Massive mortality of the giant freshwater mussel Anodontites trapesialis (Lamarck, 1819) (Bivalvia: Mycetopodidae) during a severe drought in a Neotropical reservoir

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Abstract: In 2012, a severe drought struck the southeastern of Brazil compromising the Paraná River Basin reservoirs. Here, we described how this climatic event promoted a massive mortality of the giant freshwater mussel Anodontites trapesialis in Furnas reservoir and reported the consequences of this phenomenon. In November 2012, three quarters of 100 m² were sampled in this reservoir, where 812 dead shells of A. trapesialis were analyzed and measured (33 ± 133 mm). The species showed an aggregated distribution with high density (X: 1.0 - 5.5 ind/m²). Despite the massive mortality detected in field, it was possible to find living specimens in a small channel in the studied area, allowing the species to survive the water level fluctuations. Large adult individuals (100 ± 124 mm) were more affected by drought than juveniles, accounting for about 90% of the dead mussels analyzed. Two years after the massive mortality event, water level was not reestablished and a terrestrial succession (with elevations in the concentration of organic matter and calcium in sediment) was observed in the studied area. We verified that the damming associated with extreme climatic events affect negatively the populations of A. trapesialis and should be faced as a conservationist problem.

Key words: Unionida, die-off, hydric stress, conservation, Mollusca.

INTRODUCTION

The giant freshwater mussel Anodontites trapesialis (Lamarck, 1819) is a functional simultaneous hermaphrodite bivalve with a trapezoid shell, with records of some specimens reaching over than 200 mm (Simone 1994, Callil & Mansur 2007). It is the most widely distributed bivalve in the South America basins, and shows a wide geographical distribution in Americas, occurring from some Central America basins to Argentinean Patagonian basins. This species, which occurs naturally in lotic environments has been commonly found in artificial lakes and reservoirs, especially in areas used for fish farming (Graf & Cummings 2007, Pereira et al. 2014, Torres et al. 2018). This is due to the fact that their larvae (lasidium type) are capable to use non-native fish species as hosts, as is the case of the tilapia Oreochromis spp. (Guardia-Felipi & Silva-Souza 2008, Callil et al. 2012).

However, when this species inhabits reservoirs it suffers from certain environmental restrictions due to damming, which significantly affects its population structure (L.R.P. Paschoal & D.P. Andrade, unpublished data). Reservoirs are artificial man-made environments used for multiple purposes, such as: leisure, energy production and public supply. Brazil is the country with the largest
number of reservoirs (N = 393) in the world (FAO 2016). In general, they are considered as intermediate type water bodies, since they have a retention time of two to forty days. The upstream area of these environments has lentic characteristics, while the downstream areas have lotic conditions (Henry 1999, Brasil 2012). In these environments, certain natural physical-hydrological phenomena (added or not by anthropic impacts) occur irregularly in time and space, promoting instability in benthic communities that live in these areas. The main natural limiting phenomena (factors) are the deposition of particulate matter, the action of waves on the margins and water column level fluctuations (Agostinho et al. 1992, Andrade et al. 2012, Paschoal et al. 2015).

The decrease in water level and the exposure to air caused by droughts are considered the main factors responsible for desiccation in bivalves (Collas et al. 2014, Paschoal et al. 2015). Some species of freshwater mollusks are capable of surviving to desiccation by hibernating and/or migrating to deeper or more humid aquatic zones (Darrigran & Lopez-Armengol 1998, Darrigran & Lagreca 2005). However, several studies have reported a massive mortality of bivalves caused by desiccation, when high density and biomass of individuals were transported and stranded on the coastal areas due to environmental changes caused by extreme climatic events (e.g. Ilarri et al. 2011, Sousa et al. 2012, 2018, Paschoal et al. 2015, Vaughn et al. 2015). In these studies, the authors correlated this situation mainly to the low capacity of locomotion of these animals, added to temporal variability of the physical conditions of these transitional environments.

