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JONATHAN DA ROSA

Dormant structures of aquatic microfauna: ecological aspects and perspectives on different climate temperature scenarios

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Tese apresentada ao Programa de Pós-graduação em Ecologia de Ambientes Aquáticos Continentais do Departamento de Biologia, Centro de Ciências Biológicas da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutor em Ecologia e Limnologia.
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“As nuvens mudam sempre de posição, mas são sempre nuvens no céu. Assim devemos ser todos os dias, mutantes, porém leais com o que pensamos e sonhamos.”

(PAULO BELEKI)

Estruturas dormentes da microfauna aquática: aspectos ecológicos e perspectivas sobre diferentes cenários climáticos de temperatura

RESUMO

A dormência é um processo biológico que permite que as comunidades sobrevivam em condições ambientais adversas. A produção de estruturas dormentes é uma das principais estratégias realizada pela microfauna aquática (ciliados, rotíferos, cladóceros, copépodes e ostrácodes). Avaliou-se as estruturas de dormência da microfauna aquática, abordando diferentes aspectos ecológicos como o tempo de eclosão, dispersão, influência de fungos e a viabilidade das estruturas em diferentes temperaturas. Experimentos de eclosão foram realizados com sedimentos e macrófitas aquáticas de lagoas da planície de inundação do alto rio Paraná, Brasil. Os resultados evidenciaram que os ovos resistência de ostrácodes possuem o tempo de eclosão semelhante entre espécies taxonomicamente próximas e que os traços funcionais das espécies (por exemplo: tamanho do corpo e presença de espinhos) influenciam no tempo de eclosão. Constatou-se que as raízes secas da macrófita flutuante livre (*Eichhornia crassipes*) abrigam e dispersam ovos de resistência de ostrácodes. Enquanto, para toda a microfauna aquática dormente observou-se uma relação positiva entre a presença de fungos no sedimento e riqueza de espécies da comunidade dormente, o que não influenciou negativamente a viabilidade das estruturas dormentes. O aumento da temperatura reduziu significativamente a riqueza de espécies da microfauna dormente e levou a homogeneização do banco de ovos. Mediante aos resultados, ressalta-se a importância destas estruturas dormentes na recolonização dos ambientes e o alto potencial de dispersão por vetores bióticos (plantas), entretanto também é um alerta para o processo de invasão. As mudanças climáticas responsáveis pelo aumento da temperatura são uma ameaça para as estruturas dormentes da microfauna aquática, e desse modo, é necessário criar medidas para a conservação do banco de ovos.

Palavras-chave: Banco de ovos. Eclosão. Resiliência. Aquecimento global. Lagoas Neotropicais.

Dormant structures of aquatic microfauna: ecological aspects and perspectives on different climate temperature scenarios

ABSTRACT

Dormancy is a biological process that allows communities to survive in adverse environmental conditions. The production of dormant structures is one of the main strategies performed by aquatic microfauna (ciliates, rotifers, cladocerans, copepods and ostracods). This study aimed to evaluate the dormant structures of aquatic microfauna, addressing different ecological aspects such as hatching time, dispersal, fungal influence and viability of the structures at different temperatures. The hatching experiments were performed with sediment and aquatic macrophytes of lakes from the upper Paraná River floodplain, Brazil. The results evidenced that ostracod resting eggs have similar hatching times among taxonomically close species and that the functional traits of the species (e.g. body size and presence of spines) influence the hatching time. In addition, dried roots of the free floating macrophyte (*Eichhornia crassipes*) shelter and disperse ostracod resting eggs. While, for all dormant aquatic microfauna a positive effect was observed between the presence of fungi in the sediment and species richness of the dormant communities, which did not negatively influence the viability of the dormant structures. The results also showed that the increase in temperature reduced significantly the species richness of the dormant microfauna and led to homogenisation of the egg bank. Based on the results, we highlight the importance of dormant structures in the recolonisation of environments and the high potential for dispersal by biotic vectors (plants), however this is also a warning for the invasion process. In addition, climate change responsible for temperature increase is a threat to the dormant structures of aquatic microfauna, thus it necessary to create mitigation measures for the conservation of the egg bank.

Key-words: Egg bank. Hatching. Resilience. Global warming. Neotropical lakes.

