeding habits of the crab, *Callinectes sapidus* Rathbun, in the Apalachiola estuary, Florida. *Bulletin of Marine Science*, 32: 807–822.

Magni, S.T. and Py-Daniel, V. 1989. *Aegla platensis* Schmitt, 1942 (Decapoda: Anomura) a predator of Simuliidae immature stages (Diptera: Culicomorpha). *Revista de Saúde Pública*, 23: 258–259.

Mantelatto, F.L.M. and Christofoletti, R.A. 2001. Natural feeding activity of the crab *Callinectes ornatus* (Portunidae) in Ubatuba Bay (São Paulo, Brazil): influence of season, sex, size and molt stage. *Marine Biology*, 138: 585–594.

Mao, Z.; Gu, X. and Zeng, Q. 2016. Food sources and trophic relationships of three decapod crustaceans: insights from gut contents and stable isotope analyses. *Aquaculture Research*, 47: 288–2898.

Marçal, I.C.; Ioshimura, L.M.; Rosa, J.J.S. and Teixeira, G.M. 2017. Population structure and sexual maturity of *Aegla castro* (Decapoda, Anomura), an endemic freshwater crab from Brazil. *Invertebrate Reproduction and Development*, 62: 35–42.

McClelland, J.W. and Valiela, I. 1998. Linking nitrogen in estuarine producers to land-derived sources. *Limnology and Oceanography*, 43: 577–585.

Medina, G. 1998. Seasonal variations and changes in the diet of southern river otter in different freshwater habitats in Chile. *Acta Theriologica*, 43: 285–292.

Miller, T.J.; Crowder, L.B.; Rice, J.A. and Marschall, E.A. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual-framework. *Canadian Journal of Fisheries and Aquatic Sciences*, 45: 1657–1670.

Minagawa, M. and Wada, E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relationship between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta*, 48: 1135–1140.

Nagata, R.M.; Moreira, M.Z.; Pimentel, C.R. and Morandini, A.C. 2015. Food web characterization based on $\delta^{15}N$ and $\delta^{13}C$ reveals isotopic niche partitioning between fish and jellyfish in a relatively pristine ecosystem. *Marine Ecology Progress Series*,519: 13–27.

Olson, M.H. 1996. Ontogenetic niche shifts in largemouth bass: variability and consequences for first-year growth. *Ecology*, 77: 179–190.

Pérez-Losada, M.; Jara, C.G.; Bond-Buckup, G. and Crandall, K.A. 2002. Conservation phylogenetics of Chilean freshwater crabs *Aegla* (Anomura, Aeglidae): assigning priorities for aquatic habitat protection. *Biological Conservation*, 105: 345–353.

Persson, L. 1999. Trophic cascades: abiding heterogeneity and the trophic level concept at the end of the road. *Oikos*, 85: 385–397.

Peterson, B.J. 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: A review. *Acta Oecologica*, 20: 479–487.

Peterson, B.J. and Fry, B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, 18: 293–320.

Post, D.M. 2003. Individual variation in the timing of ontogenetic niche shifts in largemouth bass. *Ecology*, 84: 1298–1310.

Purcell, J.E. and Sturdevant, M.V. 2001. Prey selection and dietary overlap among zooplanktivorous jellyfish and juvenile fishes in Prince William Sound, Alaska. *Marine Ecology Progress Series*, 210: 67–83.

Robinson, D. 2001. $\delta^{15}N$ as an integrator of the nitrogen cycle. *Trends in Ecology and Evolution*, 16: 153–162.

Santos, S.; Ayres-Peres, L.; Cardoso, R.C. and Sokolowicz, C.C. 2008. Natural diet of the freshwater anomuran *Aegla longirostri* (Crustacea, Anomura, Aeglidae). *Journal of Natural History*, 42: 1027–1037.

Santos, S.; Bond-Buckup, G.; Gonçalves, A.S.; Bartholomei-Santos, M.L.; Buckup, L. and Jara, C.G. 2017. Diversity and conservation status of *Aegla* spp. (Anomura, Aeglidae): an update. *Nauplius*, 25: 1–14.

Swiech-Ayoub, B.D.P. and Masunari, S. 2001. Reproductive biology of *Aegla castro* Schmitt (Crustacea, Anomura, Aeglidae) from Buraco do Padre, Quebra-Perna River, Ponta Grossa, Paraná State, Brazil. *Revista Brasileira de Zoologia*, 18: 1019–1030.

Tanu, F.Z.; Asakura, Y.; Takahashi, S.; Hinokidani, K. and Nakanishi, Y. 2020. Variation in foliar $\delta^{15}N$ reflects anthropogenic nitrogen absorption potential of mangrove forests. *Forests*, 11: 133.

Vannote, R.L.; Minshall, G.W.; Cummins, K.W.; Sedell, J.R. and Colbert, C.E. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, 37: 130–137.

Vogel, J.C. 1993. Variability of carbon isotope fractionation during photosynthesis. p. 29–46. In: J.R. Ehleringer; A.E. Hall and G.D. Farquhar (eds), Stable isotopes and plant carbon: water relations. Academic Press, San Diego.

Ward, G.M. and Cummins, K.W. 1979. Effects of food quality on growth of a stream detritivore, *Paratendipes albimanus* (Meigen) (Diptera: Chironomidae). *Ecology*, 60: 57–64.

Werner, E.E. and Gilliam, J.F. 1984. The ontogenetic niche and species interactions in size structured populations. *Annual Review of Ecology and Systematics*, 15: 393–425.

Werner, E.E.; Mittelbach, G.G.; Hall, D.J. and Gilliam, J.F. 1983. Experimental tests of optimal habitat use in fish: the role of relative habitat profitability. *Ecology*, 64: 1525–1539.

Williams, M.J. 1981. Methods for analysis of natural diet in portunid crabs (Crustacea: Decapoda: Portunidae). *Journal of Experimental Marine Biology*, 52: 103–113.

Wood, K.A.; O'Hare, M.T.; McDonald, C.; Searle, K.R.; Daunt, F. and Stillman, RA. 2017. Herbivore regulation of plant abundance in aquatic ecosystems. *Biological Reviews*, 92: 1128–1141.

CHAPTER II

Trophic web involving the endemic South American crab *Aegla parana* (Decapoda: Anomura: Aeglidae), in River Palmital, Paraná, Brazil

Abstract

Aeglids are endemic to the Neotropical region of South America, that play an important role in nutrient dynamics and energy flow through the trophic web in freshwater. In this study, we stablish the trophic position and food web of *Aegla parana*, identifying the juvenile-adult eating habits and their trophic position. We did not found differences for isotopic signature of juvenile-adult, and the food sources that most contributed for both life stages was leaves. Aeglids were found in a lower position (2º level) in the food web. Our study demonstrated the importance of aeglids within the trophic web. Its generalist diet, in both ontogenetic phases, and its low trophic position, demonstrated its relevance as a link between primary producers and other trophic levels. In addition, through nitrogen analyzes, we could infer that the study site already shows evidence that anthropic action, which raise the nitrogen level of the animals that consume them, thus acting on populations, which can generate instability within the trophic web.

Keywords: Aeglids, carbon, food web, generalist, nitrogen.

INTRODUCTION

Aeglids are freshwater anomuran crustacean, endemic to the Neotropical region of South America (Bond-Buckup and Buckup, 1994), living in clean and well-oxygenated water, and are negatively affects by alterations in these habitat conditions (Bond-Buckup, 2003). Research that approaches the aeglids diet is stillincipient, considering the high number of known species (Castro-Souza and Bond- Buckup, 2004), and their ecological importance, due to its role in aquatic food chains.

Aeglids are omnivorous shredders, able to feed directly on allochthones plant matter, particulate organic matter, and aquatic insect larvae (Magni and Py-Daniel, 1989; Bueno and Bond-Buckup, 2004), being important in nutrient cycling (Cogo and Santos, 2013). Comparative studies about the feeding habits of juvenile-adult aeglids have also shown that some species do not present differences between ontogenetic stages (Bueno and Bond-Buckup, 2004; Santos *et al*., 2008). However, feeding habits of aeglids can change throughout their development (Bueno and Bond-Buckup, 2004; Burress *et al*., 2013; Collins, 2019). Feeding and assimilation of nutrients are important in aeglids, due to their need to obtain energy to maintain homeostasis and facilitate the synthesis and degradation of structural macromolecules necessary for maintenance, growth, reproduction and behavior (Karasov and Martínez Del Río, 2007; Saborowski, 2015; Musin *et al*., 2017).

Stable isotope analysis has been widely used to identify the food sources and trophic position of consumers (Peterson and Fry 1987; Post, 2002) and nutrient cycling pathways in terrestrial and aquatic ecosystems (Lajtha and Michener, 1994). This method based on the premise that predators are enriched with heavy isotopes compared to their prey (Minagawa and Wada, 1984). This isotopic composition integrates the signatures of different prey consumed in longer periods of time ago and incorporated into the tissues, different to the analysis of stomach contents, which provides direct evidence of the food

eaten (Hyslop, 1980; Mao *et al.,* 2016). The carbon and nitrogen isotopic composition of consumer tissues reflect $\delta^{15}N$ and $\delta^{13}C$ of each prey species; the relative proportions of each prey species assimilated and in certain instances, foraging location. Moreover, the stable isotope signatures of tissues generally reflect the diet over the period during which the tissue was synthesized (Bearhop *et al*., 2002). Additionally, stable isotopes come as a tool to improve the analysis of food habit and trophicweb, mainly because many of the food items are at a high level of digestion, and their identification is difficult. In addition, isotope analyses require small numbers of animals,which are of great value since this group is already at various levels of threat.

Stable isotope analysis of carbon has proved be effective in the study of aquatic food webs, marked differences in the isotope signatures of the major primary sources (Huryn *et al*., 2001). Carbon incorporated in plants suffers isotopic discrimination throughout the process of photosynthesis. In C_3 plants, the $\delta^{13}C$ value ranges from -22 to -34‰, and for C_4 plants ranges from -9 to -16‰ (Vogel, 1993; Boutton, 1996; Ducatti *et al*., 2011). The carbon isotopic composition of the consumers reflects the enrichment from the diets around 1‰ (Kelly, 2000; Post, 2002). Therefore, the isotopic signature of$\delta^{13}C$ differentiates C_3 or C_4 producers in the food chains (Peterson and Fry, 1987; Clapcottand Bunn, 2003). Nitrogen can be incorporated by algae/plants through association with nitrogen-fixing bacteria or from the assimilation of nitrogen compounds in the air and in the soil (Caxito and Silva, 2015), whereas in animals it is assimilated by the incorporation of proteins (Gannes *et al*., 1998). The nitrogen is used to determine trophic niches and trophic position, because $\delta^{15}N$ of each trophic level is typically 3-5‰ higher than its source of nitrogen (Minigawa and Wada, 1984; Peterson and Fry, 1987; Fry, 1991; Robinson, 2001), in addition enriched $\delta^{15}N$ are often associated with increased carnivore (Post, 2003).

Although studies with aeglides are of paramount importance due to the rapid degradation of the environment and the disappearance of some species in South American aquatic ecosystems, studies with trophic structure involving *Aegla parana* Schmitt, 1942 were not carried out. In thiswork, we study the isotopic signature of *A. parana* comparing their life stages and the trophic web that, in the tributary of the Iguaçu River (Rio Palmital), in União da Vitória, Paraná, Brazil, using the isotopic signatures. Our hypotheses are that: a) the signature of juvenile-adult is different what suggesting a different food resources in both stages and b) this crab have a lower position in the food web, as primary consumer, being animportant link between the producers and others trophic levels.

MATERIAL AND METHODS

Aegla parana were investigated in Palmital River (sub-basin of the Iguaçu River), in União da Vitória, Paraná, Brazil (26°0'30.88 "S 51°3'28.01"W). The collection point in the "Usina Velha" waterfall, belongs to Serra da Boa Esperança Environmental Protection Area (Director Plan from União da Vitória, 2017). As it is a sampling area in the waterfall, this environment suffers from high water pressure. According to our observations on the HidroWeb Portal (National Water Agency), a tool that is part of the National Water Resources Information System (SNIRH), the Palmital River at the collection point is a second-order river, since after source of the river it meets the Rio Louro (first order). The phytogeography landscape is inserted in the micro region of the middle Iguaçu, being represented by subtropical forests, with the presence of araucarias and lowland forest (City hall from União da Vitória, 2021)

Aeglids were collected in September of 2018, using 6 traps measuring 24cm × 19.5cm × 8cm, 6 traps measuring 24cm × 14.5cm × 7cm and 6 traps measuring 17.5cm × 12.5cm × 5cm, that were randomly distributed throughout the freshwater stream. Traps

were set in the afternoon and inspected for captured animals in the following morning. Dried cat feed, containing fishmeal as one of the main ingredients, was used as bait as described by Bueno *et al*. (2007). The trap opening was place in opposite to the water flow, thus allowing the scent of the bait to be released in the surrounding water. D-frame hand nets were used to actively collect smaller aeglids (mainly juvenile individuals) and the other organisms involved in the trophic interactions of the aeglids based on the food sources already described as a food item for aeglids. Some food items, like leaves, bivalves and sediment samples were collect manually. Leaves were collected from inside the stream. All material was stored in thermal boxes with ice for further analysis. Odonata and Ephemeroptera were identified in the laboratory with the help of literature (Mugnai *et al*., 2010), but Ephemeroptera were not analyzed because de little material. The *Diplodon* sp. was confirmed with researchers working in ecology and zoology of continental waters in Brazil.

Sediment was examined for use as a basis for predicting the $\delta^{15}N$ values of aeglids. Sediment 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). As well, as a bioindicator of the environment's health observable through the values of N found that determines whether the environment may be suffering from the disposal of sewage (Gearing *et al*., 1991; Lake *et al*., 2001) and any potential biomagnification of contaminants (Cui *et al*., 2011). In addition, 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).

Abiotic variables and water quality

Microbiological analyses were performed using the multi-tube technique, according to PRC N ° 5, of 09/28/2017 (ANVISA), 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 tolerated in the absence of thermotolerant coliforms. The chemical characteristics of stream water were analyzed in laboratory according to the methods recommended by the Standard Methods for the Examination of Water and Wastewater (Rice *et al*., 2017). Abiotic variables such as pH, electrical conductivity (Ec - μS/cm), turbidity (Turb), temperature (Temp - °C), total dissolved solids (TDS - mg/L), chlorophyll (Chl - μg/L) and dissolved oxygen (DO - mg/L), were measured using a multi-parameter Eureka probe (Manta model 2-4.0).

The Palmital River according to CONAMA Resolution Nº 357 (2005) is classified as level 2, so the chemical values and abiotic variables was evaluated according to the CONAMA Resolution Nº 357 (2005).

Isotopic analyses of $\delta^{13}C$ $\delta^{15}N$

Samples of bulk sediment were collected from the river, and in the laboratory screened and a random aliquot was placed for drying. The leaves collected were washed to eliminate debris and invertebrates from their surfaces (Burress *et al*., 2013). For the animals, the material used for analyses was pedal mass for bivalves and the chelipods for the aeglids. As the Odonata were small, they were processed completely. All samples were dehydrated at 50°C, 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 $\delta^{13}C$ analyses, the samples were soaked in 1M HCl for 3 hours to remove carbonates from exoskeletons, and then they were re-dried for 24 hours (Carabel *et al*., 2006; Nagata *et al*., 2015; Bosley *et al*., 2017).

The stable isotope analysis was conducted at the Stable Isotopes Center, Institute of Biosciences, UNESP, Botucatu Campus. Aliquots of the homogeneous material (50-

70 ± 1 µg for $\delta^{13}C$ and 500-600 ± 1 µg for $\delta^{15}N$) 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). Lipid extraction was not performed in our study, so the data used were the raw values found for the organisms. 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 2000 - 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 (Rsample = $^{12}C/^{13}C$ or $^{15}N/^{14}N$) of each sample. The results are given in the relative difference of isotope ratio (δ) of a standard value (Rstandard) expressed in ‰ accordance with:

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

For $\delta^{13}C$, the $Rs_{standard}$ is V-PDB (Vienna Pee dee Belemnite) and for $\delta^{15}N$ is atmospheric air. The standard uncertainty (n = 10) of analyses was ± 0.2‰ for $\delta^{13}C$ and ± 0.15‰ for $\delta^{15}N$.

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 sequentially primary and secondary consumers. The trophic level (NT) was calculated according to the formula presented by Post (2002):

$$TL=\lambda+(\delta^{15}N_{consumidor}-\delta^{15}N_{base})/\Delta^{15}N$$

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

Statistical analyses

To determine differences between ontogenetic phases (juvenile-adult), the multivariate analysis of variance (MANOVA) was performed, with the values of carbon and nitrogen connected, because these isotopes are directly connected to each other. Following this test, a residual analysis was performed in the R program. Then, carbon and nitrogen data were analyzed separately with factorial analysis of variance (ANOVA) to identify differences for each isotope by age (juvenile-adult).