In recent years, severe droughts have dramatically altered the aquatic environments of Brazil (Nazareno & Laurance 2015). Melo et al. (2016) verified that the water supply was much compromised in reservoirs of the Paraná River Basin (southeastern of Brazil) due to a prolonged drought. In 2012, a severe drought was recorded for the Southeast region of Brazil, promoting decreases in water levels in certain reservoirs of this region up to 17 meters below the usual average for the same period (ANA 2017, ONS 2017). Considering this scenario, the present study verified how the decrease of the water level in a Neotropical reservoir promoted by drought and damming, caused a massive mortality of the giant freshwater mussel A. trapesialis. In addition, we pointed ecological and conservation implications of this phenomenon.

MATERIALS AND METHODS

Study site

The reservoir of the Furnas Hydroelectric Power Station (HPS) is composed mainly by the Grande and Sapucaí rivers (upper Paraná Basin) (Fig. 1). It is the largest reservoir in the state of Minas Gerais (flooded area: 1,440 km²) and one of the most important in Brazil, extending throughout 36 municipalities. The reservoir is used for energy production, as well as a water reserve, a recreational place and source of income for many families that live from fishing, and also directly and indirectly harbors great diversity of wildlife (Paschoal et al. 2012).

The present study was conducted at the margins of a portion of the Sapucaí River (20° 58’ 23” S, 46° 07’ 08” W), inserted in the Furnas HPS reservoir, municipality of Carmo do Rio Claro, Minas Gerais state (southeastern of Brazil) (Figs. 1 and 2). This area is characterized by high water column fluctuation levels throughout the year, clay-sandy sediment, pebbles and boulders in its margins, and predominance of the macrophyte Brachiaria sp. near the margin and Eichhornia azurea (Swartz) Kunth ten meters away from the margin (Paschoal et al. 2015).
The water column levels and accumulated precipitation patterns during the period from January 2000 to December 2015 were verified using data from ANA (2017) and ONS (2017). Mean monthly values of these variables were computed, showing that from June to October the water column level of the reservoir tends to decline due to low precipitation levels in the previous months (May to September), until it reaches its minimum level in November (Fig. 3a). However, it was possible to observe that in 2012, December was the month with the lowest water column level due to a severe drought occurred in the Southeastern region of Brazil (Fig. 3b). The storage capacity (volume of the flooded area) of the Furnas HPS reservoir fell below 13% of its total capacity in December, and during the months of November and December showed the water column level below five meters (Fig. 3b), far from the average recorded for the last 15 years for the region - 9.2 to 10.5 meters (Fig. 3a). The present study was only possible to be performed in the present condition (i.e. drought and lower water column levels), since such areas are inaccessible when the reservoir is in its normal hydrological conditions (Fig. 2).

### Data sampling

Sampling was performed on November 2\textsuperscript{nd}, 2012. The collection and analysis permissions were granted by MMA/ICMBio/SISBIO (license 36210-1). Twelve living bivalves were collected in a small channel (i.e. original river channel before impoundment) near to the sampled area (Figs. 2b and 4) and transported to the laboratory. These mussels were identified according to Simone (1994, 2006) and had its shell length (L), height (H) and width (Wi) measured (Fig. S1 - Supplementary Material). The wet weight (We) of each individual was obtained with an analytical scale (± 0.001 g), after being dried with absorbent paper. Subsequently, these animals were euthanized by freezing (-20 °C / 30 min.) and preserved in 70% ethanol. These bivalves were deposited at the Museu de Zoologia da Universidade de São Paulo (MZSP), under the number 109106.

