PANAMJAS
Pan-American Journal of Aquatic Sciences

Evaluation of density of freshwater bivalves from the Environmental Protection Area (APA) of Verde River, Campo Magro, Paraná, Brazil

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Abstract. The study of malacological fauna in hydric bodies is crucial to maintain the biodiversity in an environmental conservation area, since these organisms can interfere with water quality being potentially used as a bioindicator of environmental quality. This study aimed at identifying the diversity and population density of both native and invasive freshwater bivalves occurring in the APA of Verde River. Two sample collections were undertaken between July 2012 and March 2013. Three sampling sites (1 m^2 each) were delimited by using a PVC square in the riverbed, with a 50 m interval among them; after this procedure, the sediment contained in the sampling area was manually removed from a 10 cm depth. Then, the screening of live organisms and articulated valves was performed. The occurrence of native bivalves species was recorded as *Anodontites tenebricosus*, *Diplodon parodizi*, and the invasive mussel *Corbicula fluminea*. The higher population density was registered in March 2013 with 915 individuals (*A. tenebricosus* 4.66 ind/m^2, *D. granosus* 1.0 ind/m^2, *C. fluminea* 265.6 ind/m^2). The record of invasive species and the distribution of frequency analysis by length class frequency require continuous studies about the invasive species occurrence in the hydrographic basin of Verde River, because its spreading is closely related to the decline of the native bivalves population.

Key Words: Corbiculidae, exotic species, Hyriidae, macroinvertebrate, Mycetopodidae

Resumo. Avaliação da densidade de bivalves límnicos da Área de Proteção Ambiental (APA) do rio Verde, Campo magro, Paraná, Brasil. O estudo da fauna malacológica de corpos hídricos é fundamental para o conhecimento da diversidade de uma Unidade de Conservação. O objetivo do presente estudo foi identificar a diversidade de bivalves e a variação da densidade populacional de bivalves límnicos nativos e invasores com ocorrência na APA do Rio Verde. Em duas campanhas amostrais realizadas entre julho de 2012 e março de 2013, foram demarcados três pontos amostrais de 1m^2 utilizando-se quadrado de PVC disposto no leito do rio, em intervalos de 50 m. O sedimento contido na área demarcada foi removido, até uma profundidade de 10 cm e realizada triagem de organismos vivos e valvas articuladas. Foi registrada a ocorrência dos bivalves nativos *Anodontites tenebricosus* e *Diplodon parodizi* e do bivalve invasor *Corbicula fluminea*. Foram coletados 915 indivíduos vivos com a maior densidade registrada para março de 2013 (*A. tenebricosus* 4.66 ind./m^2, *D. parodizi* 1.0 ind./m^2 e *C. fluminea* 265.6 ind./m^2). Os resultados obtidos demonstram que a espécie invasora é predominante na área de estudo e sua densidade populacional crescente pode determinar o declínio das populações de bivalves nativos.

Palavras -chave: Corbiculidae, espécies exóticas, Hyriidae, macroinvertebrados, Mycetopodidae

Introduction

Freshwater bivalves have an important role in modifying the environment through direct or indirect alterations in both biotic and abiotic conditions. For this reason, freshwater bivalves are considered “ecosystem engineers” (Crooks 2002). Furthermore, due to their capacity of filtering large amounts of water, freshwater bivalves interfere directly with the nutriment dynamics through the filtration of organic elements that are in suspension on the water surface, in addition to both feces and pseudofeces excretion and biodeposition (Vaughn & Hakenkamp 2001). Moreover, species of freshwater bivalves are sensitive to environmental alterations, such as chemical and organic pollution. Consequently, these organisms are effective as indicators of environmental quality (Vidigal *et al*. 2005, Prather *et al.* 2012, Rodrigues *et al*. 2012).

In appropriate environmental conditions, bivalves can be found in high population densities; therefore, they have a central role in determining water quality, once they are able to filter large amounts of it (Vaughn & Hakenkamp 2001). On the other hand, the existence of invasive species in high densities can negatively affect the local biota (Werner & Rothhaupt 2007), for example, by inducing alterations in both the abundance and diversity of freshwater ecosystems because of the competition for food resources (Sousa *et al*. 2008). Also, invasive species induce modifications in the substrate composition because of the hollow valves deposition in these environments (Jones *et al*. 1994, Vaughn & Hakenkamp 2001).