To produce a biplots with the signatures of $\delta^{15}N$ and $\delta^{13}C$, to demonstrate the trophic web (*A. parana* 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 aeglidean diet, using isotopic data (Parnell and Inger, 2016). Thus, the Trophic Discrimination Factors (TDFs) used in our work, to analyze C and N together to identify in food the source of the greatest contribution for feeding of groups: aeglids juvenile (Group1) and aeglids adult (Group 2). The Trophic Discrimination Factors (TDFs) were based on different estimates proposed by: Post (2002) ($\delta^{13}C = 0.4 \pm 1.3$ - $\delta^{15}N = 3.4 \pm 1‰$) and three models proposed by McCutchan (2003) ($\delta^{13}C = 0.5 \pm 1.3$ - $\delta^{15}N = 2.3 \pm 1.5‰$; $\delta^{13}C = 1.3 \pm 0.3$ - $\delta^{15}N = 2.2 \pm 0.3‰$; $\delta^{13}C = 1.3 \pm 0.3$ - $\delta^{15}N = 3.3 \pm 0.26‰$). These TDFs were used to examine the sensitivity of the SIMMr model to variation in trophic fractionation (Riascos *et al*., 2015; Ingram *et al*., 2017). The programs used for statistical analysis were the R language (R Development Core Team, 2011; available at: www.R-project.org).

RESULTS

Abiotic variables and water quality

The results of microbiological analyses showed a of total coliforms > 23/100 mL of water and of thermotolerant coliforms 9.2/100 mL of water. Microbiological analyses indicate values of total and thermotolerant coliforms present and outside the permitted range. The results for the abiotic variables and the chemical analyses are in Table 1. According streams of level 2 (classified by CONAMA Resolution Nº 357), the water parameters of abiotic variables (pH, Ec, Turb, Temp, TDS, Chl and DO) and the water chemical (zinc, nitrite, nitrate, sulphate, chloride, copper, iron, manganese, fluoride and phosphate) presented the parameters allowed. Except aluminium, that presented values well above the allowed. For the chemical analyses, just the components that have values above the maximum allowed are shown in the table 1.

Table 1. Abiotic variables (mean ± standard deviation) and chemical assessment of Palmital River (União da Vitória), in the state of Paraná, Brazil, in comparison with CONAMA Resolution Nº357(2005), in freshwater class 2.

Abiotic Variables	**Palmital River**	CONAMA Nº357
pH	8.86 ± 0.12	6.0 to 9.0
Electric conductivity (μS/cm)	21.35 ± 0.22	-
Turbidity (NTU)	8.12 ± 1.14	Until 100 NTU
Temperature (°C)	19.69 ± 0.18	-
Total dissolved solids (mg/L)	13.65 ± 0.15	500 mg/L
Chlorophyll (μg/L)	2.34 ± 0.57	Until 30 μg/L
Dissolved oxygen (mg/L)	8.83 ± 0.01	Not less than 5 mg/L O_2
Chemical analyses	**Palmital River**	**A**
Aluminium	4.0450 $mg.L^{-1}$	0,1 $mg.L^{-1}$

Isotopic signature of juveniles-adults

Mean ± standard deviation (SD) values of isotopic signatures for the *A. parana* were -25.61‰ ± -1.03 for ^{13}C (range -26.50‰ to -23.28‰) and 8.34‰ ± 1.55 for ^{15}N (range 6.37‰ – 10.88‰). The combination of $\delta^{13}C$ and $\delta^{15}N$ did not differe between life stages (MANOVA, $F_{ontogenetic} = 0.01$, $p = 0.98$), and separated, carbon and nitrogen, did not differe regarding ontogenetic phases too (juvenile-adult) (ANOVA $\delta^{13}C$,$F_{ontogenetic} = 0.03$, $p = 0.86$; ANOVA $\delta^{15}N$, $F_{ontogenetic} = 0.24$, $p = 0.88$) (Fig. 1).

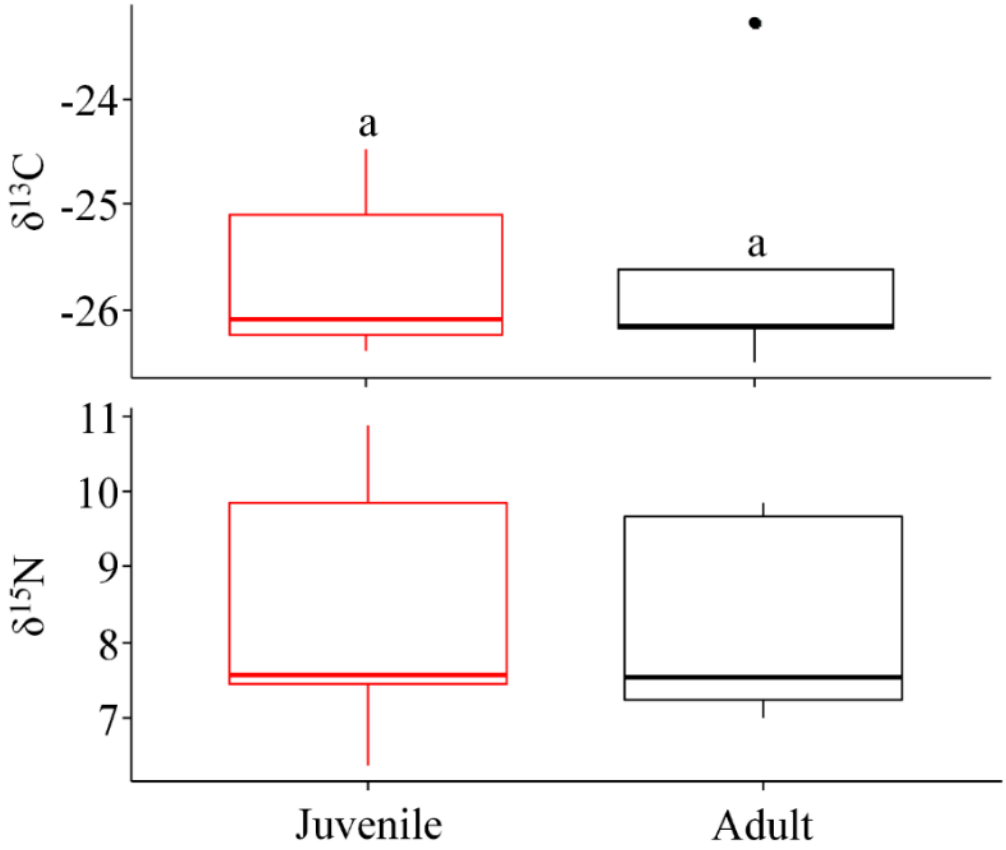

Figure 1. Variation in $\delta^{13}C$ and $\delta^{15}N$ of *Aegla parana* (União da Vitória in Paraná state). Horizontal lines inside boxes (first and third quartiles) represent median values; vertical lines represent minimum and maximum values. Differences between samples are indicated by different letters (Tukey test, $p<0.05$).

Trophic structure

We analyze *Aegla parana*, leaves, sediment, *Diplodon* sp. and Odonata. The trophic web of *A. parana* was characterized by 5 groups with distinct isotopicsignatures

(Tab. 2), classified in three trophic levels, according to the formula proposed by Post (2002). In União da Vitória, the leaves, with the lowest value of $\delta^{15}N$, representedthe chain base (1 º level) (2.21‰ ± 0.45). The second level was the *A. parana* adult ($\delta^{15}N$ 8.26‰ ± 1.38) and *A. parana juvenile* ($\delta^{15}N$ 8.42‰ ± 1.86) and the third level was composed by: sediment ($\delta^{15}N$ 9.31‰ ± 0.04), *Diplodon* sp. ($\delta^{15}N$ 9.33‰ ± 0.16) and Odonata ($\delta^{15}N$ 9.90‰ ± 0.08) (Tab. 2, Fig. 2).

The models with different trophic descrimination factor showed similarity in the results of the source thathave the greatest contribution of *A. parana*. The food source that contributed most to the *A. parana* juvenile varied between the leaves, sediment and *Diplodon* sp. with similar proportions around 10-20%, credible interval (CI = 95%) and for *A. parana* adult the foodsource that contributed was leaves around 10-30%, credible interval (CI = 95%) (Fig. 3).However, these models showed that most sources did not contribute more than 50% of the food source. This indicated that some food source used for this crab was not sampledin our study (few taxa analyzed).

Table 2: Signatures of stable carbon and nitrogen isotopes (mean ± standard deviation) and trophic level of the organisms in the community that make up the trophic web of *Aegla parana*, on the Palmital River (União da Vitória), in the state of Paraná, Brazil.

Food items	**$\delta^{13}C$‰**	**$\delta^{15}N$‰**	**Trophic level**
Leaves	-31.51 ± 0.02	2.21 ± 0.45	1.00
Aegla parana (adult)	-25.55 ± 1.30	8.26 ± 1.38	2.78
Aegla parana (juvenile)	-25.67 ± 0.82	8.42 ± 1.86	2.82
Sediment	-27.73 ± 0.04	9.31 ± 0.04	3.09
Diplodon sp.	-28.53 ± 0.02	9.33 ± 0.17	3.09
Odonata	-27.03 ± 0.08	9.90 ± 0.08	3.26

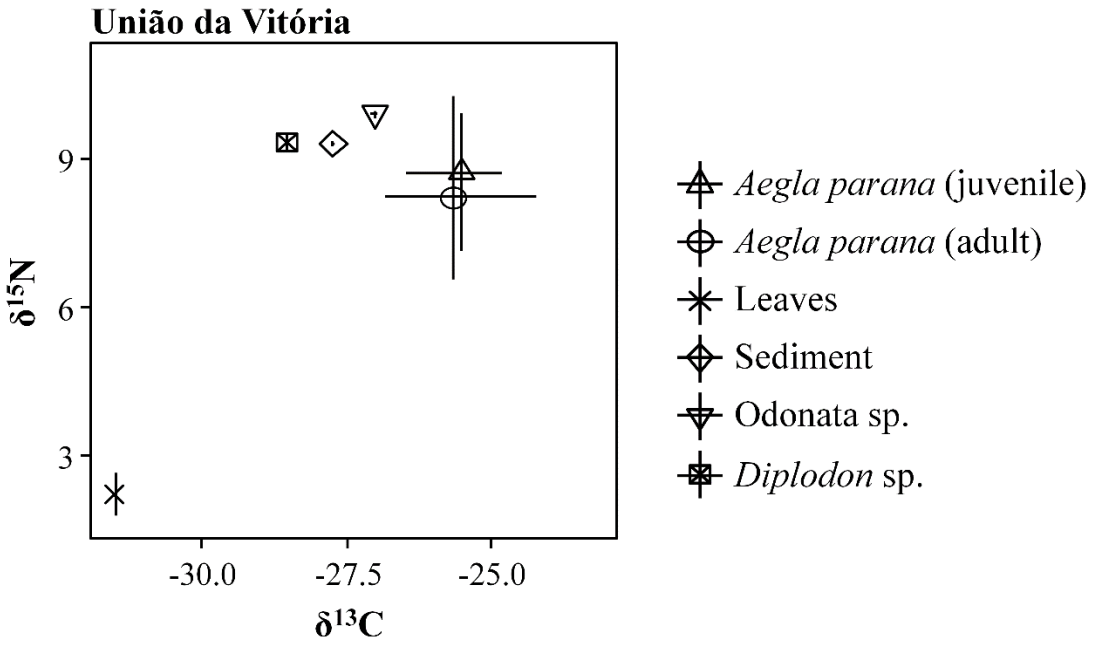

Figure 2. Biplots of stable isotopes using the mean for $\delta^{13}C$ and $\delta^{15}N$ (± standard deviation). Calculated values for *Aegla parana* juvenile-adult and potential food sources from União da Vitória, Paraná state, Brazil.

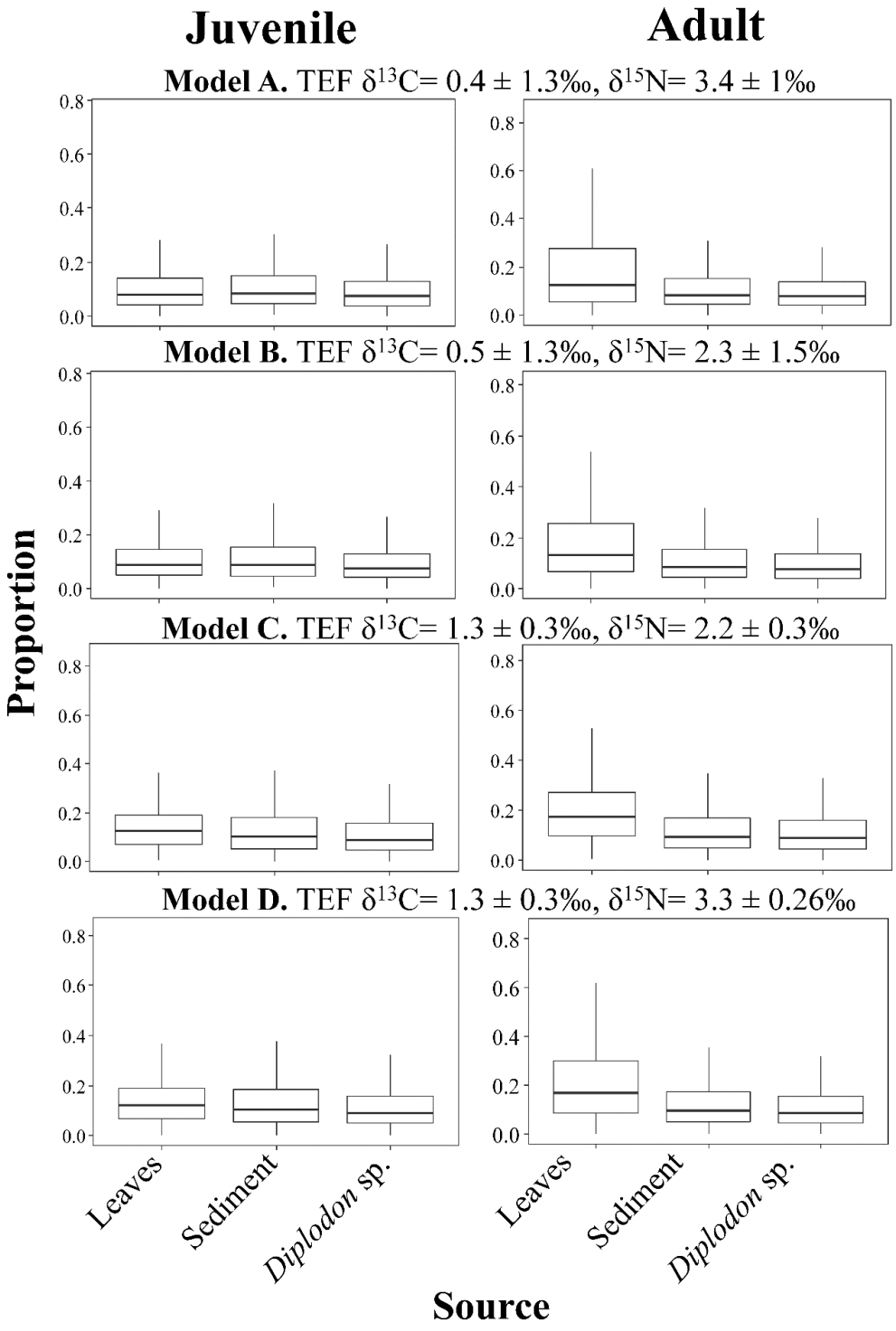

Figure 3. Median (horizontal line within the box), first and second quartile (boundaries of the box), and 95% credibility interval (whiskers of the box) of predicted proportion of food source contribution for the *Aegla parana* juvenile (Group 1) and *Aegla parana* adult (Group 2) in União da Vitória, Paraná state, Brazil. Different TDF values proposed in the literature were analyzed: (A) Post (2002) and (B, C and D) McCutchan *et al*. (2003).

DISCUSSION

The rapid degradation of South American's hydrographic basins together the anthropogenic pressure and the large number of aeglids species already threatened with extinction (Santos *et al*., 2017), leads us to intensify studies on aquatic fauna and their interactions, mainly in relation to *A. parana*, which is an important specie endemic.