In field, we analyzed three areas along the margin of the Sapucaí River channel, each one comprising a quarter of 100 m\textsuperscript{2} (subdivided into 100 quadrats of 1 m\textsuperscript{2}). These quarters were distant 40 meters from each other, and the right side of each quarter was the closest to the channel margin. There were no evidences for a
strong depth gradient along the sampled areas. Also, these areas were exposed to sunlight for a considerable amount of time. This procedure allowed to estimate the population density (ind./m²), biometric values of the shells and spatial distribution of *A. trapesialis* in the studied area. For each sampled quarter, all dead shells (i.e. without soft parts) inside the quadrats showing the two valves were identified, counted and measured (L, H and Wi) with an analogic caliper (0.05 mm) (Figs. 4 and S2). Shell fragments and buried individuals that could not be identified were disregarded and excluded from analysis.

The first quarter (I) was inserted in a grassy area (with predominance of *Zoysia* sp.) near to the river channel, with high content of organic matter in soil (61 g/kg) and clayey sediment (Fig. 4). The other quarters (II and III) showed longer exposure to sunlight due to lower depth in relation to the Quarter I, with a higher proportion of silt in its sediment composition and no grasses in their soil, indicating a lower amount of organic matter (20 g/kg) (L.R.P. Paschoal, personal data).

On November 2nd 2014, two years after the massive mortality event detected in Furnas HPS reservoir, a new sampling (using the same methodology) was performed to verify a recovery (or not) of the number of individuals in this population.

**Data analysis**

Allometric equations of $Y = aX^b$ type were adjusted for the 12 living specimens, having as independent variable ($X$) the shell length (L) and relating it to the other body dimensions of the animal (dependent variables, $Y$) (Huxley 1950). Values of the allometric constant ($b$) were tested using the $t$ test, as $H_0: b = 1$ (or 3 in the case of weight), and used to determine the growth patterns of a specific body part in relation to L. Subsequently, the equations were linearized ($Y = a \times X - b$).

The distribution pattern of *A. trapesialis* was determined using the Morisita index ($I_M$), being random when $I_M = 1$, aggregate when $I_M > 1$, and uniform when $I_M < 1$ (Krebs 1989). Analyses of variance (ANOVA’s) associated with Tukey multiple comparison tests were used to verify possible differences between the mean values of density and biometric variables of *A. trapesialis* dead shells between the quarters.

**RESULTS**

The mean (± standard deviation) shell length (L) of the 12 living specimens of *A. trapesialis* was 121.1 ± 6.0 mm, while the shell height (H) and width (Wi) were 61.3 ± 4.1 and 37 ± 4.1 mm, respectively. The mean weight (We) of these mussels was 96.32 ± 15.17 g. The smallest
individual analyzed had 113.5 mm L, 59.4 mm H, 37.5 mm Wi and 108.34 g We, while the largest animal had 133.3 mm L, 67.6 mm H, 42.6 mm Wi and 119.89 g We. Table I summarizes the linear regressions of the morphometric data and shows allometric patterns for the analyzed body portions. It is noted that only the relationship H vs. L showed positive allometry (Table I).

In field, 812 dead shells of *A. trapesialis* were analyzed and measured (Figs. 4 and S2). Statistically significant differences were registered in the total number of individuals obtained in the three quarters (F = 82.05, p < 0.001), as well as in the mean size of the body structures measured in mussels sampled in these quarters (F = 14.89, p < 0.001). Quarter I showed higher abundance (N = 546) and density ($\bar{x} = 5.52$ ind/m$^2$), besides having animals with greater body proportions in its composition ($\bar{x} = 111$ mm), when compared to the other quarters ($\bar{x} = 106$ and 109 mm). In addition, the highest absolute density (22 ind/m$^2$) recorded in this study was established in this quarter. The population of *A. trapesialis* that inhabited the Sapucaí River showed an aggregated distribution ($I_M > 1$) along the sampled area, i.e. all quarters (Fig. S2 and Table II). The biomass values estimated by extrapolating a linear regression, were: 43.45, 7.21 and 12.07 kg/quarter, for Quarter 1 to 3, respectively.