Considering the mentioned characteristics that show the ecological impact of freshwater bivalves, it is important to register their distribution, mainly in protected areas that have a priority for the conservation of continental water systems, such as the environmental protection area of Verde River. This area was established by aiming at the protection and conservation of the environmental quality and the natural systems from the Verde River watershed, which is an affluent place along the Iguaçu River that is located in the metropolitan area of Curitiba. However, to reach this goal, it is important to develop management strategies and multidisciplinary studies on the biodiversity, enabling the sustainable utilization of natural resources.

There are a few studies reporting the freshwater bivalves occurrence in the Iguaçu watershed as described by Zanardini (1965) in the rivers located in the Curitiba area: *Diplodon expansus* (Küster, 1856), *Diplodon multistriatus* (Lea, 1831), *Diplodon charruanus* (d’Orbigny, 1835), and *Diplodon suppositus* (Simpson, 1914); also, Curial & Lange (1974a, 1974b) described the occurrence of *Diplodon delodontus expansus* (Küster 1856) in the Cerne River, and Meyer *et al.* (2010) described the occurrence of *Diplodon expansus* in the Piraquara River.

Considering species that occur in the state of Paraná, no studies on the occurrence of native species were published in more than two decades. In addition, there is a need for inventories and systematic studies on local malacological fauna because of the significant environmental alterations during this timeframe, such as urbanization, pollution, implementation, and expansion of industrial areas, as well the occurrence of the invasive species *Corbicula fluminea* (Müller, 1774) (Oliveira *et al*. 2014). In order to help finding the most appropriate management and conservation strategies for this particular group of organisms, studies on these populations in areas with a low environmental impact are essential, since an expressive population decline should be seen in all continents (Vaughn & Taylor 1999; Downing *et al*. 2010).

To fill this gap, this study aimed at investigating, using shell length as a criterion, both the occurrence and distribution frequency of both native and invasive species in the environmental protection area of Verde River. Therefore, this study may contribute toward a better understanding of the malacological fauna biodiversity in a specific area in the state of Paraná.

Materials and Methods

Study area: The environmental protection area of Verde River, established by the state government (decree number 2.375/2000), is located on the west side of the metropolitan area of Curitiba, state of Paraná, Brazil. It includes four municipalities: Araucária, Campo Largo, Campo Magro, and Balsa Nova (Caneparo *et al*. 2012) (Figure 1). The Verde River sub-basin is a part of the Iguaçu River basin. Along its course, the hydric potential of the Verde River is explored, providing water resources to rural properties and to generate energy (Medeiros *et al.* 2011).

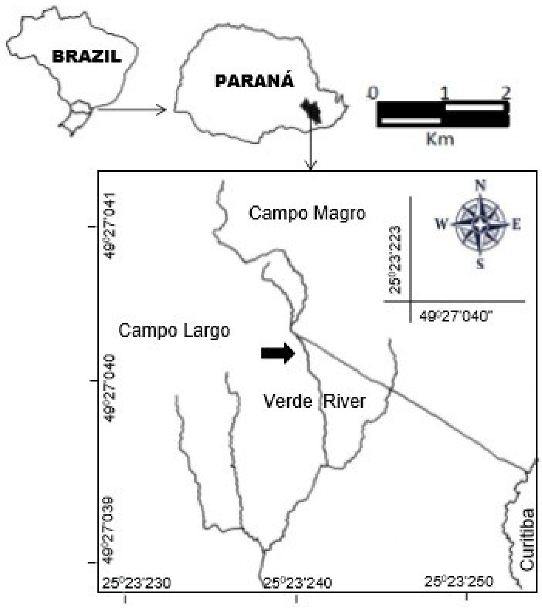

Figure 1. Map of the study area showing sampling site location. Basin of the Verde River, Campo Magro, State of Paraná, Brazil.

Two sample collections were undertaken in a portion of the Verde River, inside the environmental protection area of the Verde River (Figure 1). The first was in October/2012 (Spring), and the last was in March/2013 (Summer). Specimens were collected under the scientific collecting license (SISBIO 37400).

