



Zooplankton resting egg banks as a strategy to maintain diversity in a small tropical reservoir ecosystem

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Abstract: Small water bodies host numerous zooplankton species, which are directly influenced by their surroundings. During environmental instability, zooplanktons produce nutrient-rich eggs, via the process of diapause, that sink to the sediment and remain dormant under unfavorable environmental conditions. We aimed to observe how diapause helps maintain the richness of the zooplankton community in the small-sized Amália Reservoir, located in the municipality of Alfenas, Minas Gerais, Brazil. The studied reservoir is a well-defined microbasin with urbanization processes and a predominance of pastures and coffee plantations. The reservoir has low zooplanktonic richness despite having numerous macrophytes on its margin, which contributes to species richness. Zooplankton species were observed in dormant forms that were not recorded in the water column, demonstrating the importance of egg banks for long-term zooplanktonic survival. However, anthropogenic activities may have influenced the low number of species and dormant forms. Thus, knowing the species present in the water column in the sediment of the Amália Reservoir proved the importance of the dormant form bank for the long-term survival and structuring of the community.

Key words: dam, dormancy, community, maintenance, diapause.

Banco de ovos de resistência da comunidade zooplanctônica como estratégia para manutenção de diversidade num pequeno reservatório tropical. Resumo: Pequenos corpos d'água podem abrigar grande número de espécies zooplanctônicas, mas são influenciados pelo entorno. Durante instabilidade ambiental, o zooplâncton inicia um processo que produz ovos ricos em nutrientes, depositados no sedimento e permanecem dormentes até condições favoráveis. O objetivo deste estudo foi observar como a diapausa contribui para a manutenção da riqueza da comunidade zooplanctônica do reservatório Amália, um corpo d'água de pequeno porte, localizado no município de Alfenas, MG. O reservatório estudado pertence a uma microbacia bem definida com processos de urbanização e predominância de pastagens e lavouras de café. O reservatório possui baixa riqueza zooplanctônica apesar de possuir um grande número de macrófitas em sua margem, o que contribui para a riqueza de espécies. Espécies do zooplâncton foram observadas em formas dormentes que não foram registradas na coluna d'água, demonstrando a importância do banco de ovos para a sobrevivência zooplanctônica em longo prazo. No entanto, atividades antrópicas podem ter influenciado o baixo número de espécies e formas dormentes. Assim, conhecer as espécies presentes na coluna de água e no sedimento do reservatório da Amália comprovou a importância do banco de formas dormentes para a sobrevivência e estruturação da comunidade a longo prazo.

Palavras-chave: açude, dormência, comunidade, manutenção, diapausa.

Introduction

Small water bodies provide essential ecosystems as well as economic and social services to the population (Straskraba & Tundisi 2013). These environments have high rates of carbon sequestration and high productivity per square area and help maintain regional fauna and flora by forming “biodiversity pockets,” which serve as shelter and a food source for several species (Coelho et al. 2014; Downing 2009; Scheffer et al. 2006). These sites are sensitive to changes in their surroundings owing to their size (Scheffer et al. 2006).

The zooplankton community, which consists mainly of microcrustaceans, plays a fundamental role in maintaining ecosystem processes. These organisms act as a link between primary producers and other trophic levels and actively contribute to the nutrient cycles in the water column (Lampert & Sommer, 1997; Nogueira Júnior & Costa 2019). Zooplanktons have strategies to ensure the survival of species in unstable environments, such as the production of dormant forms through a process called diapause (Gyllström & Hansson, 2004).

Diapause, characterized by the production of dormant eggs, is induced by several stimuli such as seasonal variations, reduced availability of food, predation, exposure to chemical pollutants, drought events, and an increase in population density (Coelho et al. 2021; Santangelo 2009). Eggs produced by this process are usually deposited in the sediment, forming an “egg bank,” which serves as an essential genetic reserve of zooplanktonic organisms. Since eggs are repositories of energy and nutrients, they remain viable for long periods until they receive specific environmental stimuli for hatching (Abrusán et al. 2007). In addition, dormant forms have a refractory period before recognizing the necessary stimuli for their hatching, thus preventing the dormant forms from hatching simultaneously. This refractory period benefits the population temporarily if the first individuals to hatch are unsuccessful (De Meester et al. 1998). Therefore, the passive community is fundamental for the diversification of the active community and the repopulation of the water column after periods of environmental stress (Hairston 1996; Zadereev et al. 2019). It can also be used as a bioindicator of changes in the water column (Weider et al. 1997). However, there are few studies on the passive zooplankton community in tropical regions compared to the temperate regions (Iglesias et al. 2016), in which the first works were published by