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SUMMARY

1 GENERAL INTRODUCTION

Climate change threatens the biodiversity of aquatic ecosystems (IPCC, 2014). Some changes will be inevitable, such as the occurrence of extreme drought events and temperature increases (PALMER et al., 2009; GRILLAKIS, 2019). In temporary aquatic environments the fauna is generally adapted to predictable hydrological variability. Dormancy is one of the main strategies that occur in these environments and consists in physiological and structural change in the organism or the formation of the subsequent generation (ALEKSEEV; PINEL-ALLOUL, 2019). When dormancy is temporary, reversible, a type of hibernation of the individuals, it is referred as quiescence (GYLLSTRÖM; HANSSON, 2004). On the other hand, diapause is the stage of dormancy occurring in the subsequent generation, characterised by the production of tolerant stages of dormancy.

Extreme environmental conditions, such as long droughts, affect the metabolism of aquatic organisms, including ciliates, zooplankton and ostracods, promoting the production of dormant structures that can be cysts, spores, ephippia or resting/diapausing eggs (BROCK et al., 2003; ALEKSEEV; PINEL-ALLOUL, 2019). Thus, these communities have two distinct life phases, the active phase present under favourable conditions and the dormant phase under unfavourable environmental conditions, characterised by the presence of stages of dormancy (WALSH, 2013; ALEKSEEV; PINEL-ALLOUL, 2019).

The production, incubation, hatching and viability of the dormant structures are influenced by abiotic environmental factors (e.g. temperature, organic matter, light) and biotic factors (e.g. presence or absence of pheromones of potential predators such as fish) (BRENDONCK, 1996; ROSA et al., 2020). The dormant community (the dormant structures) responds to changes in the aquatic ecosystem. However, the hatching potential of each individual organism is specific and depends on a combination of environmental triggers, specific phenology, functional traits and individual history, including maternal effects (BRENDONCK, 1996; BRENDONCK; PINCEEL; ORTELLS, 2017; ROSA et al., 2021b).

Dormant structures play an important role in the structuring and the dynamics of populations and communities since they act as a mechanism of passive dispersal. These dormant structures have durable viability acting as inoculum in dispersal by biotic and abiotic vectors (HAIRSTON et al., 1995; HAY; JENKINS; KINGSFORD, 2018), such as wind, rain (MORENO; PÉREZ-MARTÍNEZ; CONDE-PORCUNA et al., 2016), water birds (MORAIS-JUNIOR et al., 2019), floating macrophytes (ROSA; MARTENS; HIGUTI et al., 2022), mammals (VANSCHOENWINKEL et al., 2008) and human activities (VALLS et al., 2016). Thus, they can increase the genetic and taxonomic diversity of both the dormant and the active community as individuals of different generations hatch (ELLNER & HAIRSTON, 1994; BRENDONCK; PINCEEL; ORTELLS, 2017).

The dormant structures accumulate in the sediment, forming an egg bank with species richness of different generations (BROCK et al., 2003). These structures can remain viable for decades until stimulates hatching by triggers (HAIRSTON et al., 1995). Studies on dormant communities are important to understand the functioning and dynamics of aquatic communities over time, because egg banks often harbour greater species richness when compared to the active community (CRISPIM; WATANABE, 2001; ROSA et al., 2021a). In addition, the dormant structures in the egg bank can be used as a tool in the study of ecological biogeography, biodiversity, evolutionary ecology, palaeolimnology, and population and community ecology (BRENDONCK; De MEESTER, 2003).

The investigation of different aspects of dormant structures using the species recruitment helps to understand aspects of the resilience of aquatic communities during and after extreme events, as well as their role in contributing to local diversity (ROSA et al., 2021a). In addition, studies on egg banks are essential to understand the response of the dormant community under environmental alterations, mainly climate changes, because this can provide information that can be used to create measures for the conservation of the species in the egg bank. However, the knowledge about the hatching methodology of aquatic microfauna dormant, is still insufficient, especially in the Neotropical regions (IGLESIAS et al., 2016; GERHARD et al., 2017).