For the comparative analyses related to the population density of native and invasive bivalves, a methodology was adapted from Vianna and Avelar (2010), with a 1 m x 1 m PVC square (1 m^2) delimitating an area in the riverbed. The two sample collections were undertaken in the same portion of the river, which was delimited by using a global positioning system device (GPS). In the collection area, there were three delimited sampling sites, with a distance of 50 m from each other and randomized between the bank and the central river portion. The sediment contained in the sampling area, in 10 cm of depth, was manually removed and transferred to a collection box. In addition, all the sediment was sieved by using a strainer with a 1 mm mesh opening size. Subsequently, living organisms and articulated valves were stored in plastic bags, transported to the laboratory, and kept refrigerated.

For the subsequent analyses, samples were defrosted, quantified, and the height, width, and length of the valves were measured for each specimen, by using a digital caliper with a 0.01 mm precision, according to Mansur *et al.* (1987). Both visceral mass and valves were weighted (wet weight) by using a laboratory scale with a 0.001g precision.

Population density was estimated as the number of individuals living per m^2, and the wet weight was estimated as g/m^2. Biometric data were used to obtain arithmetic mean, standard deviation, coefficient of variation, and the correlation among pairs of the following variables: valve height, width, and length as well as the visceral mass wet weight. All the statistical analyses were performed by using the Microsoft Office Excel.

In order to investigate the anthropic impact in the sampling area, the Protocol for Rapid Evaluation of Habitat Diversity (Callisto *et al.* 2002) was utilized, which allows us to visually evaluate several ecological parameters, including the environmental impact originated from the anthropic activities. According to this protocol, the total score obtained in each area represents the ecological condition, where scores from 0 to 40 indicate impacted areas, from 41 to 60 indicate altered areas, and above 61 indicate natural areas. In addition, the following environmental parameters were used: width and depth, superficial flowing speed by way of the fluctuation in the distance method, described by Bicudo and Bicudo (2004), water and ambient temperature using a mercury thermometer, and pH measurements using pieces of litmus paper.

Voucher specimens were identified by Luis R. Simone (MZUSP) and were deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP 106190; MZUSP 106198; MZUSP 106187). The *Diplodon* species had their identification confirmed by Maria C. D. Mansur and Igor C. Miyahira.

Results

In the studied area, the Verde River has in its central portion gravel rocks with different granulometry. On the other hand, its bank shows a sludgy clay soil due a mixture of silt and organic matter from the riparian forest. In the collection area, the river width ranges from 3.5 to 5 m and its depth ranges from 0.15 to 1.10 m. Abiotic data and stream flow speed measurements from both sample collections are summarized in Table I. Data obtained from the Protocol for Rapid Evaluation of Habitat Diversity showed a score of 56 points, which categorize the evaluated portions as altered.

Table I. Abiotic parameters of the Verde River, State of Paraná, Brazil, obtained during sample collection.

	Oct/12	**Mar/13**
Atmospheric temperature	24°C	26°C
Water temperature	17°C	18°C
pH	7	7
Current speed	3.9 m/s	3.6 m/s

Two native mussels were found: *Anodontites tenebricosus* (Lea, 1834) (Mycetopodidae) (Figures 2A and 2B) and *Diplodon parodizi* (Bonetto, 1962) (Hyriidae) (Figures 2C and 2D), and the invasive species *Corbicula fluminea* (Müller, 1774) (Corbiculidae) (Fig. 2E). All the three species were found to be living simultaneously at the same central portion of the riverbed and surrounding areas. Also, adjacent to the river bank, in random clusters inside the vegetation, a large amount of hollow articulated valves from native species, during both sample collections, were found. These valves showed no visceral mass on decomposition.