Crispim & Watanabe (2001) and Maia-Barbosa et al. (2003). To help understand the dynamics of the zooplankton community, we evaluated the contribution of the dormant form bank to maintaining the spatial and temporal diversity of zooplankton in a small tropical reservoir.

The Amália Reservoir is a small body of water in the rural area of Alfenas, in southern Minas Gerais, Brazil (21°S25'55" " 45°W55'25"") (Fig. 1), with approximately 80,000 inhabitants (IBGE/2019), and belongs to a well-defined microbasin. This region is characterized by growing urbanization and agricultural activities. In terms of soil use and occupation, there is a predominance of pastures and coffee, with some patches of annual crops and forests. Moreover, there is the presence of a riparian forest in the spring and all along the stream to the reservoir. The average annual temperature is approximately 23 °C, and the average annual precipitation is approximately 1500 mm (Costa et al. 1998).

Materials and Methods

The reservoir has a total area of 2,833 m² with maximum length, width, and depth of 103, 33, and 2.2 m, respectively. The water body had a perimeter of 1,38 meters and a margin development index of 1.59. It has been subjected to much dredging of the sediment and macrophytes and the introduction of fish and fingerlings in its recent history.

Sampling was conducted on March 11, 2019, near the margin for P0 to survey the zooplanktonic species and sediment, and on April 5 and August 30, 2019, for P1, P2, and P3 to sediment samples only. Climatological data were obtained from the climatological station of Machado, Minas Gerais, Brazil (approximately 30 km), by the National Institute for Space Research. Physical and chemical variables of the water column (pH, turbidity, dissolved oxygen, electrical conductivity, total dissolved solids, and temperature) were recorded using a Horiba U-50 probe on the surface and bottom of the water column. Granulometry of the sediment was carried out using the sieving technique, according to the methodology proposed by the Associação Brasileira de Normas Técnicas (2018). The suspension was subjected to gravimetric analysis (Teixeira et al. 1965). The sampling site depth was measured using a graduated rope attached to a metal weight, and water transparency was determined using the Secchi disk disappearance method. The euphotic zone limit (1%) was obtained