Abiotic variables and water quality

Microbiological analyzes showed the presence of total coliforms and thermotolerant coliforms in the studied environment. Visually in the vicinity of the collection point there is not presence of anthropic activities, however the presence of coliforms and thermotolerant coliforms in the region were indicative of waste arising from human activity. Thus, it is possible that these anthropic products are carried by the rain and end up in the river, thus, sources from other locations (Pereira *et al*., 2020), which may be directly influenced in the results of this collection point.

High values of aluminum were found dissolved in the water of the study region. The increase in aluminum in the environment can come from several sources, but we believe that because it is a deactivated plant area, the presence of metal scraps and construction waste can lead to this increase (Jordão *et al*., 2007), mainly due to the presence of iron water wheels decomposing in the region. Furthermore, in União da Vitória the soil is an oxisol type, which predominates in the region, and is composed of Al oxides, represented in the Oxisols mainly by gibbsite, its presence being related to intense weathering and intense water circulation (Ghidin *et al*., 2006), which can also lead to an increase in aluminium.

Aluminium is not one of the most toxic trace elements in relation to aquatic organisms (Kolarova and Napiórkowski, 2021), but changes in oxidative enzyme activity and lipid peroxidation have been detected in the hepatopancreas and liver of organisms

obtained from metal-polluted waters (Meyer *et al*., 1991; Bouskill *et al*., 2006; Farombi *et al*., 2007; Barim and Karatepe, 2010; Faria *et al*., 2010; Radwan *et al*., 2010). In fish, clams and crayfish, toxicity of aqueous aluminium has been attributed to its association with the gills, resulting in excessive mucus secretion (Sadler and Lynam, 1987; Weatherley *et al*., 1991; Alexopoulos *et al*., 2003), and disruption of osmoregulatory processes (Sadler and Lynam, 1987; Fjeld *et al*., 1988), sodium transport (Weatherley *et al*., 1991) and gaseous diffusion (Exley *et al*., 1996). Little aluminium is accumulated in the hepatopancreas or other crayfish organs and tissues after aqueous exposure (Alexopoulos *et al*., 2003), however, in polluted places or where their food sources are contaminated by aluminium, there is the accumulation of significant amounts of metal in the hepatopancreas (Madigosky *et al*., 1991; Walton *et al*., 2010) and in the antenal gland (Walton *et al*., 2010).

Therefore, the low values found for the abiotic variables demonstrate a low impact site, however the presence of total and thermotolerant coliforms and the changes observed for aluminium are indicative that human activities extend across the river and that even old activities, as the plant deactivated, remain influencing this point.

Isotopic signature of juveniles-adults

Aegla parana did not present in this study significant variation in the isotopic signature among juvenile and adult, even the analysis of contribution of sources in the diet showed a little difference. The high standard deviation found for food sources in both phases may indicate that the food preference does not depend on the stage of life, so we refute our hypothesis that they use different food resources.

The absence of dietary difference between the ontogenetic phases may occur because females can demonstrate parental care (Rodrigues and Hebling, 1978) and have gregarious behavior. The gregarious behavior of juvenile- adult was described, because

they were grouped in shaded places and protected from thestrong current under shelters on the banks of the rapids (Bahamonde and López, 1961; López, 1965; Rodrigues and Hebllng, 1978), in which justify the absence of differences between juvenile-adult. As the collection site consists of a waterfall, this way, the gregarious behavior can be stimulated (same distribution in the river), in addition to the juveniles being able to reflect the female isotopic signature (parental care), which there would be no difference between signatures. Santos *et al*. (2008) also found in *Aegla longirostri* Bond-Buckup & Buckup, 1994 that there are no differences in diet of juvenile-adult, justifying through the same range of distribution in the water flow. This behavior probably leads to the sharing of food items, with contributions from each different source. Resource sharing can be due to several factors,despite overlap in prey choices, for example, the same resource can be shared accordingto the selection of prey size. In any case, if the resources are sufficiently abundant and not limiting, then competition between individuals does not occur and the selection of prey is done only according to the morphological and physiological characteristics of eachspecies (Bocher *et al*., 2014).

Trophic structure

In our study at the base of the chain are the leaves (2.21 ± 0.45), followed by *A. parana* adult (δ^{15}N 8.26‰ ± 1.38) and *A. parana* juvenile (δ^{15}N 8.42‰ ± 1.86) in the second trophic level. Sediment (9.31 0.0 ± 0.04), *Diplodon* sp. (9.33‰ ± 0.17) and Odonata (9.90‰ ± 0.08) at the third level, thus proving our hypothesis that the aeglids are at lower trophic levels. However, as our results showed that possibly some food items were not sampled, mainly the diversity of macroinvertebrates, and this becomes clear when we observed the difference in δ^{15}N between plants and aeglids that differ around 6‰, therefore the trophic web in União da Vitória in Rio Palmital is more complex than sampled.

Plants as primary producers are one of the most important groups for the functioning of ecosystems, due to their role as an energy provider for the base of the trophic web (Daufresne and Loreau, 2001). This can be seen in our work, as plants may be supplying energy to primary consumers (second trophic level) and contributing as an important item in the diet of adults and along with the other items collected for juvenile aeglids. Ingestion of plant debris as the main food item in aeglids has been demonstrated. Bueno and Bond-Buckup (2004) observed plant debris in the stomachs of 56% of *Aegla platensis* Schmitt, 1942 and 45% of *Aegla ligulata* Bond-Buckup & Buckup, 1994. Castro-Souza and Bond-Buckup (2004) recorded a frequency of occurrence of plant tissues greater than 90% in the stomachs of *Aegla camargoi* Buckup & Rossi, 1977 and *Aegla leptodactyla* Buckup & Rossi, 1977. In *A. longirostri*, the proportion of plant tissues was greater than 85% (Santos *et al*., 2008).

In addition, the leaves together with the sediment offer associated organic matter to the aeglids, microorganisms (*e.g.*, algae and bacteria) (Bueno and Bond-Buckup 2004; Castro-Souza and Bond-Buckup, 2004; Graça, 2001), or crustaceans Ostracoda and the Oligochaeta, like were found in the stomachs of *A. camargoi* (Castro-Souza and Bond-Buckup, 2004). However, in our work, these items were not sampled, but they may be contributed to the isotopic signal of the aeglids, justifying the difference in the isotopic ration between the leaves and the aeglids and the level below the sediment.

These animals are also important when using leaves as food. In tropical streams with few shredder insects, decapods can be great representatives in this class of shredder (Crowl *et al*., 2001; March *et al*., 2001), playing a more important role in the decomposition of leaves. In addition, during the leaf crushing process, the aeglids ingest part of the debris and convert it into fine particulate organic matter (FPOM), cycling nutrients, making the resulting FPOM serve as food for other functional groups (Cogo

and Santos, 2013).

The sediment, bivalve *Diplodon* sp. and the Odonata macroinvertebrates presented themselves at the third trophic level, therefore at a higher trophic level then *A. parana*. Sediment has not trophic position, but it can be a long-term integrator of particles and dead organisms from the water column, and it is readily sampled (Lake *et al*., 2001). Furthermore, the sediment is readily ingested by several crustaceans, including *Aegla* sp. (Bueno and Bond-Buckup, 2004; Castro-Souza and Bond-Buckup, 2004; Santos *et al*., 2008; Burress *et al*., 2013). Its ingestion can occur accidentally during feeding or adhered to other foods (D'Incao et al., 1990; Brogim & Lana, 1997). Lake *et al.* (2001) collected sediment and obtained average values for $\delta^{15}N$ 3.8 ± 1.9, witha maximum of 6.8‰ and a minimum of -0.1‰, the sites with highest values was in the close to lots, farm drains to pond, sewage treatment and plant input. However, they did not reach the sediment values that we found (9.31‰ ± 0.04). Therefore, we believe that, even though the water parameters according to CONAMA are indicating within the values considered potable. It is possible that this place is already suffering from nitrogen changes, possiblyresulting from human activities, as we observed in the microbiological samples. Thus, this accumulation of nitrogen in the sediment may be responsible for the increase of N for the other items in the chain, such as the bivalves that are filters.

The bivalve communities (Unionida), the *Diplodon* sp. for example, are important within the trophic structure of freshwater and can relate to multiple trophic levels (Vaughn *et al*., 2008). As bivalves accumulate matter suspended from the water column, they provide a long-term integrated isotopic signal from their food sources (Gillikin *et al*., 2006; Dubois *et al*., 2007) and, therefore, their isotopic ratio will vary according to the chain bases and the ambient conditions (Lake *et al.*, 2001; Burress *et al*., 2013). In addition, as they are filters (Vaughn *et al*., 2007) and their isotopic ratio will also vary

according to the availability of food. Lake *et al.* (2001) studied *Elliptio complanata* (Lightfoot, 1786) and obtained highest values of 12.0‰ and 13.3‰ in places with sewage treatment and old septic systems, and lowest values of 4.9‰ and 5.3‰ in places with minimal or non-existent inputs of anthropic action. Burress *et al.* (2013) studied *Corbicula fluminea* (Müller, 1774) and *Neocorbicula limosa* (Maton, 1811), with an isotopic nitrogen ratio between 7-9‰, which placed them in trophic levels below the juveniles and adults of *Aegla uruguayana* Schmitt, 1942. In our study, *Diplodon* sp. showed $\delta^{15}N$ 9.33‰ ± 0.17 values, that in comparison with the Lake *et al.* (2001) study, it is equivalent to places with old septic systems and places with house occupied onshore, and which also present values close to the Burress *et al*. (2013) study. N stable-isotope ratios indicate that mussels in some oligotrophic habitats, such as subalpine lakes, are strictly primary consumers (Vander Zanden and Rasmussen, 1999), thus, positioning itself at lower trophic levels. However, mussels in more enriched habitats feed across multiple trophic levels and eat primary producers and various consumer groups (Nichols and Garling, 2000). This may be occurring in União da Vitória, causing these bivalves to occupy a trophic level above the aeglids.

The larvae of Odonata were found in our work on the third trophic level, above the aeglids. These larvae are exclusively predators (Cummins, 1973), using live animal tissue as food (Cummins *et al*., 1979). Corbet (1980) classifies that these larvae pass through three important phases during their feeding: 1) Protozoa, small crustaceans and nematodes; 2) Insect larvae, including Odonata larvae; 3) Fingerlings and small tadpoles. Due to their predatory behavior, these larvae can be found at higher trophic levels of the aeglids. Burress *et al*. (2013), also demonstrated a basal position of *A. uruguayana* compared to the hexapods Hemiptera (Belostomatidae), Trichoptera (Hydropsychidae) and Ephemeroptera (Leptophlebiidae), which presented $\delta^{15}N$ around 9-11‰, close to the

value found for Odonata in our study (9.90‰). In addition, according to isotopic signatures, we have not ruled out the hypothesis that Odonata larvae may be feeding on young aeglids, since they can feed on small crustaceans.

Our study demonstrated the importance of aeglids within the trophic web. Its generalist diet, in both ontogenetic phases, and its low trophic position, demonstrated its significance as a link between primary producers and and the higher trophic levels. Fhurthemore, through nitrogen analyzes, we could infer that the study site already shows evidence thatanthropic action, which raise the nitrogen level of the animals that consume them, thus acting on populations, which can generate instability within the trophic web. Thus, it is essential to the sustainable development and conservation of species, the increase of studies with animals from Neotropical streams, especially endemic ones such as *A. parana* in União da Vitória, understanding the importance of each food item, the links between trophic levels and how they are interrelated.

REFERENCES

Agência nacional de águas (ANA). Hidroweb: Sistemas de informações hidrológicas. Available at http://www.snirh.gov.br/hidroweb/mapa. Accessed on 15 March 2021.

Alexopoulos, E.; McCrohan, C.R.; Powell, J.J.; Jugdaohsingh, R. and White, K.N. 2003. Bioavailability and toxicity of freshly neutralized aluminium to the freshwater crayfish *Pacifastacus leniusculus*. *Archives of Environmental Contamination and Toxicology*, 45: 509–514.

ANVISA. Agência nacional de vigilância sanitária. Portaria de Consolidação nº5, de 28 de setembro de 2017. Available at www.portalarquivos2.saude.gov.br. Accessed on 03 March 2021.

Bahamonde, N. and Lopez, M.T. 1961. Estudios biológicos en lá población de *Aegla laevis* laevis (Latreille) de El Monte. *Investigaciones Zoológicas Chilenas*, 7: 21–58. Barim, O. and Karatepe, M. 2010. The effects of pollution on the vitamins A, E, C,carotene contents and oxidative stress of the freshwater crayfish, *Astacus leptodactylus*. *Ecotoxicology and Environmental Safety*, 73: 138–142

Bearhop, S.; Waldron, S.; Votier, S.C. and Furness, R.W. 2002. Factors that influence assimilation rates, and fractionation of nitrogen and carbon isotopes in avian blood and feathers. *Physiological and Biochemical Zoology*, 75: 451–458.

Bocher, P.; Robin, F.; Kojadinovic, J.; Delaporte, P.; Rousseau, P.; Dupuy, C. and Bustamante, P. 2014. Trophic resource partitioning within a shorebird community feeding on intertidal mudflat habitats. *Journal of Sea Research*, 92: 115–124.

Bond-Buckup, G. 2003. Família Aeglidae. p. 21–116. In: G.A.S Melo (ed), Manual de identificação dos Crustacea Decapoda de água doce do Brasil. Editora Loyola, São Paulo.

Bond-Buckup, G. and Buckup, L. 1994. A família Aeglidae (Crustacea, Decapoda, Anomura). *Arquivos de Zoologia*, 32: 159–346.

Bosley, K.M.; Copeman, L.A.; Dumbauld, B.R. and Bosley, K.L. 2017. Identification of burrowing shrimp food sources along an estuarine gradient using fatty acid analysis and stable isotope ratios. *Estuaries and Coasts*, 40: 1113–1130.

Bouskill, N.J.; Handy, R.D.; Ford, T.E. and Galloway, T.S. 2006. Differentiating copper and arsenic toxicity using biochemical biomarkers in *Asellus aquaticus* and *Dreissenapolymorpha*. *Ecotoxicology and Environmental Safety*, 65: 342–349.

Boutton, T.W. 1996. Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change. p. 47–82. In: T.W. Boutton and S.I. Yamasaki (eds), Mass spectrometry of soils. Marcel Dekker, New York.

Brasil, 2005. Ministério do Meio Ambiente. Conselho Nacional do Meio Ambiente - CONAMA. Resolução nº 357 de 17 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. Diário Oficial da União, Brasília, DF.

Brogim, R.A. and Lana, P.C. 1997. Espectro alimentar de *Aratus pisonii*, *Chasmagnathus granulata* e *Sesarma rectum* (Decapoda, Grapsidae) em um manguezal da Baóa de Paranaguá. *Iheringia, Série Zoologia*, 83: 35–44.

Bueno, A.A.P. and Bond-Buckup, G. 2004. Natural diet of *Aegla platensis* Schmitt and *Aegla ligulata* Bond-Buckup and Buckup (Crustacea, Decapoda, Aeglidae) from Brazil. *Acta Limnologica Brasiliensia*, 16: 115–127.

Bueno, S.L.S.; Shimizu, R.M. and Rocha, S.S. 2007. Estimating the population size of *Aegla franca* (Decapoda: Anomura: Aeglidae) by mark-recapture technique from an isolated section of Barro Preto stream, county of Claraval, state of Minas Gerais, southeastern Brazil. *Journal of Crustacean Biology*, 27: 553–559.

Burress, E.D.; Gangloff, M.M. and Siefferman, L. 2013. Trophic analysis of two subtropical South American freshwater crabs using stable isotope ratios. *Hydrobiologia*, 702: 5–13.

Carabel, S.; Godínez-Domínguez, E.; Verísimo, P.; Fernández, L. and Freire, J. 2006. An assessment of sample processing methods for stable isotope analyses of marine foodwebs. *Journal of Experimental Marine Biology and Ecology*, 336: 254–261.

Castro-Souza, T. and Bond-Buckup, G. 2004. O nicho trófico de duas espécies simpátricas de *Aegla Leach* (Crustacea, Aeglidae) no tributário da bacia hidrográficado Rio Pelotas, Rio Grande do Sul, Brasil. *Revista Brasileira de Zoologia*, 21: 805– 813.

Caxito, F.A. and Silva A.V. 2015. Isótopos estáveis: fundamentos e técnicas aplicadas à Caracterização proveniência geográfica de Produtos alimentícios. *Geonomos*, 23: 10–17.

City hall from União da Vitória. General Data. Available at http://uniaodavitoria.pr.gov.br/dados-gerais. Accessed on 15 March 2021.

Clapcott, J.E. and Bunn, S.E. 2003. Can C_4 plants contribute to aquatic food webs of subtropical streams?. *Freshwater Biology*, 48: 1105–1116.