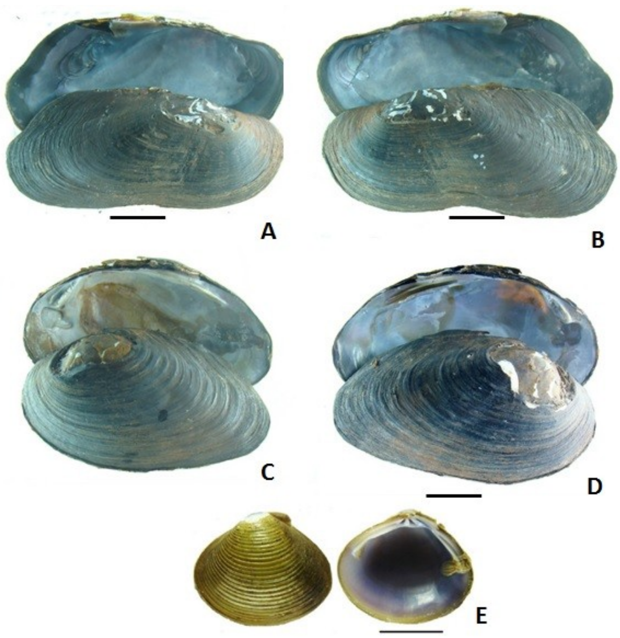

Figure 2. Shells of freshwater bivalves collected in the Verde River location, Campo Magro, Paraná, Brazil. *Anodontites tenebricosus* (Lea, 1834) (A, B); *Diplodon parodizi* (Bonetto, 1962) (C, D); *Corbicula fluminea* (Müller, 1774) (E). Scale bar = 1 cm.

During the two sample collections, 973 individuals were collected, while considering living organisms and articulated valves (*C. fluminea* n = 927; *A. tenebricosus*, n = 31 and *D. parodizi*, n= 15). The proportion of empty articulated valves was 1.3% for *C. fluminea*, 34.7% for *A. tenebricosus*, and 80.0% for *D. parodizi*. The total number of living organisms and articulated valves is shown in Table II.

The correlation observed among the valves' morphometric measures was strongly positive (r^2 > 0.7): length and height (r^2 = 0.9784), height and width (r^2 = 0.9772), as well as length and wet weight visceral mass (r^2 = 0.9624) (Table III).

Corbicula fluminea showed a population density of 39.6 ind/m^2 in October 2012 and 265.6 ind/m^2 in March 2013. Furthermore, length classes ranged from 3.0 mm to 27 mm, and modal class ranged from 9 mm to 12 mm in both sample collections. The number of collected specimens for all length classes was smaller (n = 119) in October 2012, spring season, relative to March 2013, autumn season (n = 796). Specimen population (> 7 mm) was prevalent for both sample collections, and no specimen larger than 27 mm was registered. Distribution by length class for both sample collections is shown in Fig. 3.

Population density for *A. tenebricosus* was 3.0 ind/m^2 in October 2012 and 4.6 ind/m^2 in March 2013. The length classes ranged from 61 mm to 101 mm, with modal classes between 71 mm and 91 mm. Juvenile specimens were not found during both sample collections. Distribution by length class for both sample collections is shown in Fig. 4.

Diplodon parodizi population density was 1.0 ind/m^2 in October 2012 and 0 ind/m^2 in March 2013. Length ranged from 45 mm to 63 mm. Juvenile specimens were found during both sample collections as well.

Discussion

This study showed that a particular zone with a population of bivalves is an altered area following the results of Protocol for Rapid Evaluation of Habitat Diversity (Callisto *et al*. 2002). The limited degree of alteration is limited to the studied area and cannot be applied for the entire APA of the Verde River, which has several areas with significant anthropic modifications both downstream and upstream, such as consolidated human habitation, poor sanitation conditions, drainage of pluvial and sewage water, livestock production, pesticides used in agriculture, and the presence of water dams (Cunha *et al*. 2011, Capenaro *et al*. 2012), hence inducing different forms of contamination at many different levels. Thus, it should be emphasized that environmental alterations such as those mentioned earlier are strongly related to the decline of the native bivalves population (Mansur *et al*. 1987, Bogan 1993, Vaungh & Taylor 1999, Troncon &

Avelar 2011, Paschoal *et al*. 2015). Therefore, it can be inferred that the current environmental conditions in the studied area are not appropriate to preserve the native bivalve population in both the mid and long term.