We observed the presence of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) nests near to the analyzed quarters, especially in the area of Quarter I (Fig. S3). The highest shell length (L) frequencies of dead mussels measured during the study were recorded for the size classes between 100 and 124 mm (approximately 87% of the sampled dead shells), with a modal peak at the size class 110–114 mm. Few specimens were found in the lower size classes. The smallest dead mussel analyzed in field had 30.0 mm L, 12.0 mm H and 9.8 mm Wi, while the largest had 133.0 mm L, 67.7 mm H and 42.2 mm Wi (Fig. 5).

In 2014, the second field survey cannot be performed due to the abundance of shrubs and erect macrophytes of the genera *Polygonum* and *Brachiaria* (Fig. S4), indicating that area is in an initial process of terrestrial succession (Fig. 2c). The water level was not recovered; consequently, the mussels do not come back to this area. Besides that, only shell fragments were recorded among this vegetation.
DISCUSSION

In 2012, a severe drought in Brazilian southeast region promoted a sudden change and decrease of the water levels of the reservoir analyzed here. These events have caused a great negative impact on the population of Anodontites trapesialis, evidenced by the expressive number of mussels killed by desiccation, i.e. massive mortality. Pilger & Gido (2012) demonstrated that freshwater mussels were heavily affected by rapidly receding water levels. The giant mussels could not move in the same rhythm as the rapid decrease of the water column level of the Furnas reservoir, being exposed to the environment and susceptible to desiccation and predators. Cândido & Romero (2006, 2007) demonstrated that A. trapesialis shows slow rates of burrowing, digging and displacement/locomotion. Thus, it is possible that these mussels have stranded on the sediment when exposed to drought, promoting a significant mortality in this population. This pattern is similar to that recorded for other unionoid and cirenid bivalves exposed to extreme climatic events (see Ilarri et al. 2011, Sousa et al. 2012, 2018, Bódis et al. 2014, Paschoal et al. 2015, Vaughn et al. 2015).

Despite the massive mortality verified in field during the drought, it was possible to find living specimens of A. trapesialis in a small channel in the studied area. This shows how the species is capable to adapt to different environmental conditions and to survive even in hydric stress events, as pointed out by Pereira et al. (2014). As observed here, there are indications that the plasticity of A. trapesialis population from Furnas HPS reservoir has allowed the species to survive the water levels fluctuations, which in natural environments does not occur so quickly. Callil & Mansur (2007) and Callil et al. (2018) confirmed that the reproductive cycle of A. trapesialis, as well as the energy allocation for growth of this mussel in the Pantanal wetlands are directly related to the hydrological cycle imposed by flood pulses. In this way, future studies are necessary to verify if the population analyzed in this reservoir maintains the pattern of body increment and reproductive peak influenced by higher water levels or if it is capable to reproduce and maintain constant sizes over time, as registered for Corbicula...
Anodontites trapesialis showed an aggregation behavior with high density along the stretch analyzed in the study area. The most common spatial distribution in bivalves is the aggregate. This is due to the environmental heterogeneity of the area, in which bivalves group in microenvironments more suitable for their lifestyle (Santos et al. 2012, Paschoal et al. 2015).

Anodontites trapesialis, *fluminea* (Müller, 1774) in the same reservoir by Paschoal et al. (2015).

The population of *A. trapesialis* analyzed invests energy for longitudinal shell growth. This is probably related to the growth of the foot and demibranches as the shell increases. A large foot would optimize the time spent in burrowing events and improve the anchorage of animals that inhabit clayey sediments (Cândido & Romero 2006, 2007). In field and laboratory, it was observed that large mussels were capable to bury and escape faster than smaller animals (LRP Paschoal personal data). Additionally, Callil et al. (2018) have shown that fecundity in *A. trapesialis* is directly proportional to the size of the mussel, where large animals have higher offspring than smaller individuals. In the analyzed reservoir, it is likely that large animals had more chances to escape the desiccation by burying and taking advantage of moist microhabitats, making these mussels more suitable for reproduction.