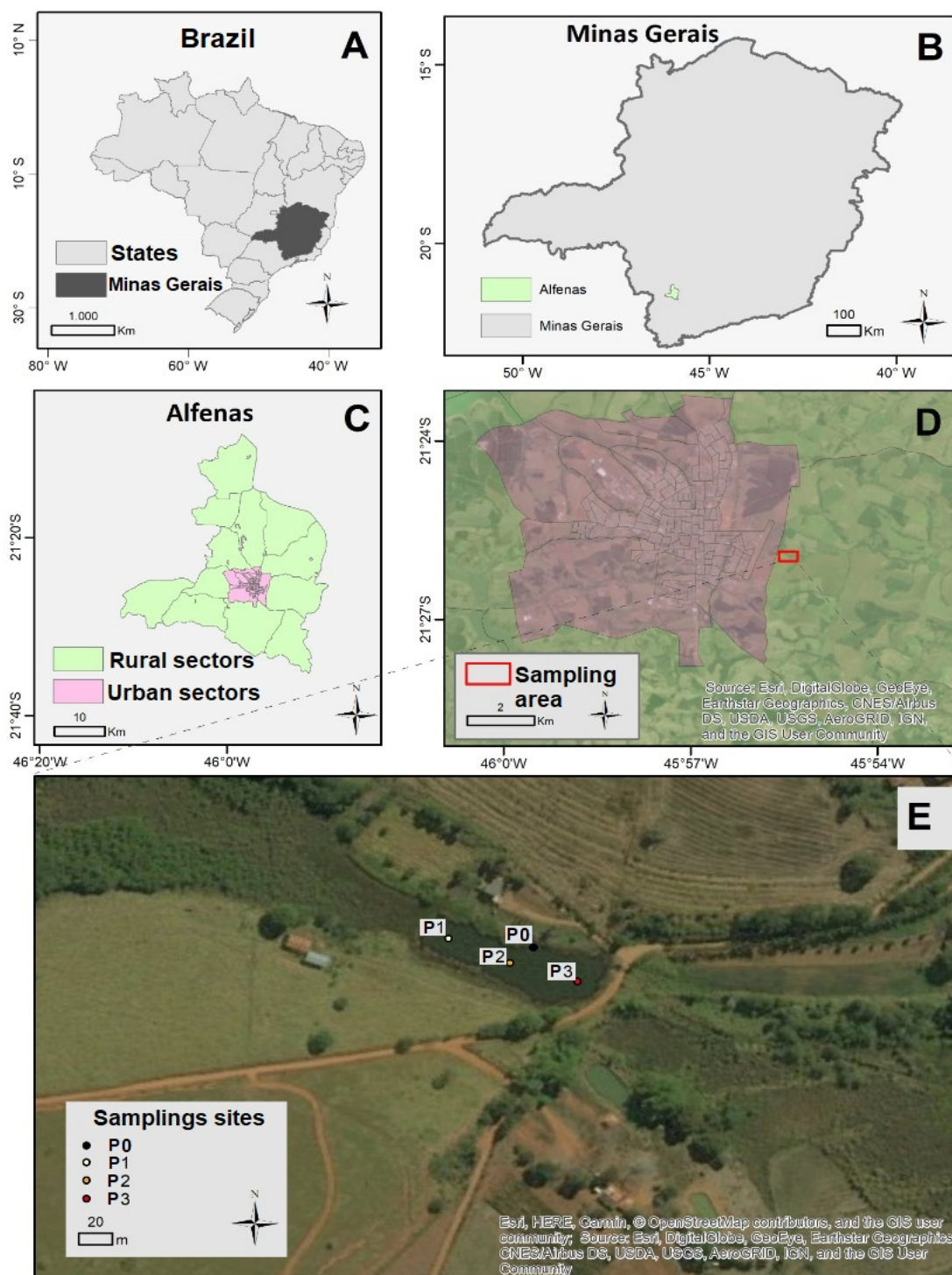


Figure 1. Location of the reservoir and sampling sites in the Amália reservoir, Alfenas, MG

by multiplying the water transparency by the coefficient 2.7, according to Margalef (1983).

Zooplankton organisms were collected from the water column surface by horizontally dragging a 68 μ m plankton net and fixed in 4% formalin. For analysis of the passive community, samples were collected using a Kajak–Brinkhurst corer. For egg separation, the sugar flotation method proposed by

Onbé (1978) and the microcosm creation method proposed by Sars (1901) and adapted by Fialek (2018) were used for the first five centimeters of the sediment. To increase the chances of reactivation, we placed the dormant forms in three different solutions: a) water reconstituted from distilled water, b) filtered and reconstituted spring water, and c) filtered water from the Amália Reservoir.

The dormant forms were separated from 100 g of sediment. Then, small amounts separated by similarity criteria were placed in 0.04 L containers to verify the dormant form species. The remainder was placed in a 1 L container solution. Each container was monitored for 30 d.

For the microcosms, sediment samples were divided into 100 g aliquots and placed in polystyrene flasks covered with aluminum foil for drying. The sediment used in the microcosm was rehydrated with 500 ml of triple-filtered water in a 20 µm net from the Amália Reservoir. Dormant forms were monitored for 148 d.

All containers were kept in a germination chamber at 25 °C ($\pm$ 2 °C) and a controlled photoperiod (12 h light/12 h dark).

Active and passive zooplankton communities were identified under a Zeiss optical microscope model Scope 20 using a specialized bibliography (Koste 1978; Reid 1985; Elmoor-Loureiro 1997; Sinev & Elmoor-Loureiro 2010; Kotov & Dumont 2000; Silva 2003; Silva & Matsumura-Tundisi 2005; Segers 2007) and were later stored in the collection of the Laboratory of Limnology at Unifal, MG. Macrophytes were sampled and identified according to the method described by Pott & Pott (2000).