Thus, the present thesis has four manuscripts about the dormant structures of aquatic microfauna in lakes of a tropical floodplain. In the first manuscript, we investigate the hatching phenology of ostracod resting eggs in temporary lakes. In the second manuscript, we evaluated the potential of a floating macrophyte as dispersal vector of ostracod resting eggs. In the third manuscript, we evaluate if the presence of fungi in sediment influences the structure of the dormant aquatic microfauna communities (ciliates, rotifers and microcrustaceans) of a tropical temporary lake. Finally, in the fourth manuscript we tested the effect of the increase of temperature on the structure (richness and species composition) of the aquatic dormant microfauna (ciliates, rotifers and microcrustaceans).

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2 SPECIES' TRAITS AND TAXONOMIC DISTANCE CAN PREDICT THE HATCHING PHENOLOGY OF OSTRACOD (CRUSTACEA) RESTING EGGS FROM TROPICAL FLOODPLAIN LAKES

Abstract

Freshwater ostracods are commonly found in temporary ponds and lakes, surviving drought periods by producing resting eggs. We investigated the hatching phenology of ostracod resting eggs from the sediments of temporary floodplain lakes, considering both the taxonomic distance between species and their functional traits, such as carapace size (length and height) and shape, valve ornamentation, and reproductive mode. In addition, we tested the hypotheses: 1) that the hatching time is more similar between congeneric ostracod species than between non-congeneric species; 2) that differences in hatching time between congeneric species are often related to differences in functional traits; 3) that both species composition and functional traits composition of hatchlings change over the incubation time, but with a reduction in the variability over time. The experiment was conducted for 98 days in the laboratory, and the microcosms were monitored weekly. The first hatching of an ostracod was recorded during the 2nd week of incubation after hydration of the sediments. A total of 12 ostracod species hatched, belonging to the families Cyprididae and Candonidae. The above three hypotheses were corroborated. The time between inundation and first hatching was mostly similar for congeneric species in the genus *Chlamydotheca* Saussure, 1858, but was different between some species of *Strandesia* Stuhlmann, 1888, which might be owing to differences in functional traits. The species composition and functional trait composition of the hatched ostracods were significantly different over the 14 weeks of incubation due to the different hatching phenology of different species. Furthermore, our results show that both taxonomic distance and functional traits can influence the hatching time of ostracod resting eggs from temporary floodplain lakes. Thus, future studies addressing the hatching phenology of ostracod resting eggs (and resting stages from other invertebrates) should also emphasize the use of functional traits.

Key words: Ostracoda. Hatching time. Functional traits. Neotropical.

2.1 Introduction

Freshwater ostracods are commonly found in temporary ponds and lakes, and their populations survive drought periods by producing resting eggs (Williams 2006; Higuti et al. 2017; Rosa et al. 2021). Such resting eggs also have additional ecological importance for the species since they act in the colonization of novel aquatic environments, reduce population oscillations, and avoid local extinction (Hairston 1996; Rossi et al. 2012). Resting eggs accumulate in the sediment and form egg banks. They require biotic and abiotic cues to trigger hatching (Gyllström & Hansson 2004; Rossi et al. 2012). Also, the time between inundation and hatching (here referred to as “hatching time”) is influenced by several environmental factors, such as temperature and hydrostatic pressure (Brendonck 1996; Bandeira et al. 2020). Moreover, the intrinsic “bet-hedging” strategy, which exists in several invertebrate groups from temporary habitats, can influence the phenology of resting eggs since some batches of eggs may remain dormant during several wet/ drought cycles of the habitat, even though environmental conditions are beneficial to hatching. This strategy increases the probability of a population persisting during stochastic events to avoid all available eggs hatching at once when conditions will not allow animals to mature and produce new eggs (Evans & Denneyi 2005; Simons 2011). Bet-hedging has thus far not been demonstrated for ostracods (Pinto et al. 2007).

Hatching time of resting eggs, as well as population growth and passive community structure, can be evaluated by laboratory experiments, simulating natural flooding after a drought event (Bhusnale et al. 2016; Freiry et al. 2020), because factors such as temperature and photoperiod can be controlled. Rehydration of temporary pool sediment is a common method to study various ecological aspects of resting eggs in several aquatic invertebrate groups, especially in crustaceans (e.g., branchiopods) (Brendonck & De Meester 2003; Bhusnale et al. 2016).