Few studies evaluated the population density of *Anodontites* mussels in Neotropical reservoirs. Nascimento-Filho et al. (2014) recorded a density up to 1 ind/m² for *A. trapesialis* in the Serrinha reservoir (Pernambuco state, northeastern Brazil). Henry & Simão (1986) registered a density of up to 0.02 ind/m² for the congener *A. trapezeus* (Spix in Wagner, 1827) in the Piraju reservoir (São Paulo state, southeastern Brazil). These values are significantly lower than those recorded in the present study. This is possibly due to the difficulty of collecting these mussels, since these animals live buried in deep substrates (up to 15 m) and have large body sizes, which makes it difficult to obtain individuals by conventional collection devices such as nets and suction or grab samplers (SEMA 2013).

Several ecological factors influence the distribution of mollusks and among them the substrate is a determinant factor in the distribution of freshwater bivalves (Harman 1972, Serrano et al. 1998, Paschoal et al. 2015). This characteristic was observed in the present study, since significant differences were observed for the three quarters, in which only the first one differed from the others. The area occupied by Quarter I was composed of clayey substrate and showed evidences of high concentration of organic matter and abundance of Nile tilapia, *Oreochromis niloticus*.
since this area was fully covered by grass and showed several nests of tilapia. An explanation for this distribution pattern and higher density, biomass and body proportions of *A. trapesialis* in the area of Quarter I would be given by the synergy of some factors: (I) the clayey substrate (i.e. fine particles) of this area would aid the colonization and burrowing process of these mussels (Pereira et al. 2000, Cândido & Romero 2006, 2007), thus avoiding predation and desiccation, (II) due to the fact of the species is a suspension feeder that preferably inhabits regions of lower river flow with high amounts of phytoplankton (food resource) and organic matter (Simone 1994, Pereira et al. 2011), these environmental features were observed in the studied area when not exposed to the drought [see Paschoal et al. (2015) and Brito et al. (2016)], and (III) the presence and possible use of the non-native fish *O. niloticus* as hosts for its larvae (Guardia-Felipi & Silva-Souza 2008), since we detected several dead shells near to the tilapia nests.

Large adult individuals\(^1\) (100 – 124 mm L) were more common during the analysis of massive mortality in the *A. trapesialis* population, while juveniles were rarely observed. Bődis et al. (2014) when evaluating the massive mortality of bivalves along the Danube River Basin (Hungary) caused by drought, verified that the individuals of the Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834) with a size range of 60 – 180 mm L were the animals more affected. Semenas & Brugni (2002) suggest that the size distribution of bivalves in limnic environments is modulated by depth, and that smaller animals inhabit deeper areas. Thus, it is possible that juveniles of *A. trapesialis* may not be affected as large adults when water levels decrease because they inhabit deeper areas.

In 2014, a new survey was carried out to verify if the population presented or not recovery in the number of individuals. However, it was not possible to be performed due to the abundance of vegetation in the initial stage of terrestrial succession at the sampled area (Fig. S4), showing that the flooded area of Furnas reservoir did not recover from the drought (Fig. 2). As verified by Bődis et al. (2014), the massive mortality of bivalves promotes the input of

\(^1\) Here, we considered mature mussels all animals with > 47 mm L (size at the onset of physiological maturity, sensu Callil & Mansur 2007). Mussels with small shells (< 47 mm L) were considered immature individuals.