According to Pereira *et al*. (2013), *A. tenebricosus* were recorded in seven countries in South America, showing 21% of occurrence of inventoried hydrographic areas. In Brazil, this particular species was observed by Zanardini (1965) in the Atuba River, which belongs to the Iguaçu River basin; in the Sinos River, which is a part of the Guaíba River basin (Mansur & Pereira, 2006); in the Sapucai River basin (Troncon & Avelar 2011); and in the Ribeira do Iguape River, which is a part of the Ribeira basin by Rodrigues *et al*. (2012). It is also considered by Simone (2006) as a widespread species; besides this wide distribution, this species does not occur homogeneously in a given basin.

Table II. Articulated valves and living specimens collected in the Verde River area, Paraná, Brazil, in October 2012 and March 2013.

	Corbicula fluminea		*Anodontites tenebricosus*		*Diplodon parodizi*	
	Living specimens	**Articulated valves**	**Living specimens**	**Articulated Valves**	**Living specimens**	**Articulated valves**
Oct	119	0	9	2	3	3
Mar	796	12	14	6	0	9
Total	915	12	23	8	3	12

Table III. Descriptive statistical analyses of visceral mass and valves' wet weight, length, height, and width of *Corbicula fluminea*, *Diplodon parodizi*, and *Anodontites tenebricosus* collected in the Verde River, Paraná, Brazil, in October 2012 and March 2013. SD : standard deviation; Min: minimum; Max: maximum; CV: coefficient of variation**.**

Species	Variable	Collect	Average	SD	Min	Max	CV
Corbicula fluminea	Visceral mass (g)	Oct/12	0.07	0.27	0.01	1.6	0.1
		Mar/13	0.16	0.17	0.01	1.4	0.0
	Valves (g)	Oct/12	0.16	0.49	0.03	3.0	0.2
		Mar/13	0.38	0.37	0.01	2.7	0.1
	Length (mm)	Oct/12	11.0	3.60	5.0	25.0	13.2
		Mar/13	13.0	3.20	3.0	24.0	10.5
	Height (mm)	Oct/12	6.52	3.62	2.0	20.0	13.3
		Mar/13	10.0	2.86	1.0	20.0	8.2
	Width (mm)	Oct/12	6.4	2.84	3.7	22.0	8.1
		Mar/13	7.0	2.01	2.0	14.0	4.1
Anodontites tenebricosus	Visceral mass (g)	Oct/12	9.30	4.60	6.5	17.4	21.4
		Mar/13	11.6	4.63	9.3	22.2	21.4
	Valves (g)	Oct/12	18.0	8.98	11.0	34.0	80.7
		Mar/13	30.5	9.71	15.3	44.4	94.4
	Length (mm)	Oct/12	78.0	8.30	61.0	84.4	69.0
		Mar/13	82.0	8.91	65.0	96.0	79.4
	Height (mm)	Oct/12	21.0	3.76	17.0	33.0	27.0
		Mar/13	36.0	4.04	29.0	43.0	16.3
	Width (mm)	Oct/12	32.0	3.74	22.0	33.0	14.0
		Mar/13	26.0	3.32	18.0	31.0	11.0
Diplodon parodizi	Visceral mass (g)	Oct/12	7.70	1.98	4.4	8.0	3.92
		Mar/13	-	-	-	-	-
	Valves (g)	Oct/12	12.7	6.36	6.5	24.0	40.4
		Mar/13	18.0	8.23	4.0	30.0	67.7
	Length (mm)	Oct/12	59.3	9.39	45.0	71.0	88.2
		Mar/13	67.0	7.08	54.0	75.0	50.0
	Height (mm)	Oct/12	19.0	4.58	10.0	24.0	24.0
		Mar/13	39.0	4.04	29.0	42.0	19.0
	Width (mm)	Oct/12	34.0	3.65	28.0	40.0	13.3
		Mar/13	22.0	3.12	16.0	25.0	9.8