Cogo, G.B. and Santos, S. 2013. The role of aeglids in shredding organic matter in Neotropical streams. *Journal of Crustacean Biology*, 33: 519–526.

Collins, P. 2019. Trophic ecology. p. 97–132. In: S. Santos and S.L.S. Bueno (eds), Aeglidae: Life History and Conservation Status of Unique Freshwater Anomuran

Decapods, CRC Press, Taylor and Francis Group, New York.
Corbet, P. 1980. Biology of Odonata. *Annual Review of Entomology*, 25: 189–217.
Crowl, T.A.; McDowell, W.H.; Covich, A.P. and Johnson, S.L. 2001. Freshwater shrimp effects on detrital processing and nutrients in a tropical headwater stream. *Ecology*, 82: 775–783.
Cui, B.; Zhang, Q.; Zhang, K.; Liu, X. and Zhang, H. 2011. Analyzing trophic transfer of heavy metals for food webs in the newly-formed wetlands of the Yellow River Delta,China. *Environmental Pollution*, 159: 1297–1306.
Cummins, K.W. 1973. Trophic relations of aquatic insects. *Annual Review of Entomology*, 18: 183–206.
Cummins, K.W. and Klug, M.J. 1979. Feeding ecology of stream invertebrates. *Annual Review of Entomology*, 10: 147–172.
Daufresne, T. and Loreau, M. 2001. Ecological stoichiometry, primary producer–decomposer interactions, and ecosystem persistence. *Ecology*, 82: 3069–3082.
D'Incao, F.; Silva, K.G.; Ruffino, M.L. and Braga, A.C. 1990. Hábito alimentar do caranguejo *Chasmagnathus granulata* Dana, 1851 na barra do Rio Grande, RS (Decapoda, Grapsidae). *Atlântica*, 12: 85-93.
Dubois, S.; Blin, J.L.; Bouchaud, B. and Lefebvre, S. 2007. Isotope trophic-step fractionation of suspension-feeding species: implications for food partitioning in coastal ecosystems. *Journal of Experimental Marine Biology and Ecology*, 351: 121–128.
Ducatti, C.; Martins, C.L.; Arrigoni, M.B.; Martins, M.B.; Vieira Jr., L.C. and Denadai,
J.C. 2011. Utilização de isótopos estáveis em ruminantes. *Revista Brasileira de Zootecnia*, 40: 68–75.
Exley, C.; Wicks, A.J.; Hubert, R.B. and Birchall, J.D. 1996. Kinetic constraints in acute aluminium toxicity in the rainbow trout (*Ocorhynchus mykiss*). *Journal of TheoreticalBiology*, 179: 25–31.
Faria, M.; Huertas, D.; Soto, D.X.; Grimalt, J.O.; Catalan, J.; Riva, M.C. and Barata, C. 2010. Contaminant accumulation and multi-biomarker responses in field collected zebra mussels (*Dreissena polymorpha*) and crayfish (*Procambarus clarkii*), to evaluate toxicological effects of industrial hazardous dumps in the Ebro River (NE Spain). *Chemosphere*, 78: 232–240.
Farombi, E.O.; Adelowo, O.A. and Ajimoko, Y.R. 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River. *International Journal of Environmental Research and Public Health*, 4: 158–165
Fjeld, E.; Hessen, D.O.; Roos, N. and Taugbøl, T. 1988. Changes in gill ultrastructure and haemolymph chloride concentrations in the crayfish, *Astacus astacus*, exposed tode-acidified aluminum-rich water. *Aquaculture*, 72: 139–150
Fry, B. 1991. Stable isotope diagrams of freshwater food webs. Ecology, 72: 2293–2297. Gannes, L.Z.; Del-Rio, C.M. and Koch, P. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comparative Biochemistry Physiology*, 119: 725–737.
Gearing, P.J.; Gearing, J.N.; Maughan, J.T. and Oviatt, C.A. 1991. Isotopic distribution of carbon from sewage sludge and eutrophication in the sediments and food web of estuarine ecosystems. *Environmental Science & Technology*, 25: 295–301.
Ghidin, A.A.; Melo, V.D.F.; Lima, V.C. and Lima, J.M.J.C. 2006. Toposseqüências de Latossolos originados de rochas basálticas no Paraná: I - mineralogia da fração argila.*Revista Brasileira de Ciência do Solo*, 30: 293–306.
Gillikin, D.P.; Lorrain, A.; Bouillon, S.; Willenz, P. and Dehairs, F. 2006. Stable carbon

isotopic composition of *Mytilus edulis* shells: relation to metabolism, salinity, $\delta^{13}C$ DIC and phytoplankton. *Organic Geochemistry*, 37: 1371–1382.
Graça, M.A.S. 2001. The role of invertebrates on leaf litter decomposition in streams - a review. *International Review of Hydrobiology*, 86: 383–393.
Huryn, A.D.; Riley, R.H.; Young, R.G.; Arbuckle, C.J.; Peacock, K. and Lyon G. 2001. Temporal shift in contribution of terrestrial organic matter to consumer production ina grassland river. *Freshwater Biology*, 46: 213–226.
Hyslop, E.J. 1980. Stomach contents analysis - a review of methods and their application. *Journal of Fish Biology*, 17: 411–429.
Ingram, B.A.; Pitt, K.A. and Barnes, P. 2017. Stable isotopes reveal a potential kleptoparasitic relationship between an ophiuroid (*Ophiocnemis marmorata*) and theSemaeostome jellyfish, *Aurelia aurita*. *Journal of Plankton Research*, 39: 138–146.
Jordão, C.P.; Ribeiro, P.R.S.; Matos, A.T. and Fernandes, R.B.A. 2007. Aquatic Contamination of the Turvo Limpo River Basin at the Minas Gerais State, Brazil. *Journal of Brazilian Chemical Society*, 18: 116–125.
Karasov, W. and Martínez Del Río, C.M. 2007. Physiological ecology: How animals process energy, nutrients, and toxins. Princeton University Press, Princeton, NJ, 744p.
Kelly, J.F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology*, 78: 1–27.
Kolarova, N. and Napiórkowski, P. 2021. Trace elements in aquatic environment. Origin, \distribution, assessment and toxicity effect for the aquatic biota. Ecohydrology &Hydrobiology, 1: 14.
Lajtha, K. and Michener, R.H. 1994. Stable isotopes in ecology and environmental science. Oxford: Blackwell Scientific Publications, 316p.
Lake, J.L.; McKinney, R.A.; Osterman, F.A.; Pruell, R.J.; Kiddon, J.; Ryba, S.A. and Libby, A.D. 2001. Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. *Canadian Journal of Fisheries and Aquatic Sciences*, 58:870–878.
López, M.T. 1965. Estudios biológicos en *Aegla odebrechtti paulensis*, Schmitt. *Boletim da Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo, Zoologia*, 25: 301–314.
Madigosky, S.R.; Alvarez-Hernandez, X. and Glass, J. 1991. Lead, cadmium and aluminium accumulation in the red swamp crayfish *Procambarus clarkii* G. collectedfrom roadside drainage ditches in Louisiana. *Archives of Environmental Contamination and Toxicology*, 20: 253–258.
Magni, S.T. and Py-Daniel, V. 1989. *Aegla platensis* Schmitt, 1942 (Decapoda: Anomura) a predator of Simuliidae immature stages (Diptera: Culicomorpha). *Revistade Saúde Pública*, 23: 258–259.
Mao, Z.; Gu, X. and Zeng, Q. 2016. Food sources and trophic relationships of three decapod crustaceans: insights from gut contents and stable isotope analyses. *Aquaculture Research*, 47: 288–2898.
March, J.G.; Benstead, J.P.; Pringle, C.M and Ruebel, M.W. 2001. Linking shrimp assemblages with rates of detrital processing along an elevational gradient in a tropical stream. *Canadian Journal of Fisheries and Aquatic Sciences*, 58: 470–478.
McCutchan, Jr. J.H.; Lewis, Jr, W.M.; Kendall, C. and McGrath, C.C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, 102: 378–390.

Meyer, W.; Kretschmer, M.; Hoffmann, A. and Harisch, G. 1991. Biochemical and histochemical observations on effects of low-level heavy metal load (lead, cadmium)in different organ systems of the freshwater crayfish, *Astacus astacus* L. (Crustacea: Decapoda). *Ecotoxicology and Environmental Safety*, 21: 137–156.

Minagawa, M. and Wada, E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relationship between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta*, 48: 1135–1140.

Mugnai, R.; Nessimian, J.L. and Baptista, D.F. 2010. Manual de Identificação de Macroinvertebrados Aquáticos do Estado do Rio de Janeiro. Rio de Janeiro, TechnicalBooks Editora, 174p.

Musin, G.E.; Rossi, A.; Diawol, V.P.; Collins, P.A. and Williner, V. 2017. Dynamic metabolic pattern of *Aegla uruguayana* (Schmitt, 1942) (Decapoda: Anomura: Aeglidae): responses to seasonality and ontogeny in a temperate freshwater environment. *The Journal of Crustacean Biology*, 37: 436–444.

Nagata, R.M.; Moreira, M.Z.; Pimentel, C.R. and Morandini, A.C. 2015. Food web characterization based on $\delta^{15}N$ and $\delta^{13}C$ reveals isotopic niche partitioning between fish and jellyfish in a relatively pristine ecosystem. *Marine Ecology Progress Series*,519: 13–27.

Nichols, S.J. and Garling, D. 2000. Food-web dynamics and trophic-level interactions in a multispecies community of freshwater unionids. *Canadian Journal of Zoology*, 78:871–882.

Parnell, A. and Inger, R. 2016. Stable isotope mixing models in R with simmr. Available at https://cran. r-project.org/web/packages/simmr/vignettes/simmr. Accessed on 20 de October de 2020.

Pereira, M.A.B.; dos Santos Alves, W.; de Oliveira, L.D.; Morais, W.A.; Lima, L.O. and da Costa Nunes, N. 2020. Qualidade Hídrica da Cachoeira do Rio São Tomás, no Município de Rio Verde, Goiás, Brasil. *Revista Brasileira de Geografia Física*, 13: 377–390.

Peterson, B.J. and Fry, B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, 18: 293–320.

Post, D.M. 2003. Individual variation in the timing of ontogenetic niche shifts in largemouth bass. *Ecology*, 84: 1298–1310.

Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83: 703–718.

Radwan, M.A.; El-Gendy, K.S. and Gad, A.F. 2010. Oxidative stress biomarkers in the digestive gland of *Theba pisana* exposed to heavy metals. *Archives of EnvironmentalContamination and Toxicology*, 58: 828–835

Riascos, J.M.; Docmac, F.; Reddin, C. and Harrod, C. 2015. Trophic relationships between the large scyphomedusa *Chrysaora plocamia* and the parasitic amphipod *Hyperia curticephala*. *Marine Biology*, 162: 1841–1848.

Rice, E.W.; Baird, R.B. and Eaton, A.D. 2017. Standard Methods for the Examination of Water and Wastewater; American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF).

Robinson, D. 2001. $\delta^{15}N$ as an integrator of the nitrogen cycle. *Trends in Ecology and Evolution*, 16: 153–162.

Rodrigues, W. and Hebling, J.N. 1978. Estudos biológicos em *Aegla perobae* Hebling & Rodrigues, 1977 (Decapoda: Anomura). *Revista Brasileira de Biologia*, 38: 383–390.

Saborowski, R. 2015. Nutrition and digestion. p. 285–319. In: E.S. Chang and M. Thiel (eds), The natural history of the Crustacea. Oxford University Press, Vol. 4.

UK, Oxford.
Sadler, K. and Lynam, S. 1987. Some effects on the growth of brown trout from exposure to aluminium at different pH levels. *Journal of fish biology*, 31: 209–219.
Santos, S.; Ayres-Peres, L.; Cardoso, R.C. and Sokolowicz, C.C. 2008. Natural diet of the freshwater anomuran *Aegla longirostri* (Crustacea, Anomura, Aeglidae). *Journalof Natural History*, 42: 1027–1037.
Santos, S.; Bond-Buckup, G.; Gonçalves, A.S.; Bartholomei-Santos, M.L.; Buckup, L. and Jara, C.G. 2017. Diversity and conservation status of *Aegla* spp. (Anomura, Aeglidae): an update. *Nauplius*, 25: 1–14.
UNIÃO DA VITÓRIA. Director plan from União da Vitória. União da Vitória: City hall of União da Vitória, 2017. Available at http://uniaodavitoria.pr.gov.br/wp-content/uploads/2017/08/pdm-uniao-da-vitoria-2.pdf. Accessed on 03 March 2021.
Vander Zanden, M.J. and Rasmussen, J.B. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. *Ecology*, 1395–1404.
Vaughn, C.C.; Nichols, S.J. and Spooner, D.E. 2008. Community and foodweb ecology of freshwater mussels. *Journal of the North American Benthological Society*, 27: 409–423.
Vaughn, C.C.; Spooner, D.E. and Galbraith, H.S. 2007. Context-dependent species identity effects within a functional group of filter-feeding bivalves. *Ecology*, 88: 1664–1662.
Vogel, J.C. 1993. Variability of carbon isotope fractionation during photosynthesis. p. 29–46. In: J.R. Ehleringer; A.E. Hall and G.D. Farquhar (eds), Stable isotopes and plant carbon: water relations. Academic Press, San Diego.
Walton, R.C., McCrohan, C.R., Livens, F., White, K.N. 2010. Trophic transfer of aluminium through an aquatic grazer-omnivore food chain. *Aquatic Toxicology*, 99: 93–99.
Weatherley, N.S., Rutt, G.P., Thomas, S.P. and Ormerod, S.J. 1991. Liming acid stream: aluminium toxicity to fish in mixing zones. *Water Air Soil Pollution*, 55: 345–353.

CHAPTER III

Environment and anthropic influences on *Aegla castro* Schmitt, 1942 (Crustacea: Anomura) trophic interactions

Abstract

The advance of anthropogenic activities constantly threatens freshwater ecosystems. The endemic freshwater aeglids are sensitive species to a disturbance and/or abrupt environmental variations and it can be a bioindicator of water quality. In this way,this study investigated the trophic position and food web for *Aegla castro*, in four streams with different anthropogenic disturbance, attesting how the environmental scenarios can modulate trophic relationships through stable isotope analyzes. Aeglids were found as a primary consumer; however, we found differences in isotopic signature for this crustacean in the studied areas, indicating that environment and anthropogenic action can be the factors that modulate the trophic webs. This promoting different isotopic signature, consequently changing the rates of carbon and nitrogen between areas. This factor generates different trophic interactions and different food items. For example, in the Iapó stream results, that through that the highest value of $\delta^{15}N$ (11.54‰), it was possible to detect the higher amount of anthropic organicwaste differently to the others studied tributaries with better conservation status, *e.g.* in Quebra Perna stream ($\delta^{15}N$ = 4.03‰). Studies like this are becoming increasingly important due to the rapid degradation of freshwater environments and the lack of trophicknowledge about these endemic animals. Just as it becomes important to understand howthe anthropic action is interfering in the trophic relationships, and how it can affect the permanence of species like *Aegla* spp.

Keywords: Aeglids, carbon, anthropic pollution, food web, isotope stable, nitrogen.

INTRODUCTION

Impacts of anthropogenic disturbance are especially severe in freshwater ecosystems. In particular, land use disturbance can lead to increased levels of pollution, including elevated nutrient and sediment loads whose negative impacts range from the community to the individual level (Britton *et al*., 2019). Therefore, this rapid degradation of hydrographic basins in South America, where the decline in water quality observed, requires intensification in studies on continental aquatic fauna and their interactions (Bond-Buckup and Buckup, 1994).

The aeglids have 89 described species, 56 of which are present in Brazilian continental waters (Bueno et al., 2017; Santos et al., 2017; Páez et al., 2018; Trombetta et al., 2019; Marçal et al., 2020), being important representatives of low order streams. These crustaceans preferably live in environments with low temperatures and high levels of oxygen (Feldmann et al., 1998; Bueno et al., 2007; Francisco et al., 2007; Oliveira et al., 2007). Environmental disturbances are the main factors that can cause the reduction or disappearance of populations of *Aegla* spp. (Bond-Buckup and Santos, 2007; Trevisan et al., 2009). Aeglids are endemic, which severely affected by anthropic effects as they have a restricted distribution, and, therefore, they must consider essential for the determination of biodiversity hotspots in studies of conservation and management of native species (Pimm et al., 1995; Caldecott et al., 1996). In addition, this crustacean have great ecological importance, due to its 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 areable to feed on allochthones plant matter, particulate organic matter and aquatic insect larvae (Magni and Py-Daniel 1989; Bueno and Bond-Buckup, 2004), thus being a key species in nutrient cycling (Cogo and Santos, 2013). Feeding and assimilation for aeglids are of paramount importance, due to their need to obtain energy for their

homeostasis and to facilitate the synthesis and degradation of macromolecules necessary for maintenance, growth, reproduction and behavior (Karasov and Martínez Del Río, 2007; Saborowski; , 2015; Musin et al., 2017).