Results

Granulometric analysis revealed that the sediment mainly consisted of clay and silt (Fig. 2).

During sampling, differences in depth, water transparency, and euphotic zone were observed between the dry and rainy seasons, as shown in Figure 3. The water body also contains a significant amount of inorganic material, as shown in Figure 4. Table I presents the results of the physical and chemical variables temperature, pH, conductivity, turbidity, dissolved oxygen concentration and total suspended solids.

The reservoir had a high density of macrophytes on its margins and extended through the water column in the places where it had shallow depth with representatives of *Nymphaea* sp., *Utricularia gibba*, *Nitella cernua*, *Mayaca fluviatilis*, *Hydrocotyle* sp., *Elodea* sp., and *Salvinia auriculata*.

Few representatives of the main active zooplankton community groups were observed with four families of Cladocera (Daphniidae, Ilyocryptidae, Macrothricidae, and Sididae), three families (Brachionidae, Lecanidae, Synchaetidae), and one class (Bdelloidea) of Rotifera, and two Copepoda orders (Calanoida and Cyclopoida), as shown in Table II. From the dormant forms collected

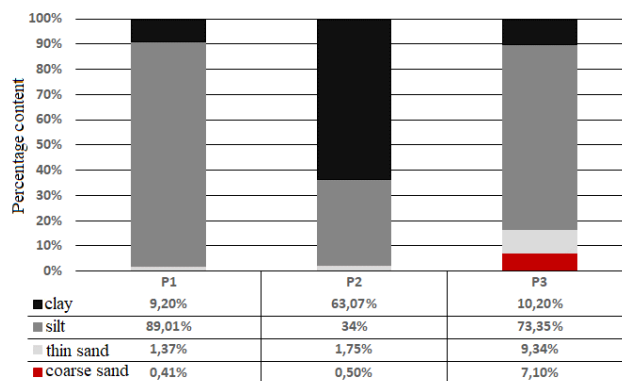


Figure 2. Amália Reservoir's granulometry.

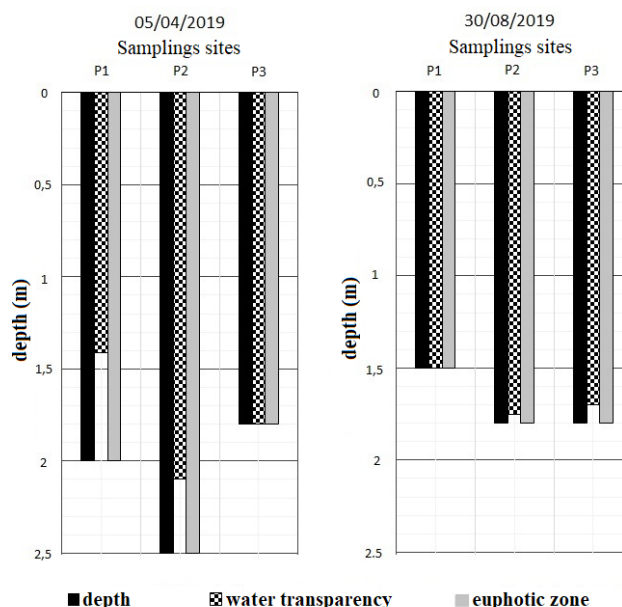


Figure 3. Depth, water transparency, and euphotic zone observed in the Amália Reservoir.

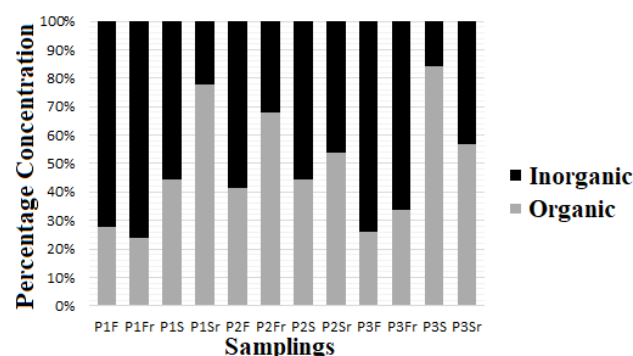


Figure 4. Amount of inorganic and organic material observed on the Amália Reservoir.