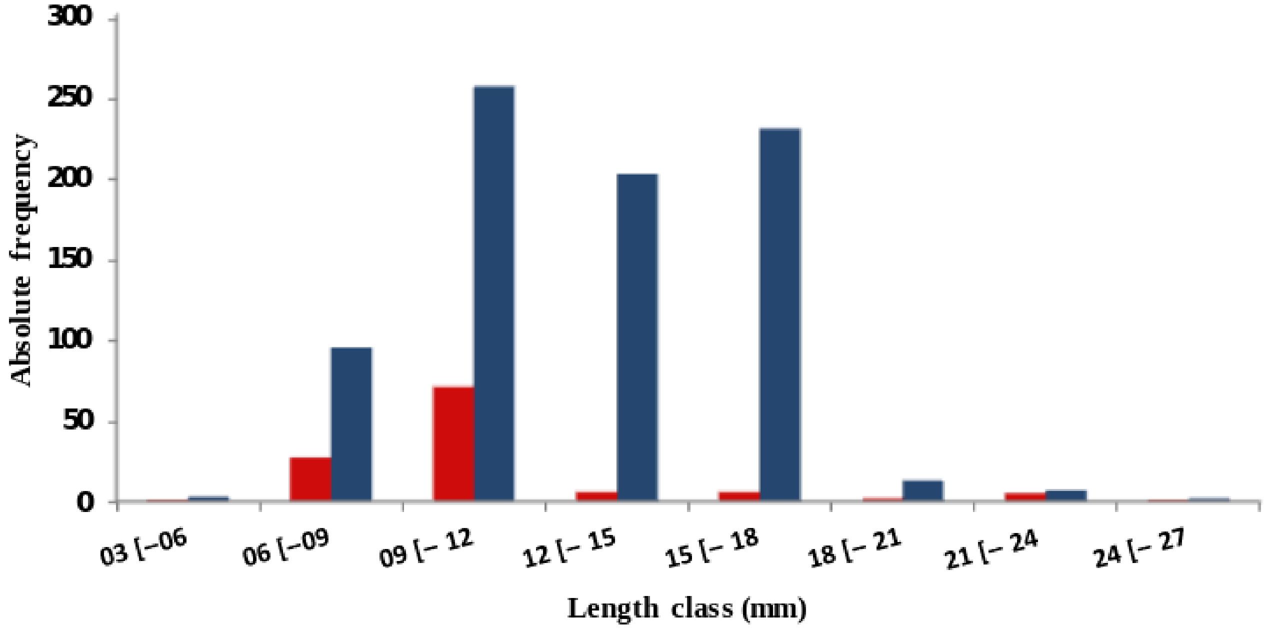

Figure 3. *Corbicula fluminea* (Müller, 1774). Frequencies by length classes (mm) in the Verde River, State of Paraná, Brazil. Sample collection in October 2012 (Red bars) and March 2013 (Blue bars).