Stable isotope analysis has been widely used to identify the food sources and trophic position of consumers, in recent years (Peterson and Fry, 1987; Post, 2002) and nutrient cycling pathways in terrestrial and aquatic ecosystems (Lajtha and Michener, 1994). This method based on the premise that predators enriched with heavy isotopes compared to their prey (Minagawa and Wada, 1984). This isotopic composition integrates the signatures of different prey consumed in longer periods and incorporated intothe tissues, different from what sampled in the analysis of stomach contents, which provides direct evidence of the food eaten at that particular moment (Hyslop, 1980; Mao*et al*., 2016). Therefore, we chose to use in our study the analysis of stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) to study the trophic web that *Aegla castro* Schmitt, 1942. The carbon and nitrogen isotopic composition of consumer tissues are thus a function to analyze the prey species $\delta^{15}N$-$\delta^{13}C$'s signatures, the relative proportions of each prey species assimilated and in certain instances, and the foraging location. Moreover, the stable isotope signatures of tissues generally reflect the diet over the periodthat the tissue was synthesized (Bearhop *et al*., 2002).

The carbon incorporated in plants suffers isotopic discrimination throughout the process of photosynthesis. In C_3 plants, the $\delta^{13}C$ value ranges from -22 to -34‰, and for C_4 plants ranges from -9 to -16‰ (Vogel, 1993; Boutton, 1996; Ducatti *et al*., 2011), and makes it possible to characterize an animal's diet through isotopic analysis of the carbon in tissues (Ducatti, 2011). Plants are the primary source of food for animals, so the isotopic signature of $\delta^{13}C$ differentiates C_3 or C_4 producers in the food chains (Peterson and Fry, 1987; Clapcott and Bunn, 2003). Nitrogen can be incorporated by algae/plants

through association with nitrogen-fixing bacteria or from the assimilation of nitrogenous compounds in the air and in the soil (Caxito and Silva, 2015), whereas in animals it is assimilated by the incorporation of proteins (Gannes *et al*., 1998). The nitrogen is use to determine trophic niches and trophic position, because $\delta^{15}N$ of each trophic level is typically 3 higher than its source of nitrogen (Minagawa and Wada, 1984), in addition enriched $\delta^{15}N$ are often associated with carnivore behavior (Post, 2003).

Furthermore, $\delta^{15}N$ can used as an indicator of environmental pollution. According to Tanu *et al*. (2020) studying mangroves and land use around them, estimated that $\delta^{15}N$ can vary from 5-14‰ in those mangroves that have their surroundings with human activities (livestock and agriculture), while places with less human action, this value does not exceed 3‰, suggesting that the N from anthropogenic action is regulating the nitrogen concentration in the leaves in these locations. In coastal environments, a change in $\delta^{15}N$ was also recorded in plants and animals, where sewage in port locations promoted higher levels of nitrogen (Lassauque *et al*., 2010). Dolenec *et al*. (2006) recorded a $\delta^{15}N$ enrichment of up to 4.7‰ in environments influenced by fish farming, compared to the unaffected reference area.

There are few studies on trophic interactions in tropical-subtropical freshwater comunnities, mainly with eglids species ussualy found in environment degraded by human disturbance activity (Burress *et al*., 2013; Santos et al., 2017). Invertebrates. Thus, it is necessary to intensify studies on aquatic fauna and their interactions (Bond-Buckup and Buckup, 1994) and to assess the quality of the environments in whichendemic species, like *A. castro,* live. There are no studies with the trophic structure of *A. castro,* therefore, our objective was to study the trophic web that A. castro in the southeastern and southern basins of Brazil.

Therefore, our hypotheses are: a) These crabs have a lower position in the food chain, as primary consumers, being an important link between producers and other trophic levels; b) Due to the differences between the sampling areas, it is expected to find a difference in the isotope ratios of carbon and nitrogen by *A. castro* and other items of the food web.

MATERIAL AND METHODS

Study area

We chose four different area of the sampling to collect *A. castro* based on its geographic distribution and the state of preservation, *i.e.*: Paranapanema sub-basin (São Paulo state), (1) Itaúna stream (ITA) (Itatinga city); and Tibagi sub-basin (Paraná state), (2) Preto stream (PRE) (Mauá da Serra city), (3) Iapó stream (IAP) (Castrocity) and (4) Quebra Perna stream (QPE) (Ponta Grossa city). The locations weredescribed through personal observations and using google maps to view the surroundingarea of the sampling areas:

_ ITA tributary (23°09’38”S 48°37’56”W): the local vegetation is Atlantic forest and Cerrado biome (Montana semi-deciduous), with a lot of eucalyptus reforestation. The sampling area is located at a private farm and the stream has preserved and continuous riparian forest with a good tree canopy and creeping plants (grass and herbaceous).

_ PRE tributary (23°57'00"S 51°07'00"W): the local vegetation is Atlantic forest (Dense Ombrophilous Forest with remnants of Mixed Ombrophilous Forest). It is located behind a restaurant and a road with small dirt roads and constructions surrounding the area. Riparian forest is present as well areas composed of creeping plants (grass), tree canopy.

_ IAP tributary (24º48'37"S 49º51’30"W): the local vegetation is Atlantic Forest (Dense Ombrophilous Forest with remnants of Mixed Ombrophilous Forest). The location 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 area specifically is composed by a deforested area with creeping plants (grass), close to a road.

_ QPE tributary (25°10'19"S 49°58'08"W): the local vegetation is Atlantic Forest (Dense Ombrophilous Forest and natural field), and the sampling area is inside the private park “Buraco do Padre”. It is a touristic park where the main attraction is a cave with a waterfall being accessed through an approximately 730 meters trail. Visitors usually go there for outdoor activities as walking, bathing, rappelling, hiking and climbing. Around the park, places with plantations and deforestation are observed.

Biological samples

Aeglids were collected during August (PRE), September (IAP and QPE) and December (IAP) 2018, using 6 traps measuring 24cm × 19.5cm × 8cm, 6 traps measuring 24cm × 14.5cm × 7cm and 6 traps measuring 17.5cm × 12.5cm × 5cm, 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 aeglides (mostly young individuals) and the other items involved in aeglide trophic

interactions, based on the food sources already described as a food item for aeglides. Leaves, algae and moss were collected manually. All material was stored in thermal boxes with ice for further analysis. Macroinvertebrateswere identified in the laboratory with the help of literature (Mugnai *et al*., 2010), but somemacroinvertebrates were not analyzed because they didn't have enough weight to performthe C and N analysis (Lepidoptera and Hemiptera in PRE, Ephemeroptera in QPE and ITA, Trichoptera in IAP and Odonata, Plecoptera and Diptera in ITA). 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 (Rice *et al*., 2017). Microbiological analyzes were performed using the multi-tube technique, according to PRC N ° 5, of 09/28/2017 (ANVISA), 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 - µS/cm), turbidity (Turb), temperature (Temp - °C), total dissolved solids (TDS - mg/L), chlorophyll (Chl - µg/L) and dissolved oxygen (DO - mg/L), were measured using a multi-parameter Eureka probe (Manta model 2-4.0).

Stable isotope analysis

Stable isotope analyses provide a picture of diet integrated over a period of time, while conventional dietary analysis by stomach contents only 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 may also be biased toward particular types of prey (Hobson *et al*., 1994). In addition, isotope analyzes require small numbers of animals, which are of great value since this group is already at various levels of threat. Samples of bulk sediment were collected from the middle of river. The $\delta^{15}N$ values of sediment were examined for use as a basis for predicting the $\delta^{15}N$ values of aeglids. Sediment has no trophic position, but it can be a long-term integrator of particles and dead organisms from the water column, and it is readily sampled (Lake *et al*., 2001). In addition, 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 the chelipods for the aeglids, muscles between the dorsal and ventral fin of fish and abdomen muscle of the shrimp. All the macroinvertebrates were small in size, so they were processed whole. All samples were dehydrated at 50°C, 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 $\delta^{13}C$ analyses, the samples were soaked in 1M 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).

The stable isotope analysis was conducted at the Stable Isotopes Center, Institute of Biosciences, UNESP, Botucatu. Aliquots of the homogeneous material (50-70 ± 1μg for $\delta^{13}C$ and 500-600 ± 1μg for $\delta^{15}N$) were analyzed in duplicate and placed in tin capsules (5mm, Sercon), except for material with HCL, which were placed in silver capsules inside

tin capsules (Carabel *et al*., 2006). Lipid extraction was not performed in our study, so the data used were the raw values found for the organisms. 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 2000 - 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 (Rsample = $^{12}C/^{13}C$ or $^{15}N/^{14}N$) of each sample. The results are given in the relative difference of isotope ratio (δ) of a standard value (Rstandard) expressed in ‰ accordance with:

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

For $\delta^{13}C$, the $Rs_{standard}$ is V-PDB (Vienna Pee dee Belemnite) and for $\delta^{15}N$ is atmosphericair. The standard uncertainty (n = 10) of analyzes was ± 0.2‰ for $\delta^{13}C$ and ± 0.15‰ for$\delta^{15}N$.

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 sequentially primary and secondary consumers. he estimated of the trophic level (TL) was calculated according to the formula presented by Post (2002):

$$TL=\lambda+(\delta^{15}N_{consumidor}-\delta^{15}N_{base})/\Delta^{15}N$$

Where: λ value 1 was used, since the organisms used in $\delta^{15}N_{base}$ were considered primary producers; $\delta^{15}N_{consumidor}$ is the value of each consumer; and $\Delta^{15}N$ is the enrichment in $\delta^{15}N$ 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 permanova analysis was done to test the differences between the areas of sampling. The programs used for statistical analysis were the R language (R Development Core Team, 2011; available at: www.R-project.org).

To determine differences between area of the sampling areas (ITA, PRE, IAP and QPE) regarding to the stable isotopes, the multivariate analysis of variance (MANOVA) was performed, with the values of carbon and nitrogen connected, because these isotopes are directly connected to each other. Then, carbon and nitrogen data were analyzed separately with factorial analysis of variance (ANOVA) to identify differences for each isotope by the area of the sampling areas (ITA, PRE, IAP and QPE).

To produce a biplot with the signatures of $\delta^{15}N$ e $\delta^{13}C$, 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), program R (R Development Core Team 2011). SIMMr adapts to a Bayesian mixture model to determine estimates of contributions from dietary sources in the aeglidean diet, using isotopic data (Parnell and Inger, 2016). The programs used for statistical analysis were the R language (R Development Core Team, 2011; available at: www.R-project.org).

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. 1), in all area of the sampling there are indications of human waste. Chemical analyzes showed parameters as aluminium, iron and cuprum outside the maximum allowed value (Tab. 1). For the aluminum, the all area of the sampling showed values above the maximum allowed value (0.2 $mg.L^{-1}$), *i.e.*, values between 2.59 $mg.L^{-1}$ to 6.65 $mg.L^{-1}$. In IAP, iron (0.70 $mg.L^{-1}$) showed values above maximum allowed value (0.3 $mg.L^{-1}$) and in QPE, cuprum (1.71 $mg.L^{-1}$) showed values above the maximum allowed value (1.5 $mg.L^{-1}$), but in this case the values were not so different from the maximum allowed values (Tab. 1). 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 area of sampling according to the environmental factors (Fig. 1). The Permanova showed differences between the areas of sampling and abiotic factors ($F= 2.46$, $df = 3$, $p < 0.05$).

Table 1. Abiotic factors (mean ± standard deviation), water quality through the abiotic factors, microbiological and chemical analysis in in 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. Legend of abiotic factors: pH, Ec (Conductivity), Turb (Turbidity), Temp (Temperature), TDS (Total dissolved solids), Chl (Chlorophyll), DO (Dissolved oxygen).

Abiotic factors				
	ITA	**PRE**	**IAP**	**QPE**
pH	8.55 ± 0.06	8.94 ± 0.23	8.96 ± 0.04	8.95 ± 0.24
Ec (μS/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 (°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 (μg/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				
Number more probable of total coliforms	7.3/100 mL of water	3.6/100 mL of water	>23/100 mL of water	>23/100 mL of water
Number more probable of thermotolerant coliforms	7.3/100 mL of water	3.6/100 mL of water	9.2/100 mL of water	16.1/100 mL of water
Chemical analysis	Aluminum (6.65 $mg.L^{-1}$)	Aluminum (3.28 $mg.L^{-1}$)	Iron (0,70 $mg.L^{-1}$) Aluminum (2.59 $mg.L^{-1}$)	Cuprum (1.71 (m $mg.L^{-1}$) Aluminum (4,36 $mg.L^{-1}$),

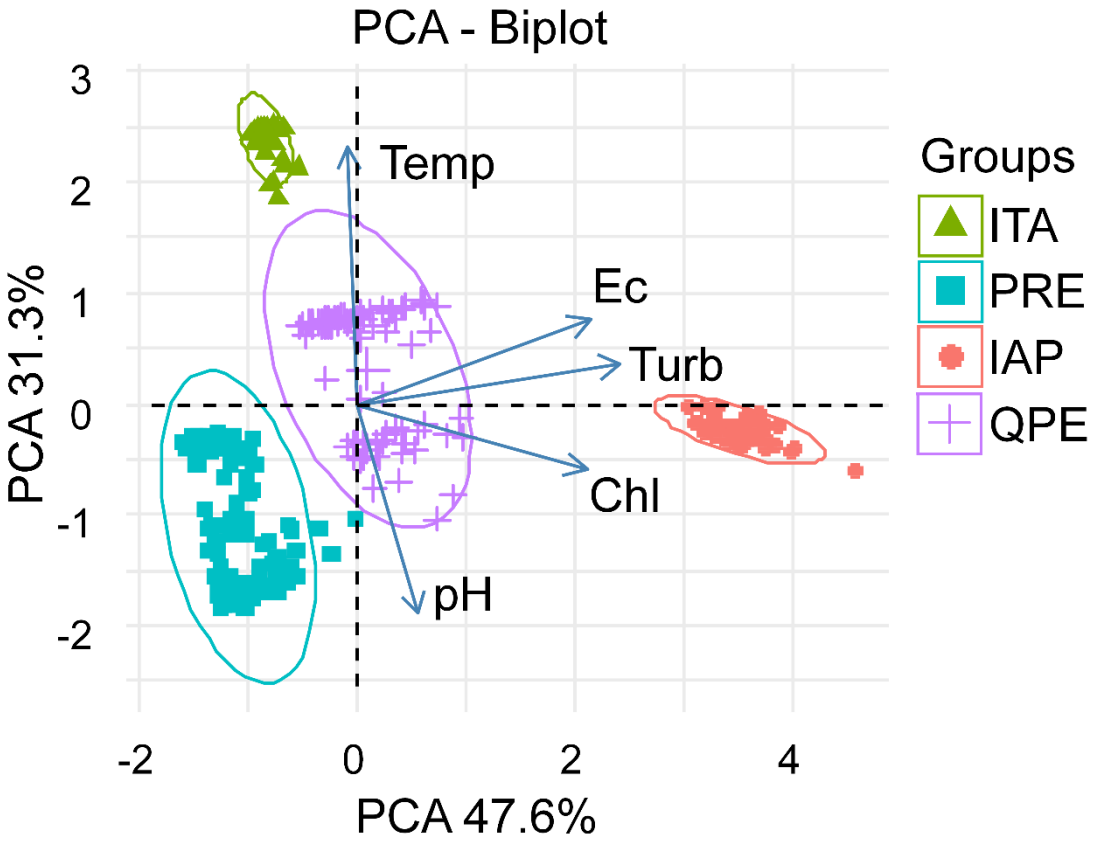

Figure 1. Results from the Principal Components Analysis (PCA) in the differential sampling areas: in Itatinga from São Paulo state (ITA: Itaúna stream, Paranapanema sub-basin) and Mauá da Serra (PRE: Preto stream, Tibagi sub-basin), Castro (IAP: Iapó stream, Tibagi sub-basin) and Ponta Grossa (QPE: Quebra Perna stream, Tibagi sub-basin) in Paraná state. According to the abiotic factors: pH, Ec (Conductivity), Turb (Turbidity), Temp (Temperature), Chl (Chlorophyll). The canonical axes explained78.9% of the variation.