from the sediment, three families of Cladocera (Daphniidae, Ilyocryptidae, and Macrothricidae), one family (Lecanidae), one class of rotifers (Bdelloidea), and one order of Copepods (Harpacticoida) were recorded. Some species were

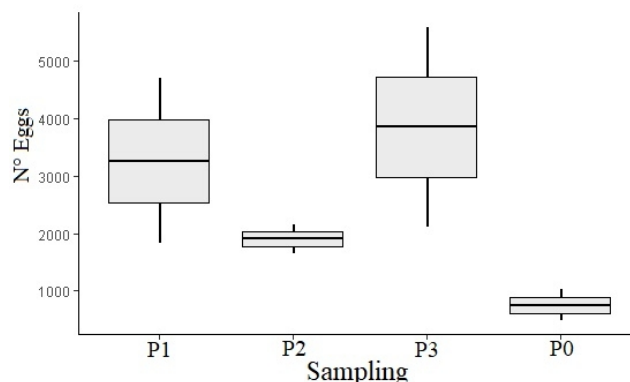


Figure 5. Boxplot with mean, variance, and standard deviation of the number of dormant forms in the Amália Reservoir, Alfenas, MG.

observed only in the passive community, such as rotifers Bdelloidea, *Lecane lunaris* and Copepoda Harpacticoida.

During the sampling of zooplankton, a high number of fingerlings and different fish species were

observed in the water body. In the analysis of collected samples, representatives of the genus *Aphyocharax* and fingerlings of different species were observed.

The number of dormant forms recorded was between 480 and 5595 forms per 100 g of sediment for each sampling site, as shown in Figure 5. Figure 6 shows some dormant forms of the zooplankton community found in the reservoir sediment.

Discussion

The sampled water body had small dimensions and depth and was classified as a small reservoir. These reservoirs are greatly influenced by margins in the supply of nutrients and environmental heterogeneity, which in turn influences the productivity and species richness of the environment (Tundisi & Matsumura-Tundisi 2008). External factors such as wind velocity and vegetal cover around the reservoir can accelerate silting (Singh et al. 2013). The sediment of the Amália Reservoir

Table I. Physical and chemical variables of the sampled water body. OD - dissolved oxygen concentration and STD - Total dissolved solids

Date	Point	Zone	Temperature (°C)	pH	Conductivity (mS.cm ⁻¹)	Turbidity (NTU)	OD (mg. L ⁻¹)	STD (mg. L ⁻¹)
04/05/19	P1	Surface	22.84	4.89	0.026	3.70	5.92	0.017
04/05/19	P1	Bottom	20.40	4.23	0.047	2.25	1.47	0.031
04/05/19	P2	Surface	23.88	4.69	0.026	3.60	5.74	0.017
04/05/19	P2	Bottom	20.64	3.98	0.025	16.40	1.43	0.016
04/05/19	P3	Surface	25.40	4.63	0.024	3.80	5.91	0.016
04/05/19	P3	Bottom	21.05	4.11	0.025	14.03	3.21	0.017
04/12/19	P1	Surface	22.10	4.34	0.022	4.10	5.92	0.014
04/12/19	P1	Bottom	20.90	4.05	0.020	3.90	1.83	0.013
04/12/19	P2	Surface	22.61	4.34	0.021	4.00	4.30	0.013
04/12/19	P2	Bottom	21.00	4.00	0.023	11.60	3.90	0.015
04/12/19	P3	Surface	22.70	5.00	0.022	4.10	6.25	0.014
04/12/19	P3	Bottom	21.50	4.17	0.022	4.30	4.47	0.016
08/30/19	P1	Surface	17.60	5.90	0.017	3.90	10.52	0.011
08/30/19	P1	Bottom	16.03	4.41	0.020	16.00	4.60	0.013
08/30/19	P2	Surface	19.00	5.10	0.016	3.40	10.00	0.010
08/30/19	P2	Bottom	16.00	3.90	0.016	4.20	5.43	0.010
08/30/19	P3	Surface	21.00	4.40	0.016	3.10	12.00	0.010
08/30/19	P3	Bottom	16.10	4.70	0.017	4.90	4.80	0.011
09/06/19	P1	Surface	19.29	4.65	0.019	3.90	8.74	0.012
09/06/19	P1	Bottom	18.42	3.27	0.022	7.00	4.38	0.017
09/06/19	P2	Surface	19.08	3.60	0.016	4.20	6.58	0.010
09/06/19	P2	Bottom	17.24	3.16	0.019	7.80	2.43	0.012
09/06/19	P3	Surface	19.28	3.83	0.017	3.90	5.40	0.011
09/06/19	P3	Bottom	17.72	3.54	0.019	4.10	2.61	0.014

Table II. Taxon composition of the active and passive zooplankton community of the Amália Reservoir.