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**Table II.** Total number of shells (N), mean, minimum and maximum density (Ind/m²), distribution pattern (I, \(M\)) and mean (± standard deviation) values of the analyzed biometric variables for the dead valves of *Anodontites trapesialis* at the three quarters analyzed.

| Quarter | N     | Ind/m²        | \(I_M\) | L        | H        | Wi       |
|---------|-------|---------------|---------|----------|----------|----------|
| I       | 546   | 5.52\(^a\) (1 - 22) | 1.39    | 111.25\(^a\) (± 7.50) | 56.00\(^a\) (± 4.05) | 35.40\(^a\) (± 2.35) |
| II      | 104   | 1.05\(^a\) (1 - 5)    | 1.64    | 106.55\(^a\) (± 10.75) | 53.45\(^a\) (± 5.80) | 33.90\(^a\) (± 3.40) |
| III     | 162   | 1.64\(^b\) (1 - 6)    | 1.13    | 109.45\(^b\) (± 9.10) | 55.00\(^b\) (± 4.95) | 34.80\(^b\) (± 2.85) |

Different letters represent significantly different means. H, shell height. L, shell length. Wi, shell width.
nutrients and biomass into the area where it occurs, increasing nutrient concentration and, consequently, primary productivity. In the analyzed area, the concentration of organic matter in sediment increased between two to six times (128 g/kg) after the massive mortality. In addition, dead shells probably increased calcium concentration in the analyzed area (0.76 g/kg Ca), since nearby areas without *A. trapesialis* had concentrations of this element, three times lower (~ 0.25 g/kg Ca) (L.R.P. Paschoal, personal data). This shows that the shells can persist in the environment, providing calcium and nutrients that will be incorporated into the sediment (Strayer & Malcom 2007). It can be noted that this phenomenon promoted an ecological impact in the analyzed area, altering the environmental scenario of the reservoir. Future studies should be carried out in this area, in order to verify how nutrient and calcium cycling occur from dead shells and how the ecological succession will impact the reservoir after the flooding and reestablishment of the normal water column levels.

The habitat modification promoted by dams (i.e. water level control) associated with climatic changes may restrict populations of *A. trapesialis* and should be faced as an ecological problem, since it is considered one of the causes of bivalve extinction (Bogan 1993, Haag & Williams 2014). The present study shows the first record of massive mortality of native bivalves in South America promoted by synergic events. A management proposal for *A. trapesialis* and other mussels, would be the relocation of these bivalves to nearby deeper areas with perennial and resident water, aiming the conservation and maintenance of these native species. We suggest monitoring of the populations and verification of the effectiveness of the adopted measures, being the responsibility of the competent organs the adoption of mitigating measures. In addition, it was observed in field the coexistence of the giant freshwater mussel to the Asian clam *C. fluminea* (L.R.P. Paschoal, personal observation).
This invasive species has the capacity to alter the environment that inhabits and is capable to displace native species. Moreover, when exposed to climatic disturbances it shows a fast recovery and can overlap and repel native species in the same area of occurrence (Sousa et al. 2008, Paschoal et al. 2015). Until now, seven non-native species of mollusks were registered in reservoirs of Brazilian basins (Pereira et al. 2018). These authors suggested that invasive species can promote a drastic reduction of native mollusk populations, so an increased attention should be given to the monitoring of the A. trapesialis population that inhabits this reservoir.

The development of conservation strategies for bivalves faces challenges, including the lack of quantitative data and/or assessments, selection of priority species and populations for conservation, strategic decisions on habitat restoration and propagation in captivity, as well as the participation of academic, governmental and general public (Règnier et al. 2009, Santos et al. 2013, Lopes-Lima et al. 2017, Torres et al. 2018). Thus, future studies related to temporal changes in the patterns of transitional environments are of extreme importance, since the bivalves are also a faunal group with a high rate of extinction (Bailie et al. 2004, Lydeard et al. 2004), as well as the mycetopodids (Pereira et al. 2012). The results presented here described the effects of extreme climatic events in Neotropical reservoirs, creating a basis for future studies on ecology and conservation of freshwater native bivalves.

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SUPPLEMENTARY MATERIAL

Figure S1.
Figure S2.
Figure S3.
Figure S4.

How to cite

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