Aegla castro samples

Five aeglids in ITA (13.09 mm ± 10.41 of CC), 10 in PRE (CC 13.92 mm ± 5.47), 8 in IAP (CR 12.51 mm ± 4.71mm) and 9 in QPE (CC 11.63mm ± 3.47) were collected. The lowest value found for *A. castro* for $\delta^{15}N$ was 3.54‰ and for $\delta^{13}C$ -33.04‰, and the highest value for $\delta^{15}N$ was 13.96‰ and for $\delta^{13}C$ -13.53‰. The combination of $\delta^{13}C$ and $\delta^{15}N$ showed differences between area of the sampling (MANOVA, $F_{areaofsampling} = 32.35$, df = 1, $p < 0.05$) for *A. castro*. In addition, separated, $\delta^{13}C$ and $\delta^{15}N$ showed differences

(ANOVA $\delta^{13}C$, $F_{areaofsampling} = 17.52$, df = 3, p value < 0.05; ANOVA $\delta^{15}N$, $F_{areaofsampling} = 92.43$, df = 3, p value < 0.05), respectively, for *A. castro*. The differences and similarities of $\delta^{13}C$ and $\delta^{15}N$ in the area of the sampling according to the Tukey test are shown in Figure 2.

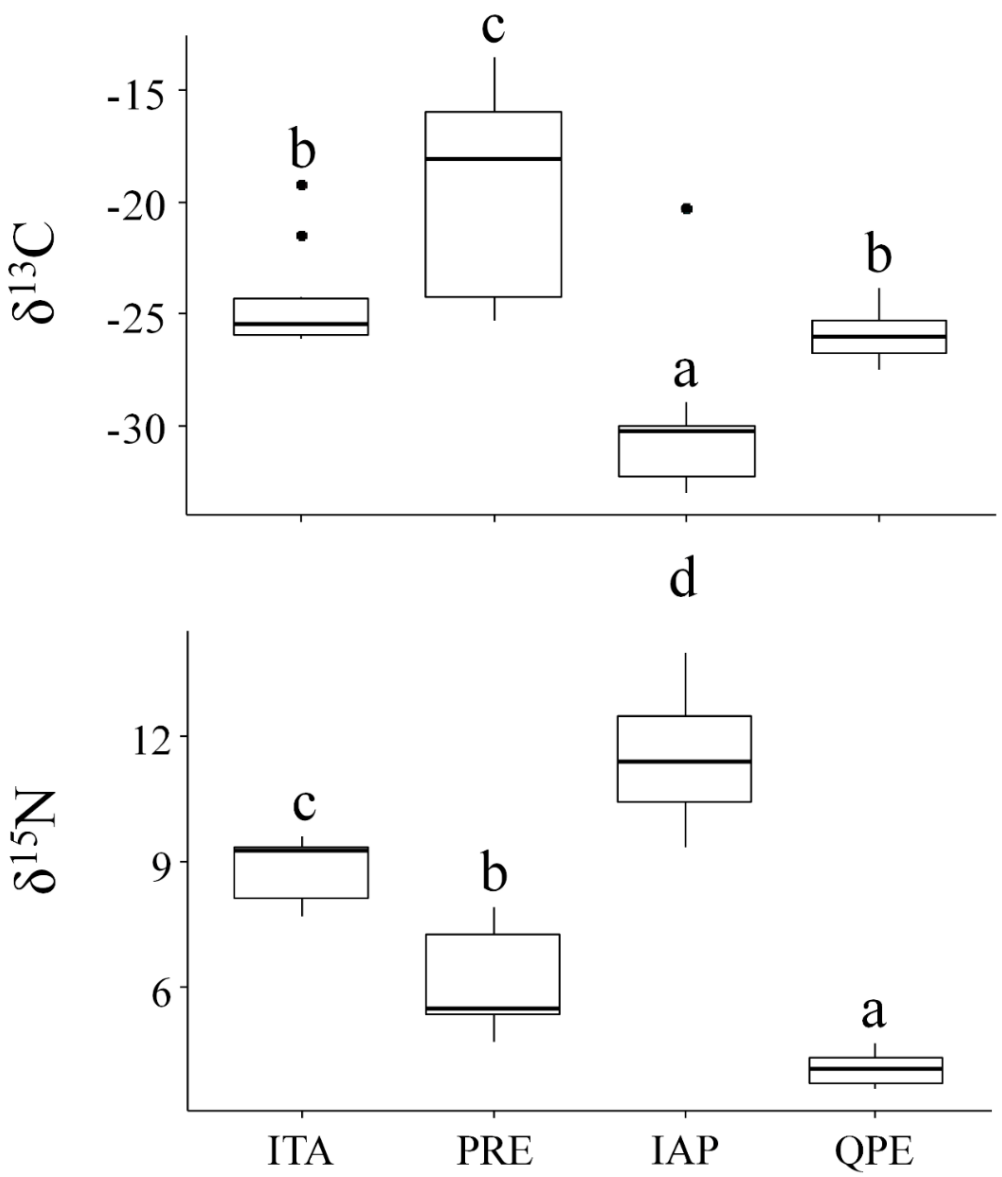

Figure 2. Variation in $\delta^{13}C$ and $\delta^{15}N$ of *Aegla castro* in different area of the sampling: in Itatinga from São Paulo state (ITA: Itaúna stream, Paranapanema sub-basin) and Mauá da Serra (PRE: Preto stream, Tibagi sub-basin), Castro (IAP: Iapó stream, Tibagi sub-basin) and Ponta Grossa (QPE: Quebra Perna stream, Tibagi sub-basin) in Paraná state. Median values represented by central bars between first quartile and third quartile, extremities representing minimum and maximum. Letters denote significant comparisons, by analysis of variance (ANOVA), followed by Tukey's post-hoc tests.

Trophic structure

Nineteen distinct isotopic signatures of the representative organisms of the communities were estimated (*A. castro*, fish, macroinvertebrates, leaves, moss, shrimp, algae and sediment) (Tab. 2). Leaves (ITA), Sediment (PRE and IAP) and algae (QPE) were the first level. *Aegla castro* was the primary consumers, together with others organisms, *e.g.* macroinvertebrates, *Astyanax bockmanni* Vari & Castro, 2007 and *Macrobrachium* sp. The secondary consumers were Characiformes and Siluriformes fishes (PRE), *Phalloceros harpagos* Lucinda, 2008 in IAP and *Phalloceros pellos* Lucinda, 2008 in QPE. We did not find any top predators (TL above 4) in our study (Tab. 2, Fig. 3).

It was possible to see that *A. castro* could feeding on items like: leaves, algae, moss and sediment, that perhaps is the same niche as Megaloptera in ITA and Coleoptera in PRE (Fig. 3). However, the value of the base of the food chain was different between the areas of the sampling. This can be seen when comparing the values of the leaves, for example, of each sampling area, in ITA $\delta^{15}N$ 5.68‰, in PRE $\delta^{15}N$ 3.28‰, in IAP $\delta^{15}N$ 10.03‰ and in QPE $\delta^{15}N$ 0.81‰. We emphasize the base value of IAP that starts with $\delta^{15}N$ 8.35‰, a high value compared to the others area of the sampling, as: leaves $\delta^{15}N$ 5.68‰ in ITA, sediment $\delta^{15}N$ 2.63‰ in PRE and algae $\delta^{15}N$ 0.44‰ in QPE (Table 2, Fig. 3).

Table 2: Stable isotope signatures of carbons and nitrogen (mean ± SD) and the estimated trophic level of the organisms, from freshwater community that compound the trophic web with *Aegla castro* in in 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.

Localities	Sources	$\delta^{13}C‰$	$\delta^{15}N‰$	Estimated Trophic Level
ITA - Itatinga city	Leaves	-33.01 (± 0.23)	5.68 (± 0.13)	1.0
	Algae	-29.96 (± 0.30)	7.67 (± 0.85)	1.58
	Megaloptera	-25.54 (± 0.08)	8.73 (± 0.00)	1.89
	Aegla castro	-24.46 (± 2.33)	8.85 (± 0.77)	1.93
	Sediment	-27.83 (± 0.07)	10.61 (± 0.31)	2.45
	Astyanax bockmanni	-26.75 (± 1.46)	11.27 (± 0.04)	2.64
	Oligochaeta	-25.20 (± 0.04)	11.69 (± 0.01)	2.76
	Macrobrachium sp.	-25.79 (± 0.08)	11.70 (± 0.10)	2.77
PRE - Mauá da Serra	Sediment	-27.97 (± 0.13)	2.63 (± 0.37)	1.0
	Leaves	-31.11 (± 0.26)	3.28 (± 0.01)	1.19
	Moss	-26.53 (± 0.32)	4.08 (± 0.08)	1.43
	Coleoptera	-21.54 (± 0.11)	5.80 (± 0.16)	1.93
	Aegla castro	-19.59 (± 4.65)	6.12 (± 1.17)	2.03
	Trichoptera	-25.46 (± 0.01)	6.13 (± 0.13)	2.03
	Algae	-25.68 (± 0.06)	6.30 (± 0.60)	2.08
	Ephemeroptera	-24.06 (± 0.11)	6.51 (± 0.11)	2.14
	Megaloptera	-24.28 (± 0.04)	6.99 (± 0.39)	2.28
	Plecoptera	-23.41 (± 0.14)	7.93 (± 0.25)	2.56

	Odonata	-22.50(± 0.21)	8.26 (± 0.03)	2.66
	Diptera (Chironomidae)	-24.61 (± 0.24)	8.29 (± 0.22)	2.66
	Peixe Characiformes	-22.93 (± 0.07)	10.20 (± 0.36)	3.23
	Peixe Siluriformes	-24.49 (± 0.06)	11.07 (± 0.09)	3.48
IAP - Castro	Sediment	-26.75 ± 0.14	8.35 ± 0.13	1.00
	Leaves	-29.48 ± 0.54	10.03 ± 0.03	1.49
	Algae	-35.57 ± 3.01	11.29 ± 0.91	1.86
	Aegla castro	-29.99 ± 3.70	11.54 ± 1.54	1.93
	Odonata	-34.81 ± 0.05	13.03 ± 0.01	2.37
	Phalloceros harpagos	-35.04 ± 0.13	15.17 ± 0.01	3.00
QPE - Ponta Grossa	Algae	-32.39 ± 0.54	0.44 ± 0.13	1.00
	Leaves	-30.89 ± 0.46	0.81 ± 0.13	1.11
	Sediment	-26.07 ± 0.66	3.96 ± 0.65	2.04
	Aegla castro	-25.96 ± 1.14	4.03 ± 0.40	2.06
	Phalloceros pellos	-24.00 ± 0.42	8.94 ± 0.25	3.50

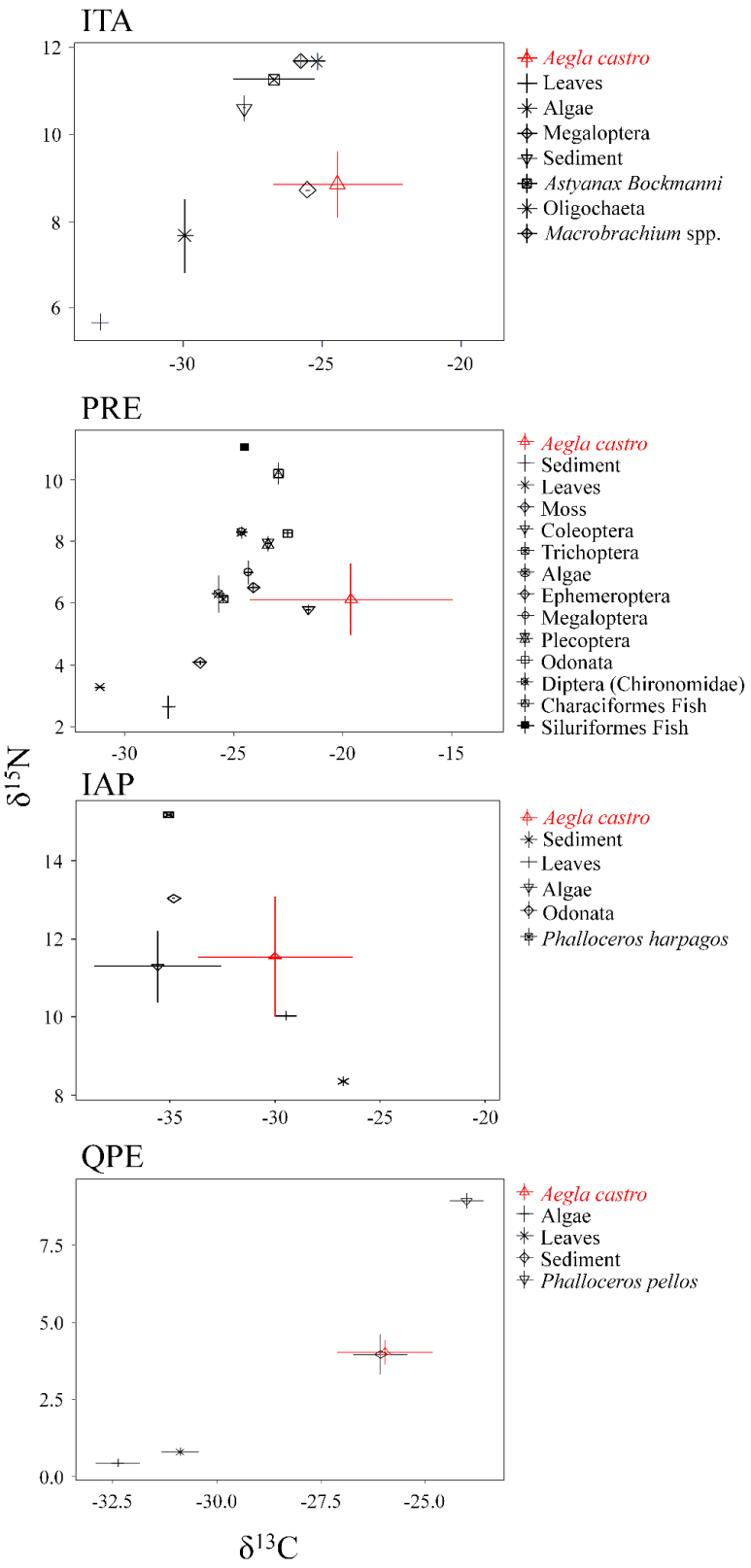

Figure 3. Biplots of stable isotopes using the mean for $\delta^{13}C$ and $\delta^{15}N$ (± SD). Calculated values for *Aegla castro* and potential food sources from in Itatinga from São Paulo state (ITA: Itaúna stream, Paranapanema sub-basin) and Mauá da Serra (PRE: Preto stream, Tibagi sub-basin), Castro (IAP: Iapó stream, Tibagi sub-basin) and Ponta Grossa (QPE: Quebra Perna stream, Tibagi sub-basin) in Paraná state.

DISCUSSION

The lower trophic position of studied aeglids corroborated our hypothesis, as primary consumer, being an important link between the producers and others trophic levels. Furthermore, due to the difference in the sampling areas, *A. castro* and the other items of the trophic web presented different isotopic signatures of N and C, and those places with the greatest anthropic action presented nitrogen.

Water quality and abiotic factors

The presence coliforms, aluminum (in all locations), iron (in IAP) and copper (in QPE) can be explained by natural or anthropic actions. Due to natural actions, it can be the presence of wild animals (Silva *et al*., 2017) or the type of soil found in all regions studied, which is an alic soil type, with aluminum saturation above 50% or of rocks (EMBRAPA, 2006), for example. Anthropogenic actions, on the other hand, can come from waste from the farm (ITA), restaurant (PRE) and from agricultural practices and animal production (IAP). Moreover, waste deposited around streams or that reach it improperly (Nriagu , 1990; Brown and Peake, 2006; Jordão *et al*., 2007; Kinsella and Crowe, 2016), elements in water treatment plants (Gobena *et al*., 2017; Sillanpää *et al*., 2018), creation from animal's farm (Green and Kramer, 1979; Asih, 2020), metallurgical industries (Douay *et al*., 2008), among other sources, could be anthropogenic disturbance actions examples.

In addition, organic matter as well as chemical elements could be carried by rain and by the river, coming from other locations, and be influencing the results at these collection's streams (Pereira *et al*., 2020). Our objective with these analyzes was not to demonstrate exactly the origins of these alterations, but to demonstrate that these alterations are indicative of modifications in the natural environment and, consequently, eglids are also sensitive and vulnerable by anthropogenic alterations.

Areas of study were different according to the environmental factors, except pH that did not show great numerical variation between locations, remaining within the range of protection of aquatic life with values between 6-9, according to CONAMA Resolution 357/05.