	Active community				Passive community			
	P0	P1	P2	P3	P0	P1	P2	P3
Cladocera								
<i>Ceriodaphnia</i> sp.	X	–	–	–	–	–	–	X
<i>Diaphanosoma birgei</i>	X	–	–	–	–	–	–	–
<i>Ilyocryptus spinifer</i>	X	–	–	–	–	–	X	X
<i>Macrothrix</i> sp.	X	–	–	X	–	–	–	X
Rotifera								
<i>Bdelloidea</i>	–	–	–	–	X	–	–	–
<i>Euchlanis dilatata</i>	–	–	X	–	–	–	–	–
<i>Lecane bulla</i>	–	X	X	X	–	–	–	–
<i>Lecane leontina</i>	X	–	–	–	–	–	–	–
<i>Lecane lunaris</i>	–	–	–	–	X	X	X	–
<i>Lecane signifera</i>	–	–	X	–	–	–	–	–
<i>Synchaeta jollyae</i>	–	–	X	X	–	–	–	–
Copepoda								
Calanoida	–		–	–	–	–	–	–
Nauplius	–	X	–	–	–	–	–	–
Cyclopoida	–	–	–	–	–	–	–	–
<i>Ectocyclops</i> sp.	X	–	–	–	–	–	–	–
<i>Tropocyclops proximus</i>	X	–	–	–	–	–	–	–
Adult	–	X	–	X	–	–	–	–
Copepodito	–	–	X	X	–	–	–	–
Nauplius	–	X	–	X	–	–	–	–
Harpacticoida	–	–	–	–	X	–	–	–
Other			–					
<i>Arcella gibbosa</i>	–	X	X	–	–	–	–	–
<i>Arcella vulgaris</i>	–	–	X	X	–	–	–	–
<i>Centropyxix aculeata</i>	–	–	–	X	–	–	–	–
<i>Chironomidae</i>	X	X	–	X	–	–	–	–
Nematoda	–	X	–	–	–	–	–	–
Oligochaeta	–	X	–	–	–	–	–	–
<i>Ostracoda</i> sp.	–	–	–	–	–	X	–	X

mostly consists of clay and silt, which may affect the dynamics of abiotic variables and consequently influence the distribution of aquatic communities (Loverde-Oliveira et al. 2007).

The reservoir receives discharges of pesticides and other compounds from agricultural activities from the surroundings, which can contaminate sediment through subsidence. Sediments are one of the biggest pollutants in water and serve as catalysts, carriers, and fixing agents for other polluting agents. Thus, sediment particles act as vehicles for pesticides, chemical agents, toxic residues, nutrients, pathogenic bacteria, and viruses (Carvalho 2008).

Further, the xenobiotics in sediments can be toxic to the zooplankton community organisms (Gillis et al. 2006; Terra & Gonçalves 2013). Studies carried out with dormant forms have shown considerable resistance of eggs to pollutants, corroborating the importance of egg banks in the repopulation of the water column after periods of environmental stress (Oskina et al. 2018; Zadereev et al. 2019).