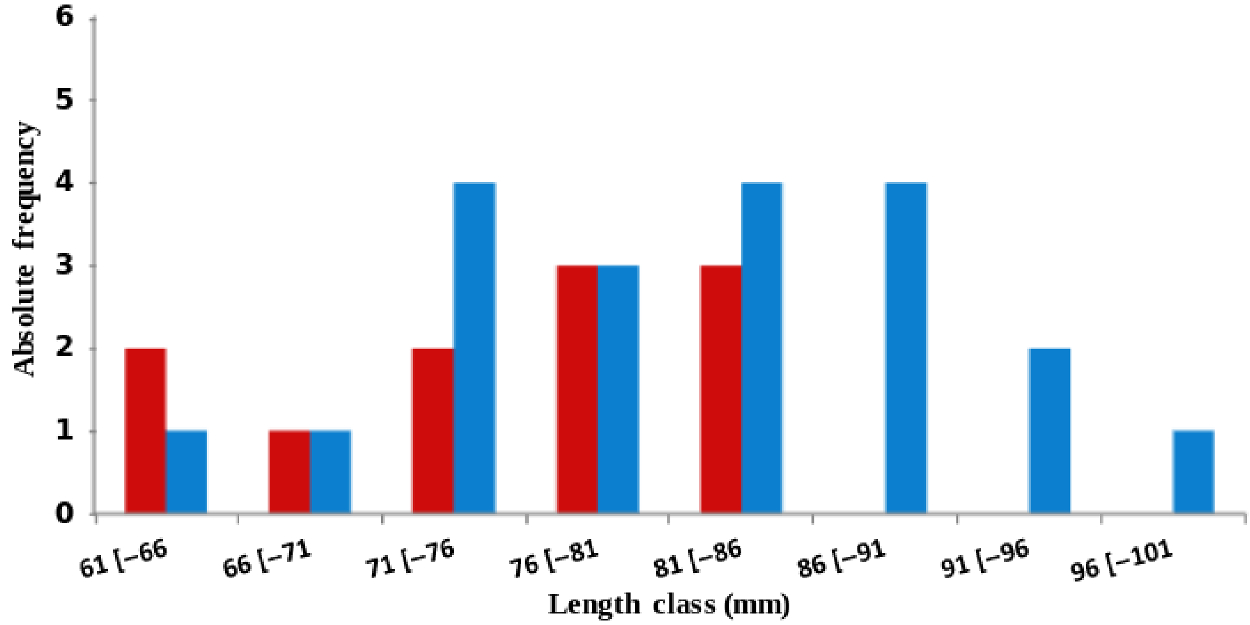

Figure 4. *Anodontites tenebricosus* (Lea, 1834). Frequencies by length classes (mm) in the Verde River, State of Paraná, Brazil. Sample collection in October 2012 (Red bars) and March 2013 (Blue bars).

The species *Anodontites tenebricosus* collected in the Verde River showed similar characteristics as those described for species with a reniform-elliptic-shaped valve, a matted and worn periostracum, and a dark and slightly iridescent nacre (Mansur & Silva 1990, Mansur & Pereira 2006, Troncon & Avelar 2011). The valves of the analyzed specimens showed strong correlations among the height, width, and length of valves, in accordance with the observations by Troncon and Avelar (2011). The mean value of proportion between length and height was 26.9% for specimens collected in October 2012 and 43.9% for those collected in March 2013, whereas Troncon and Avelar (2011) reported 43% and Mansur and Pereira (2006) observed 40% for this proportion analysis.

These discrepant results may be explained due to the number of analyzed specimens, because when the total collected samples are considered (n= 23), the proportion between length and height is 30.7%. In addition, the natural variations of species must be considered, observed as a result of the different ratios of the juvenile body growth, and ecological variations because of its wide-range territorial distribution (Mansur & Silva 1990, Mansur & Pereira 2006, Troncon & Avelar 2011).

In the Verde River, *A. tenebricosus* was found in areas with high streamflow speed, and substratum constituted by gravel, as commonly observed in areas with occurrence of bivalves described by Troncon and Avelar (2011) and Mansur & Pereira (2006). However, some aspects are not mentioned in these studies, such as the occurrence of species along the bank, where a substrate constituted predominantly by silt is observed. Also, the species association with roots from riparian forest trees is not mentioned. The length classes of *A. tenebricosus* analyzed by Troncon and Avelar (2011) ranged from 33.2 mm to 74.7 mm; these variations were lower than those observed for the Verde River, which ranged from 61 mm to 101 mm. This difference may be explained due to propitious environmental abiotic conditions. The increase in body growth due to the high organic particle availability was demonstrated in the laboratory by Parada et al (2008) for *Diplodon chilenses* (Gray, 1928) species. As in the studied area, the Verde River receives organic matter from the domestic animals breeding and pisciculture facilities. Therefore, it is possible to infer that the water enrichment with nutrients and dissolved solids contributes to bivalves' body growth, once they are remarkable filter feeders of suspended matter (Vaughn & Hakenkamp 2001). Rodrigues *et al*. (2012) observed a population with a mean length of 40 mm in the Ribeira do Iguape River (SP), which is 40 mm, and where the mean is lower than the mean obtained in our study; however, these authors found high levels of heavy metal contamination, which can interfere in the species development.

According to Pereira *et al* (2013), the species *D. parodizi* has occurrence records in South America, specifically in Argentina, Paraguay, and Brazil. Simone (2006) mentions the occurrence in the basins of the Amazon and Paraná, with a paratype of 53 mm. Despite these reports, the knowledge about the species is restricted to the morphological description provided by Bonetto (1962). In spite of the lack of information on length classes and density for this particular species, which directly affects intraspecific comparisons, data from our study are in accordance with data obtained for populations of mussels from the genus *Diplodon*, with the modal class being of an intermediary size and no juvenile being collected (Henry & Simão 1984, Meyer *et al*. 2010, 2014).