The temperature found in all locations were within the range already described for *A. castro*, from 15ºC to 22º, in which the authors did not indicate a relationship with the annual fluctuation of temperature and their abundance variation (Swiech-Ayoub and Masunari, 2001). Thus, these factors would be less influencing the presence of *A. castro* in the localities, and maybe they are within a tolerable range for the species.

Factors such as Ec, Turb and Chl, important parameters in the observation of polluted stream, indicated QPE with better environmental conditions than IAP, . IAP shows signs of being the most polluted region, for example: values greater than 100 $\mu S.cm^{-1}$ are indicative of an impacted environment (CETESB, 2007). Therefore, IAP is the region of greatest impact and the increase in turbidity that was related to the presence of animal and plant organic matter, in addition to sediments from rocks and soil (Perdigão *et al*., 2018). Due to the conditions of the collection area in the IAP, we believe that the highest turbidity value found in the IAP during the collection period was due to organic and inorganic matter, coming from the removal of natural forest for insertion of agriculture or for pasture, in addition to solid matter in suspension, a scenario opposite to that could be observed in the PRE, where we have better rates of Ec, Turb and DO.

Trophic structure

We found differences among organisms in the sampling areas for $\delta^{13}C$ and $\delta^{15}N$, and distinct isotopic signatures characterized their trophic structure. These differences are due to environmental differences, which change the rates of these components between the sampling areas, a factor that generated different trophic interactions, besides

the anthropic action. So, in different streams these animals can behave similarly, in low trophic position, but within different webs trophic molded by human action.

Leaves (ITA), sediment (PRE and IAP) and algae (QPE) were in the first level in the trophic web. Plants as primary producers, as well as algae and mosses, 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 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). Therefore, in our work these items supplying energy to primary consumersand may be contributing as an important item in the diet of *A. castro*.

Our sampled rivers ranged in width and degree of overhanging riparian vegetation. In our results, most the $\delta^{13}C$ values were found within C_3 plants, except in PRE, that Coleoptera (-21.54 ± 0.11 and *A. castro* -19.59 ± 4.65), showed values above the -22‰, maybe because consume some type of item C_4 that produce intermediate values. Consequently, the diversity of carbon inputs into each river probably played a large role influencing the resulting $\delta^{13}C$ signature. Furthermore, the most negative C values may indicate feeding from terrestrial carbon sources (Rounick and Winterbourn, 1986) which is typical of a shredder (Vannote *et al*., 1980). We did not find aeglids out of the water, but it is possible that these animals can feed on items C coming from outside the stream, as they act on leaves of allochthonous origin, brought by the wind or that fall directly from the riparian forest.

In our study, the $\delta^{15}N$ value of the base of the food chain (C sources and sediment) was different among sampling areas, which indicates that there are spatial nitrogen's enrichment difference, providing distinct trophic webs. The $\delta^{15}N$ values of primary producers increase with the anthropic waste discharge (McClelland and Valiela, 1998) 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‰ ± 0.13) and ITA (10.61‰ ± 0.31) compared with PRE (2.63‰ ± 0.37) and QPE (3.96‰ ± 0.65), as well as the highest leaves values (10.03‰ ± 0.03) and algae (11.29‰ ± 0.91) from IAP compared to the other sampling areas, it is evident that the base of the chain in IAP is enriched with N, as well as for ITA that had less enriched values. So, the sediment was enriched around ~7‰. Tanu *et al*. (2020) reported that plants in areas facing human disturbances had $\delta^{15}N$ values of 5–14‰, while unaffected areas had values up to 3‰, suggesting that anthropogenic N may be regulating $\delta^{15}N$.

Nitrate (NO_3) from human and animal waste is enriched in $\delta^{15}N$, 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 could consider that IAP is the location with the greatest anthropogenic interference through the enrichment of N in several items, and ITA with the beginning of an increase in that N, through what we could observed in the sediment.

The factors that could be altered the $\delta^{15}N$ values in this environment were sewage and fertilizer discharge (Tanu *et al*., 2020), of the variation in $\delta^{15}N$ values of primary consumers is due to anthropogenic impacts (Cabana and Rasmussen 1996) state that 68%, that result of isotope fractionation during ammonification (Heaton, 1986). In IAP, where animal farming and agriculture are the main economic activity, the high $\delta^{15}N$ value is clearly influenced by discharge from these activities, and the same starting to occur in ITA.

As primary consumers were found aeglids together with others items (moss, macroinvertebrates, *A. bockmanni* and *Macrobrachium* sp). There are few studies that build the trophic web and certainly indicate the trophic position of *Aegla* spp., but we know that as a primary consumer, they are an important link between producers, and the

others consumers. They act directly in the reduction of organic particles, facilitating their transport to other environments (Castro-Souza and Bond-Buckup, 2004; Santos *et al*., 2008; Cogo e Santos, 2013). Burress *et al*. (2013) showed that *Aegla uruguayana* Schmitt, 1942 occupies a much lower trophic position than is typical for crayfishes, with isotopic signature around 9‰. Biasi *et al*. (2019) demonstrated that the shredders, like *Aegla* spp., might have feeding preferences for grass species that do not contribute significantly to higher trophic levels due to the low enrichment in the tissues. Other studies that did not contain *Aegla* spp. as a focus, but studied them as belonging to the trophic web described their isotopic ratios: 7‰ (Bonato *et al*., 2018) in the Patos Lagoon drainage (Rio Grande do Sul, Brazil), 8.4‰ in Moreno East lake and 5.9‰ in Moreno West lake, at Nahuel Huapi Nacional Park (Northwest Patagonia, Argentina) (Arcagni *et al*., 2013) and 5‰ (Arcagni *et al*., 2015) in Lake Nahuel Huapi at Nahuel Huapi National Park (Northwest Patagonia, Argentina). The values found for *Aegla* spp. were similar to those found for

A. castro, only IAP presented values above those already reported. We point out that *A. castro* may be feeding on items found below in the trophic web, such as leaves, algae, moss and sediment. Ingestion of plant debris as the main fooditem in aeglids has been demonstrated. Plants debris were 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 had also found sediment as part of the aeglids diet (Bueno & Bond-Buckup, 2004; Castro-Souza and Bond-Buckup, 2004; Santos *et al*., 2008; Burress *et al*., 2013). Its ingestion can occur accidentally during feeding (D'Incao *et al*., 1990) or due to the large supply of organic matter (an easy sourceof energy) and by the colonies of microorganisms, algae and bacteria, which can serve asfood for the aeglids (Graça, 2001; Bueno and Bond-Buckup, 2004; Castro-Souza and Bond-Buckup, 2004). According to the high standard deviation for carbon for the aeglids,we indicated wider range of food

sources used for this eglid in our study.

Furthermore, as we assume that these animals are omnivores, *A. castro* probably have an overlap for the food resources with Megaloptera in ITA and Coleoptera in PRE, for being close to each other in the trophic web. Megaloptera is predators, feeding whole animals or part of them (Merritt *et al*., 2017) and Coleoptera is a scraper, feeding periphyton (attached algae and associated material) or herbivores (grazing scrapers of mineral and organic surfaces) (Merritt *et al*., 2017). Thus, the food items may be available in a similar way for these groups, with this overlapping and presenting them close to each other in the trophic web.

In ITA, the *A. bockmanni* and *Macrobrachium* sp. in ITA were primary consumer. Characiformes and Siluriformes fishes (PRE), *P. harpagos* in IAP and *P. pellos* in QPE were secondary consumers. These fishes and shrimp consumers did not present as possible prey for aeglids. However, aeglids can serve as a food source for these animals, especially for fishes as shown in the trophic web, which occupied higher trophic levels. Fish have a plasticity in food items consumed, *e.g.*, *Astyanax* sp. was classified as an omnivore with a tendency to herbivory (Schneider *et al*., 2011). Moreover, *P. harpagos* has several classifications as to its feeding habit, *i.e.*, classified as invertivorous, detritivore, iliophage-algivore and insectivore (Rocha *et al*., 2009; Schneider *et al*., 2011). For the species *P. pellos*, we did not find any studies that report their feeding habits, so we consider the general feeding behavior of Siluriformes, as feeding on macrocrustaceans, *Aegla* sp., macroinvertebrates larvae and plants (Bonato and Fialho, 2014). *Macrobrachium* sp. is considered omnivorous predators, but with an important carnivorous component (Lima *et al*., 2014). In addition, for the *Macrobrachium nipponense* (De Haan, 1849) species, a high value for high $\delta^{15}N$ and its trophic position also high (TL = 3.38) has also been reported, along with fish (Mirzajani *et al.*, 2020). As

demonstrated in our study by *Macrobrachium* sp. and the fish presented a high versatility in their diet, ingesting the items that were found below them in trophic web.

In addition, the high standard deviation found for aeglids in all locations in our study could indicate a range of other food sources that we did not estimate. Thus, the food consumption depends on what was availability in each environment. Omnivorous behavior is considered a key component for regulate energy flow and nutrient recycling (Evans-White *et al*., 2001; Buck *et al*., 2003). Omnivore is favorable within systems, combining different conversion efficiency features (Krivan and Diehl 2005).

Our study demonstrated a difference in the isotopic ratios of carbon and nitrogen by *A. castro* also for others items of the trophic web among sampling areas, showing the higher differences in ^{15}N values at the base of the food web between the sampled areas, indicating anthropic activity that can modify the local enrichment of the streams, and interfere in trophic relationship. So, 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. Just as it becomes important to understand how the anthropic action is interfering in the trophic relationships, and how it can affect the permanence of species like *Aegla*, which are considered water quality bioindicator.

REFERENCES

ANVISA. Agência nacional de vigilância sanitária. Portaria de Consolidação nº5, de 28 de setembro de 2017. Available at www.portalarquivos2.saude.gov.br. Accessed on 15 May 2021.

Arcagni, M.; Campbell, L.M.; Arribére, M.A.; Kyser, K.; Klassen, K.; Casaux, R.; Miserendino, M. L. and Guevara, S.R. 2013. Food web structure in a double-basin ultra-oligotrophic lake in Northwest Patagonia, Argentina, using carbon and nitrogenstable isotopes. *Limnologica-Ecology and Management of Inland Waters*, 43: 131– 142.

Arcagni, M.; Rizzo, A.; Campbell, L.M.; Arribére, M.A.; Juncos, R.; Reissig, M.; Kyser, K.; Barriga, J.P.; Battini, M. and Guevara S.R. 2015. Stable isotope analysis of trophicstructure, energy flow and spatial variability in a large ultraoligotrophic lake in Northwest Patagonia. *Journal of Great Lakes Research*, 41: 916–925.

Asih, A.Y.P. 2020. Levels of Cuprum (Cu) and Plumbum (Pb) in shrimp in the traditional

market and their impacts towards the environmental health. *Journal of Physics: Conference Series*, 1469: 012118.

Bearhop, S.; Waldron, S.; Votier, S.C. and Furness, R.W. 2002. Factors that influence assimilation rates, and fractionation of nitrogen and carbon isotopes in avian blood and feathers. *Physiological and Biochemical Zoology*, 75: 451–458.

Bearhop, S.; Thompson, D.R.; Waldron, S.; Russell, I.C.; Alexander, G. and Furness, R.W. 1999. Stable isotopes indicate the extent of freshwater feeding by cormorants *Phalacrocorax carbo* shot at inland fisheries in England. *Journal of Applied Ecology*,36: 75–84.

Biasi, C.; Cogo, G.B.; Hepp, L.U. and Santos, S. 2019. Grass species as a source of allochthonous energy for shredders and fungal decomposers in a subtropical stream. *Fundamental and Applied Limnology*, 192: 331–341.

Bonato, K.O.; Burress, E.D.; Fialho, C.B. and Armbruster, J.W. 2018. Resource partitioning among syntopic Characidae corroborated by gut content and stable isotope analyses. *Hydrobiologia*, 805: 311–324.

Bonato, K.O. and Fialho, C.B. 2014. Evidence of niche partitioning under ontogenetic influences among three morphologically similar Siluriformes in small subtropical streams. *PloS one*, 9: e110999.

Bond-Buckup, G. and Buckup, L. 1994. A família Aeglidae (Crustacea, Decapoda, Anomura). *Arquivos de Zoologia*, 32: 159–346.

Bond-Buckup, G. and Santos, S. 2007. Crustáceos Anomuros de Águas Continentais: Diversidade e Aspectos Biológicos. *Ciência e Ambiente*, 35: 47–54.

Bosley, K.M.; Copeman, L.A.; Dumbauld, B.R. and Bosley, K.L. 2017. Identification of burrowing shrimp food sources along an estuarine gradient using fatty acid analysis and stable isotope ratios. *Estuaries and Coasts*, 40: 1113–1130.

Boutton, T.W. 1996. Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change. p. 47–82. In: T.W. Boutton and S.I. Yamasaki (eds.), Mass spectrometry of soils. New York: Marcel Dekker.

Brasil, 2005. Ministério do Meio Ambiente. Conselho Nacional do Meio Ambiente - CONAMA. Resolução nº 357 de 17 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. Diário Oficial da União, Brasília, DF.

Britton, A.W.; Murrell, D.J.; McGill, R.A.; Doble, C.J.; Ramage, C.I. and Day, J.J. 2019. Os efeitos da perturbação do uso da terra variam com a posição trófica nas comunidades de peixes ciclídeos do litoral do Lago Tanganica. *Freshwater Biology*, 64: 1114–1130.

Brown, J.N. and Peake, B.M. 2006. Sources of heavy metals and polycyclic aromatic hydrocarbons in urban stormwater runoff. *Science of the total environment*, 359: 145–155.

Buck, T.L.; Breed, G.A.; Pennings, S.C.; Chase, M.E.; Zimmer, M. and Carefoot, T.H. 2003. Diet choice in an omnivorous saltmarsh crab: Different food types, body size, and habitat complexity. *Journal of Experimental Marine Biology and Ecology*, 292: 103–116.

Bueno, A.A.P. and Bond-Buckup, G. 2004. Natural diet of *Aegla platensis* Schmitt and *Aegla ligulata* Bond-Buckup & Buckup (Crustacea, Decapoda, Aeglidae) from Brazil. *Acta Limnologica Brasiliensia*, 16: 115–127.

Bueno, S.L. and Shimizu, R.M. 2008. Reproductive biology and functional maturity in females of *Aegla franca* (Decapoda: Anomura: Aeglidae). *Journal of Crustacean Biology*, 28: 652–662.

Bueno, S.L.S.; Shimizu, R.M. and Rocha, S.S. 2007. Estimating the population size of *Aegla franca* (Decapoda: Anomura: Aeglidae) by mark-recapture technique from an isolated section of Barro Preto stream, county of Claraval, state of Minas Gerais, southeastern Brazil. *Journal of Crustacean Biology*, 27: 553–559.

Bueno, S.L.S.; Camargo, A.L. and Moraes, J.C.B. 2017. A new species of stygobitic aeglid from lentic subterranean waters in southeastern Brazil, with an unusual morphological trait: short pleopods in adult males. *Nauplius*, 25: 1–18.

Burress, E.D.; Gangloff, M.M. and Siefferman. 2013. Trophic analysis of two subtropical South American freshwater crabs using stable isotope ratios. *Hydrobiologia*, 702: 5–13.

Cabana, G. and Rasmussen, J.B. 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences*, 93: 10844–10847.

Caldecott, J.O.; Jenkins, M.D.; Johnson, T.H. and Groombridge, B. 1996. Priorities for conserving global species richness and endemism. *Biodiversity and Conservation*, 5:699–727.

Carabel, S.; Godínez-Domínguez, E.; Verísimo, P.; Fernández, L. and Freire, J. 2006. An assessment of sample processing methods for stable isotope analyses of marine foodwebs. *Journal of Experimental Marine Biology and Ecology*, 336: 254–261.

Castro-Souza, T. and Bond-Buckup, G. 2004. O nicho trófico de duas espécies simpátricas de *Aegla Leach* (Crustacea, Aeglidae) no tributário da bacia hidrográficado Rio Pelotas, Rio Grande do Sul, Brasil. *Revista Brasileira de Zoologia*, 21: 805– 813.

Caxito, F.A. and Silva A.V. 2015. Isótopos estáveis: fundamentos e técnicas aplicadas à Caracterização proveniência geográfica de Produtos alimentícios. *Geonomos*, 23: 10–17.

CETESB: Significado sanitário: parâmetros de qualidade. São Paulo, 2007. Accessed on 25 May 2021.

Clapcott, J.E. and Bunn, S.E. 2003. Can C_4 plants contribute to aquatic food webs of subtropical streams?. *Freshwater Biology*, 48: 1105–1116.

Clarke, K.R. and Ainsworth, M. 1993. A method of linking multivariate community structure to environmental variables. *Marine Ecology-Progress Series*, 92: 205–205.