The variation in the depth between the dry and rainy periods was greater than 25%, which, combined with a considerable increase in suspended material and the topography and coverage characteristics of the hydrographic basin, results in a

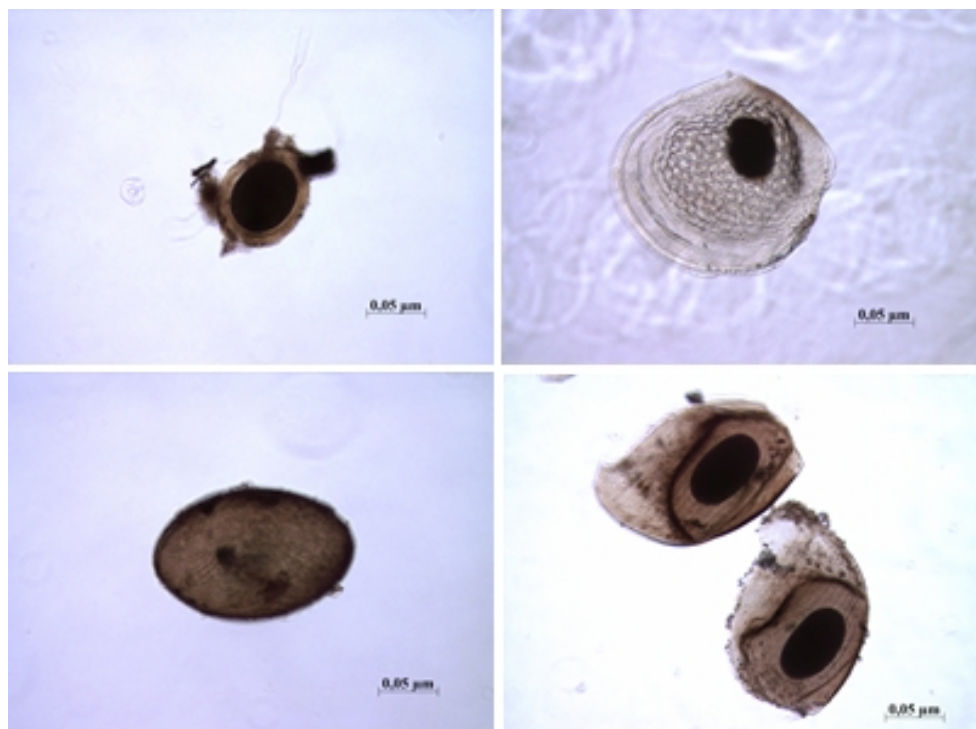


Figure 6. Typical dormant forms of the zooplankton community from the Amália Reservoir sediment egg bank.

large volume of allochthonous material and low water residence time. The reservoir also had a high amount of inorganic material in the water column, which can lead to variations in environmental characteristics and coverage of dormant forms, which reduces stimuli for reactivation and affects the active community over time.

The high density of macrophytes observed in both the littoral and limnetic zones of the reservoir, in addition to being influenced by the greater input of nutrients, may be related to the shallow depth of the water column, benefitting the rooting of several species of macrophytes (Thomaz & Bini 2003). Among the macrophyte species recorded, *Nymphaea* sp., *Utricularia gibba*, and *Nitella cernua* species associated with oligotrophic environments (Tavares 2003) were observed at high densities. At lower densities, species indicative of transitional environments, such as *Mayaca fluviatilis* and *Hydrocotyle* sp., and some species generally associated with eutrophic environments, such as *Elodea* sp. and *Salvinia auriculata* were also recorded.

The values of the physical and chemical variables observed in the reservoir were similar to those recorded by other authors in small tropical water bodies, such as Silva & Santos-Wisniewski (2016) in two water bodies in Guaxupé, Minas Gerais and Coelho et al. (2014) in a body of water in

Alfenas, Minas Gerais. These small reservoirs are located within a radius of less than 100 km and share a good part of similar climatological characteristics. They are artificial and are characterized as small, shallow, and oligotrophic. Macrophytes are present in all of them. However, when analyzing the species richness data, we noticed a significant difference: in the two reservoirs studied by Silva & Santos-Wisniewski (2016) 34 and 56 taxa were observed; in the reservoir evaluated by Coelho et al. (2014), 50 taxa were identified, and in the Amália reservoir we detected only 16 taxa. Despite the many similarities, the number of species in these other bodies of water was 112.5 to 250% greater than that found in this study.