Population densities of *A. tenebricosus* (3.0 ind/m^2) and *D. parodizi* (1.0 ind/m^2) are lower than those observed in other bivalves from the order Unionoida. Lara and Parada (1988) reported different population densities, depending on substratum type and climatic season; numbers ranging from 10 to 58.7 ind/m^2 for *Diplodon chilenses* were observed in the Villarrica Lake. In addition, Henry and Simão (1984) reported 36.6 ind/m^2 for *Diplodon delodontus expansus* (Küster, 1856), in the Pardo River reservoir. However, freshwater bivalves are organisms with aggregate distribution (Henry & Simão 1984, Araujo & Ramos 2000, Lara & Parada 1988, Meyer *et al*. 2010), which contribute toward improving reproduction (Amyot & Downing 1988) and results in some areas with a high density. It is possible that the portion of the Verde River analyzed is located in a low-density area or the low population density for the native species is related to the presence of *C. fluminea*. In the Verde River, the low density of *D. parodizi* and the higher percentage of empty articulated valves (80%) can be emphasized compared with *A. tenebricosus* (34.7%). Considering the large amount of articulate valves found on the Verde River banks, these data suggest that this species shows high rates of mortality in the studied area, even though the valves may have been carried out from a different river portion or may be a result of selective predation.

The *C. fluminea* length range, between 3 and 27 mm, is significantly lower than that observed by Duarte and Diefenbach (1994), who reported specimens with 41,1 mm in Arroio Imbá (RS), and lower than that observed by Rodrigues *et al*. (2007), reporting organisms with 39,63 mm in Paranoá Lake (DF). In contrast, this length range is similar to the one reported by Bagatini *et al*. (2007) in the Rosana reservoir (SP), Vianna and Avelar (2010) in the Sapucai River (RS), and Oliveira *et al*. (2014) in the Passaúna River, in the metropolitan area of Curitiba. These different results are related to the biotic and abiotic conditions that contribute toward improving development and species phenotypic plasticity (Barret & Richardson 1986, Martins *et al*. 2004).

Population density analyses of *C. fluminea* showed a different range between the two sample collections, with a higher density in March 2013 (265.33 ind./m^2). This high population density may be explained as a result of a larger number of specimens from intermediary classes, resulting in more juvenile subjects, associated with high survival rates and the absence of predators.

As for the species *C. fluminea*, it was found to be of a relatively lower population density compared with observations by Mansur and Garces (1988) in the Estação Ecológica do Taim (5.195 ind./m^2), França *et al*. (2007) in the Baixo Tiête River (SP) (6.154 ind./m^2), and Vianna and Avelar (2010) in the Sapucai River (SP) (1.282 ind./m^2). However, according to Mansur and Garces (1988), population densities from 200 ind./m^2 have an important negative impact on native mussels population, mainly because the population settlement can induce benthic fauna alterations due to feces and pseudofeces biodeposition, as well as as alterations in the substratum as a result of hollow valves deposition (Jones *et al*. 1994, Vaughn & Hakenkamp 2001).

Therefore, the occurrence of these two native and one invasive species does not mean that there are no others species in the APA of the Verde River area, once this particular portion of the Verde River where the organisms were found is limited. However, the record for these populations is expressive because of its ecological relevance in maintaining the quality of hydric environments (Vaughn & Hakenkamp 2001) as well as in improving management strategies for a protected area, which must consider the entire fauna and flora (Gaston 2000).

To sum up, these data show the necessity to expand the studied area, as well as the implementation of monitoring programs to investigate native and invasive species population density; in addition, studies on taxonomy, and biological and ecological studies of the species were found.

Acknowledgments

The authors would like to thank Universidade Positivo for the institutional support; they are also grateful to Dra. Maria Cristina D. Mansur and Igor C. Miyahira (Universidade Federal do Estado do Rio de Janeiro) for the taxonomic review, and also to Dr. Luiz Simone, curator of the Malacology collection of the Museu de Zoologia da Universidade de São Paulo, for his technical support, taxonomic identification, and deposit of voucher specimens.

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Received: August 2016
Accepted: February 2017
Published: April 2017