Cogo, G.B. and Santos, S. 2013. The role of aeglids in shredding organic matter in Neotropical streams. *Journal of Crustacean Biology*, 33: 519–526.

Collins, P. 2019. Trophic ecology. p. 97–132. In: S. Santos and S.L.S. Bueno (eds), Aeglidae: Life History and Conservation Status of Unique Freshwater Anomuran Decapods, CRC Press, Taylor and Francis Group, New York.

Daufresne, T. and Loreau, M. 2001. Ecological stoichiometry, primary producer–decomposer interactions, and ecosystem persistence. *Ecology*, 82: 3069–3082.

D'Incao, F.; Silva, K.G.; Ruffino, M.L. and Braga, A.C. 1990. Hábito alimentar do caranguejo *Chasmagnathus granulata* Dana, 1851 na barra do Rio Grande, RS(Decapoda, Grapsidae). *Atlântica*, 12: 85–93.

Dolenec, T.; Lojen, S.; Lamba Z. and Dolenec, M. 2006. Effects of fish farm loading on sea grass *Posidonia oceanica* at Vrgada Island (Central Adriatic): a nitrogen stable isotope study. *Isotopes in Environmental and Health Studies*, 42: 77–85.

Douay, F.; Pruvot, C.; Roussel, H.; Ciesielski, H.; Fourrier, H.;Proix, N.; Waterlot, C. 2008. Contamination of urban soils in an area of northern France polluted by dust emissions of two smelters. *Water Air Soil Pollution*, 188: 247–260.

Ducatti, C.; Martins, C.L.; Arrigoni, M.B.; Martins, M.B.; Vieira Jr., L.C. and Denadai, J.C. 2011. Utilização de isótopos estáveis em ruminantes. *Revista Brasileira de*

Zootecnia, 40: 68–75.
EMBRAPA Solos. 2006. Sistema Brasileiro de Classificação de Solos. Rio de Janeiro: EMBRAPA Produção de informação, 306p.
Evans-White, M.; Dodds, W.K.; Gray, L.J. and Fritz, K.M. 2001. A comparison of the trophic ecology ofthe crayfishes *Orconectes nais* (Faxon) and *Orconectes neglectus* (Faxon) and the central stoneroller minnow *Campostoma anomalum* (Rafinesque): omnivory in a tall- grass prairie stream. *Hydrobiologia*, 462: 131–144.
Fasolo, P.J.; Carvalho, A.P.; Potter, R.O.; Bognola, I.A.; Bhering, S.B. and Martorano, L.G. 2002. Caracterização dos solos do Município de Castro, PR. Embrapa Solos-Boletim de Pesquisa e Desenvolvimento 09 (INFOTECA-E).
Feldmann, R.M.; Veja, F.J.; Applegate, S.P. and Bishop, G.A. 1998. Early Cretaceous arthropods from the Tlayúa formation at Tepexi de Rodríguez, Puebla, México. *Journal of Paleontology*, 72: 79–90.
Francisco, D.A.; Bueno, S.L.S. and Kihara, T.C. 2007. Description of the first juvenile of *Aegla franca* Schmitt, 1942 (Crustacea, Decapoda, Aeglidae). *Zootaxa*, 1509:17–30.
Gannes, L.Z.; Del-Rio, C.M. and Koch, P. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comparative Biochemistry Physiology*, 119: 725–737.
Gobena, B.; Adela, Y. and Alemayehu, E. 2017. Evaluation of mix-chemical coagulants in water purification process. *International Journal of Engineering Research & Technology*, 7: 431–435.
Graça, M.A.S. 2001. The role of invertebrates on leaf litter decomposition in streams - a review. *International Review of Hydrobiology*, 86: 383–393.
Green, J.G. and Kramer, A. 1979. Food processing wast management. Westport, AVI Publising Company, 629p.
Hansson, S.; Hobbie, J.E.; Elmgren, R.; Larsson, U.; Fry, B. and Johansson, S. 1997. The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration.*Ecology*, 78: 2249–2257.
Heaton, T.H. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. *Chemical Geology*, 59: 87–102.
Hobson, K.A.; Piatt, J.F. and Pitocchelli, J. 1994. Using stable isotopes to determine seabird trophic relationships. *Journal of animal ecology*, 63: 786–798.
Hoffman, J.C.; Kelly, J.R.; Peterson, G.S.; Cotter, A.M.; Starry, M.A. and Sierszen, M.E. 2012. Using $\delta^{15}N$ in fish larvae as an indicator of watershed sources of anthropogenicnitrogen: Response at multiple spatial scales. *Estuaries and Coasts*, 35: 1453–1467.
Hyslop, E.J. 1980. Stomach contents analysis - a review of methods and their application. *Journal of Fish Biology*, 17: 411–429
Jordão, C.P.; Ribeiro, P.R.S.; Matos, A.T. and Fernandes, R.B.A. 2007. Aquatic contamination of the Turvo Limpo river basin at the Minas Gerais State, Brazil. *Journal of Brazilian Chemical Society*, 18: 116–125.
Karasov, W. and Martínez Del Río, C.M. 2007. Physiological ecology: How animals process energy, nutrients, and toxins. Princeton, Princeton University Press, 744p.
Kinsella, C.M. and Crowe, T.P. 2016. Separate and combined effects of copper and freshwater on the biodiversity and functioning of fouling assemblages. *Marine pollution bulletin*, 107: 136–143.
Krivan, V. and Diehl, S. 2005.Adaptive omnivory and species coexistence in tri-trophic food webs. *Theoretical Populatoon Biology*, 67: 85–99.

Lajtha, K. and Michener, R.H. 1994. Stable isotopes in ecology and environmental science. Oxford: Blackwell Scientific Publications, 316p.

Lake, J.L.; McKinney, R.A.; Osterman, F.A.; Pruell, R.J.; Kiddon, J.; Ryba, S.A. and Libby, A.D. 2001. Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. *Canadian Journal of Fisheries and Aquatic Sciences*, 58:870–878.

Lassauque, J.; Lepoint, G.; Thibaut, T.; Francour, P. and Meinesz, A. 2010. Tracing sewage and natural freshwater input in a Northwest Mediterranean bay: evidence obtained from isotopic ratios in marine organisms. *Marine Pollution Bulletin*, 60: 843–851.

Lima, J.D.F.; Garcia, J.D.S. and Silva, T.C.D. 2014. Natural diet and feeding habits of a freshwater prawn (*Macrobrachium carcinus*: Crustacea, Decapoda) in the estuary ofthe Amazon River. *Acta Amazonica*, 44: 235–244.

Magni, S.T. and Py-Daniel, V. 1989. *Aegla platensis* Schmitt, 1942 (Decapoda: Anomura) a predator of Simuliidae immature stages (Diptera: Culicomorpha). *Revistade Saúde Pública*, 23: 258–259

Mao, Z.; Gu, X. and Zeng, Q. 2016. Food sources and trophic relationships of three decapod crustaceans: insights from gut contents and stable isotope analyses. *Aquaculture Research*, 47: 288–2898.

Marçal, I.C.; Páez, F.P.; Souza-Shibatta, L.; Sofia, S.H. and Teixeira, G.M. 2020. Taxonomic revision of *Aegla lata* Bond-Buckup & Buckup, 1994 (Decapoda: Anomura: Aeglidae), with the description of a new species of *Aegla Leach*, 1820 fromthe Upper Paraná Ecoregion, Brazil. *Journal of Crustacean Biology*, 40: 425–434.

McClelland, J.W. and Valiela, I. 1998. Linking nitrogen in estuarine producers to land-derived sources. *Limnology and Oceanography*, 43: 577–585.

Merritt, R.W.; Cummins, K.W. and Berg, M.B. 2017. Trophic relationships of macroinvertebrates. p. 413–433. In: F.R. Hauer and G.A. Lamberti (eds), Methods inStream Ecology. Academic Press, Vol. 1.

Minagawa, M. and Wada, E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. *Geochimica et CosmochimicaActa*, 48: 1135–1140.

Mirzajani, A.; Ghane, A.; Bagheri, S.; Abbasi, K.; Sayadrahim, M.; Salahi, M. and Lavajoo, F. 2020. Diet survey and trophic position of *Macrobrachium nipponense* inthe food web of Anzali Wetland. *Wetlands*, 40: 1229–1239.

Mugnai, R.; Nessimian, J.L. and Baptista, D.F. 2010. Manual de Identificação de Macroinvertebrados Aquáticos do Estado do Rio de Janeiro. Rio de Janeiro, TechnicalBooks Editora, 174p.

Musin, G.E.; Rossi, A.; Diawol, V.P.; Collins, P.A. and Williner, V. 2017. Dynamic metabolic pattern of *Aegla uruguayana* (Schmitt, 1942) (Decapoda: Anomura: Aeglidae): responses to seasonality and ontogeny in a temperate freshwater environment. *The Journal of Crustacean Biology*, 37: 436–444.

Nagata, R.M.; Moreira, M.Z.; Pimentel, C.R. and Morandini, A.C. 2015. Food web characterization based on $\delta^{15}N$ and $\delta^{13}C$ reveals isotopic niche partitioning between fish and jellyfish in a relatively pristine ecosystem. *Marine Ecology Progress Series*,519: 13–27.

Nriagu, J.O. 1990. Rise and fall of leaded gasoline. *Science of the total environment*, 92: 13–28.

Oliveira, G.T.; Fernandes, F.A.; Bueno, A.A. and Bond-Buckup, G. 2007. Seasonal variations in the intermediate metabolism of *Aegla platensis* (Crustacea,

Aeglidae). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 147: 600–606.

Páez, F.P.; Marçal, I.C.; Souza-Shibatta, L.E.N.I.C.E.; Gregati, R.A.; Sofia, S.H. and Teixeira, G.M. 2018. A new species of *Aegla* Leach, 1820 (Crustacea, Anomura) fromthe Iguaçu River basin, Brazil. *Zootaxa*, 4527: 335–346.

Parnell, A. and Inger, R. 2016. Stable isotope mixing models in R with simmr. Available at https://cran. r-project.org/web/packages/simmr/vignettes/simmr. Accessed on 20 de October de 2020.

Perdigão, G.S.; Cordeiro, J.; Calazans, G.M.; Ferreira, D.L.G.; Nascimento, F.D.; Brandão, J.M.S. and Guimarães, J.C.S. 2018. Análise da qualidade da água de atrativos turísticos naturais localizados na comunidade de Cabeça de Boi, Itambé do Mato Dentro (MG). *Research, Society and Development*, 7: 1–25.

Pereira, M.A.B.; dos Santos Alves, W.; de Oliveira, L.D.; Morais, W.A.; Lima, L.O. and da Costa Nunes, N. 2020. Qualidade Hídrica da Cachoeira do Rio São Tomás, no Município de Rio Verde, Goiás, Brasil. *Revista Brasileira de Geografia Física*, 13: 377–390.

Peterson, B.J. and Fry, B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, 18: 293–320.

Pimm, S.L.; Russel, G.J.; Gittleman, J.L. and Brooks, T.M. 1995. The future of biodiversity. *Science*, 269: 347–350.

Post, D.M. 2003. Individual variation in the timing of ontogenetic niche shifts in largemouth bass. *Ecology*, 84: 1298–1310.

Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83: 703–718.

Rice, E.W.; Baird, R.B. and Eaton, A.D. 2017. Standard Methods for the Examination of Water and Wastewater; American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF).

Rocha, F.C.D.; Casatti, L.; Carvalho, F.R. and Silva, A.M.D. 2009. Fish assemblages in stream stretches occupied by cattail (Typhaceae, Angiospermae) stands in SoutheastBrazil. *Neotropical Ichthyology*, 7: 241–250.

Rounick, J.S. and Winterbourn, M.J. 1986. Stable carbon isotopes and carbon flow in ecosystems. *Bioscience*, 36: 171–177.

Saborowski, R. 2015. Nutrition and digestion. p. 285–319. In: E.S. Chang and M. Thiel (eds), The natural history of the Crustacea. Oxford University Press, Vol. 4. UK, Oxford.

Santos, S.; Ayres-Peres, L.; Cardoso, R.C. and Sokolowicz, C.C. 2008. Natural diet of the freshwater anomuran *Aegla longirostri* (Crustacea, Anomura, Aeglidae). *Journalof Natural History*, 42: 1027–1037.

Santos, S.; Bond-Buckup, G.; Gonçalves, A.S.; Bartholomei-Santos, M.L.; Buckup, L. and Jara, C.G. 2017. Diversity and conservation status of *Aegla* spp. (Anomura, Aeglidae): an update. *Nauplius*, 25: 1–14.

Schneider, M.; Aquino, P.D.P.U.D.; Silva, M.J.M. and Fonseca, C.P. 2011. Trophic structure of a fish community in Bananal stream subbasin in Brasília National Park, Cerrado biome (Brazilian Savanna), DF. *Neotropical Ichthyology*, 9: 579–592.

Sillanpää, M.; Ncibi, M.C.; Matilainen, A. and Vepsäläinen, M. 2018. Removal of natural organic matter in drinking water treatment by coagulation: A comprehensive review.*Chemosphere*, l: 54–71.

Silva, J.M.; Santos, G.O.; de Almeida Júnior, M.C.D.; Guimarães, A.G. and Domingos, G.S. 2017. Qualidade da água de captação para abastecimento público no município de Maurilândia, Goiás. *Caminhos de Geografia*, 18: 199–213.

Swiech-Ayoub, P.B. and Masunari, S. 2001. Flutuações temporal e espacial de abundância e composição de tamanho de *Aegla castro* Schmitt (Crustacea, Anomura,Aeglidae) no Buraco do Padre, Ponta Grossa, Paraná, Brasil. *Revista Brasileira de Zoologia*, 18: 1003–1017.

Tanu, F.Z.; Asakura, Y.; Takahashi, S.; Hinokidani, K. and Nakanishi, Y. 2020. Variation in foliar $\delta^{15}N$ reflects anthropogenic nitrogen absorption potential of mangrove forests. *Forests*, 11: 133.

Trevisan, A.; Hepp, L.U. and Santos, S. 2009. Abundância e distribuição de Aeglidae (Crustacea: Anomura) em função do uso da terra na bacia hidrográfica do Rio Jacutinga, Rio Grande do Sul, Brasil. *Zoologia (Curitiba)*, 26: 419–426.

Trombetta, A.S.; Páez, F.P.; Santos, S. and Teixeira, G.M. 2019. *Aegla nebeccana* n. sp. (Crustacea, Aeglidae) from Ivaí Basin, Paraná, Brazil. *Zootaxa*, 4712: zootaxa–4712.

Vannote, R.L.; Minshall, G.W.; Cummins, K.W.; Sedell, J.R. and Cushing, C.E 1980. River continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, 37:130–137.

Vogel, J.C. 1993. Variability of carbon isotope fractionation during photosynthesis. p. 29–46. In: J.R. Ehleringer; A.E. Hall and G.D. Farquhar (eds), Stable isotopes and plant carbon: water relations. Academic Press, San Diego.

Votier, S.C.; Bearhop, S.; MacCormick, A.; Ratcliffe, N. and Furness, R.W. 2003. Assessing the diet of great skuas, Catharacta skua, using five different techniques. *Polar Biology*, 26: 20–26.

Zuur, A.F.; Ieno, E.N. and Elphick, C.S. 2010. A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, 1: 3–14.

CONSIDERAÇÕES FINAIS

As perguntas propostas na presente dissertação foram respondidas nos três capítulos, futuros artigos a serem publicados. Destaca-se abaixo, resumidamente, nossas principais conclusões:

(1) Nosso estudo não mostrou diferença nas dietas durante as fases ontogenéticas para *A. castro* e para *A. parana*, podendo ambas as espécies se alimentarem dos mesmos itens alimentares e forragearem nos mesmos locais, indicando baixa competição ou alta disponibilidade de recursos para ambas as fases.

(2) Demonstramos a importância dos aeglídeos nas teias tróficas dos cinco riachos, pois sua dieta onívora-generalista e sua baixa posição trófica, são indicativos do seu elo entre produtores primários e outros níveis tróficos.

(3) Através das análises de nitrogênio, pudemos observar a diferença existente nas bases de cadeia de cada localidade, indicando que os riachos estão sob diferentes pressões antrópicas, que elevam o nível de nitrogênio dos animais que os consomem, agindo assim sobre as populações, o que pode gerar instabilidade dentro da teia trófica.

(4) Visto que *Aegla* spp. ocorrem em habitats com boas condições ambientais e, portanto, são susceptíveis a mudanças ou perturbações ambientais, é de suma importância estudos que visem a conservação e o monitorando, principalmente deespécies endêmicas, visto que os riachos Neotropicais estão passando por rápida degradação nos últimos anos.