However, unlike the reservoir evaluated here, where there is a predominance of pastures and coffee growing with some annual crops, the aforementioned environments had less anthropic pressure from its surroundings owing to the presence of preserved areas, with forest fragments on at least one bank and pastures on the other. These characteristics are considerable because, because they are smaller, these places are sensitive to changes in their surroundings (Scheffer et al., 2006)

The highest species richness was observed in the rotifer group of the active zooplankton community in the reservoir. Four families (Brachionidae, Lecanidae, Synchaetidae) and one

class (Bdelloidea) of Rotifera are usually found in eutrophic environments and have a high rate of reproduction (Serafim-Júnior et al. 2010). The cladoceran families Daphnidae and Sididae are usually observed in the pelagic region of water bodies, and the families Ilyocryptidae and Macrothricidae in the littoral region are associated with macrophytes (Castilho-Noll et al. 2010).

Among the analyzed physical and chemical parameters, no extreme values that could have influenced the low species richness were detected. In addition to the anthropic influence, restoration efforts, low water residence time, and the predatory pressure exerted by the ichthyofauna on the zooplankton community may also contribute to the low zooplankton richness and diversity. Water bodies had a high density of small fish and fingerlings of the genus *Aphyocharax* during sampling, which, among other species, uses the zooplankton community as a food source (Maia-Barbosa & Matsumura-Tundisi 1984; Lazzaro 1987).

The occurrence of fish in lentic environments strongly influences the density of zooplanktonic organisms, especially in small water bodies (Knapp et al. 2016), which may be responsible for the low diversity of cladocerans in the active community. Cladocerans have higher nutritional value and digestibility (Sipaúba-Tavares & Bachion 2002; Sarma et al. 2005; Ismail et al. 2011). Thus, the dormant form bank becomes an important genetic reserve fundamental for the repopulation and structuring of the zooplankton community, as these forms accumulate in the sediment for years and can remain viable for a long time (Brendonck & de Meester 2003). Among the dormant forms collected in the sediment, some species were observed only in the passive community, such as rotifers of the Bdelloidea class, Copepoda Harpacticoida, and *Lecane lunaris*.

In the sediment samples, the highest number of dormant forms was observed in P3 and the lowest in P2. The highest number of dormant forms in P3 may be related to its higher fine sand concentration in the sediment compared to the other sampling sites. Maia-Barbosa & Eskinazi-Sant'Anna (2003) observed a relationship between the abundance of dormant forms and the concentration of fine sand, with a higher abundance at sites with the highest concentration of fine sand. This may be related to the shape and fall velocity of dormant forms, which resemble silt particles (Marcus 1989)

Furthermore, the low number of dormant forms observed in P2 may also be related to the sediment composition and sampling site with the highest clay content compared to the other sampling sites. Moreover, the constant dredging of the sediment in this reservoir may have affected the bank of dormant forms. The resuspension and dredging of sediments can suspend and drag organisms and dormant forms through the water column, damaging or displacing them (Maia-Barbosa and Eskinazi-Sant'anna 2003).

In a comparative study of 26 lakes from four regions of Brazil, Santangelo et al. (2015) observed a large variation in the number of hatches (1–295 with a median of 30) and the richness of hatched species (1–18 with a median of 7). The bank of dormant forms in each environment depends on a particular combination of stimulus factors (Santangelo 2009). Araújo et al. (2013) pointed out that active and passive zooplanktonic communities, due to their relative environmental stability, may present a greater response to biotic stimuli such as predation and competition than to abiotic factors, such as controlled laboratory variables. Temperature fluctuations are considered one of the major factors contributing to the activation of dormant forms (Jersabek, Schabetsberger 1995).

Therefore, despite the low number of dormant forms hatched in the laboratory, from 0 to 12 individuals per experiment, the potential of the sediment dormant form bank to maintain the diversity of zooplankton species in the water column is evident. The hatching of two taxa that were not observed in the active community highlights the importance of diapause for the repopulation of the zooplankton community and the maintenance of ecosystem processes in the water column during periods of environmental instability.

The sampled water body was small and had numerous macrophytes in its surroundings, which allowed for compartmentalization of the environment and a greater number of microhabitats. However, the richness of the active zooplankton community was low, which besides being influenced by anthropic changes in the water column, may also be related to predatory pressure in the water column due to the presence of fish and fingerlings. In the hatching tests, the presence of taxa in the sediment that were not in the water column was observed, which demonstrated the importance of the dormant form bank of this water body in the long-term repopulation and structure of the community.

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Ethical statement

The present investigation did not involve the use of regulated animals and did not require approval by an ethical Committee

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