Functional traits and phylogenetic distance approaches (often with taxonomic classifications as proxies - Warwick & Clarke 1995) have been used to answer ecological questions (Saito et al. 2015). Functional traits are phenotypic characteristics that can influence ecosystem functioning through the survival of organisms and populations (Tilman 2001). Recent studies have used functional trait analyses to explain phenologies of species of several biological groups (König et al. 2018; Stemkovski et al. 2020; Wang et al. 2020). This is, for example, common in plants, where there are different flowering phenologies in trees, shrubs, herbs, and grasses in relation to certain functional traits, such as growth form (Wang et al. 2020). Thus far, there are no studies using a functional trait approach to explain the hatching phenology of drought-resistant eggs from aquatic microcrustaceans.

Ostracods are small bivalved crustaceans, which range in length from 0.3 to 5.0 mm in the Neotropics, and which can have different body morphologies (e.g., flattened or rounded) (Meisch 2000). There are about 2300 living species of non-marine ostracods presently known (Meisch et al. 2019), 331 of which have been reported from the Neotropical region (Higuti & Martens 2020). Carapace shape and presence/absence of swimming setae are important functional traits in the life history and dispersal of ostracod species (Matsuda et al. 2015). Some species can also have pointed or rounded external structures on their carapaces (e.g., spines, dorsal helmets), which might serve to avoid predators (e.g., fish and other invertebrates) (Barnhisel 1991). In addition, the reproductive mode might strongly influence the dispersal and successful establishment of populations. There are three main modes of reproduction in non-marine ostracod species: fully sexual, mixed reproduction (males, sexual and asexual females), or fully asexual (parthenogenesis, males are fully absent) (Horne et al. 1998).

Few studies on the phenology of ostracod resting eggs have thus far been performed in Neotropical regions (Rosa et al. 2020; Rosa et al. 2021). The phenology of eggs of some ostracod species, such as *Heterocypris incongruens* (Ramdohr, 1808), is relatively well-known, but these studies are almost exclusively done in temperate regions (Rossi et al. 2004; 2012).

Here, we investigate the hatching phenology of ostracod resting eggs, considering the taxonomic distance and functional traits of the ostracod species, which hatched from sediments of temporary lakes in a tropical floodplain (southern Brazil). We tested the following hypotheses:

1) That hatching time is more similar between the congeneric ostracod species than between the non-congeneric species. Since similarity in life cycle and growth between congeneric species had already been observed in other invertebrate groups (Towns 1983), e.g., in larvae of the genus *Wyeomyia* Theobald, 1901 (Culicidae, Diptera) (Dezerald et al. 2017), we test if this is also the case for non-marine ostracods.

2) That differences in hatching time between congeneric species are often related to differences in functional traits.

3) That both species composition and functional traits composition of hatchlings change over the incubation time, but with a reduction in the variability over time (expressed in weeks).

2.2 Material and methods

In our experiment, we used sediment from five temporary lakes located on the Porto Rico island (Lake Clara, 22°45'20.7"S/053°15'27.7"W; Lake Figueira, 22°45'22.7"S/053°15'34.0" W; Lake Pontal, 22°45 '05.7" S/053°15'23.6"W) and Mutum island (Lake Osmar, 22°46'28.6"S/053°19'58.8"W; Lake Pousada, 22°44'43.4"S/053°14'06.9W) in the Upper Paraná River floodplain in southern Brazil (Figure 1). This floodplain belongs to the Environmental Protection Area of the Varzea Islands of the Paraná River (Agostinho et al. 2004). The regional climate is tropical–subtropical with distinct rainy (November–March) and dry (April–October) seasons (Köppen Cfa; Eletrosul 1986; Agostinho et al. 2004).

The studied lakes are isolated, shallow (not exceed 2.2 meters of depth) and relatively small (all with a maximum inundatable surface area of c. 0.15 hectares), with dense riparian vegetation. The flooding time between lakes is uniform because the connection between the lakes and the main river channel occurs when the river rises above the 3.5 m level (Souza Filho et al. 2004). Flood events are responsible for the hatching of resting eggs after drought events in temporary lakes (Rosa et al. 2021). The characteristics of water, sediment and emergent vegetation cover are similar and uniform amongst the lakes. The sediment of the lakes is composed basically of very fine sand and little organic matter. The water is slightly acid, with low oxygen saturation (< 50%) and relatively high concentrations of dissolved salts (> 50 $\mu S.cm^{-1}$).

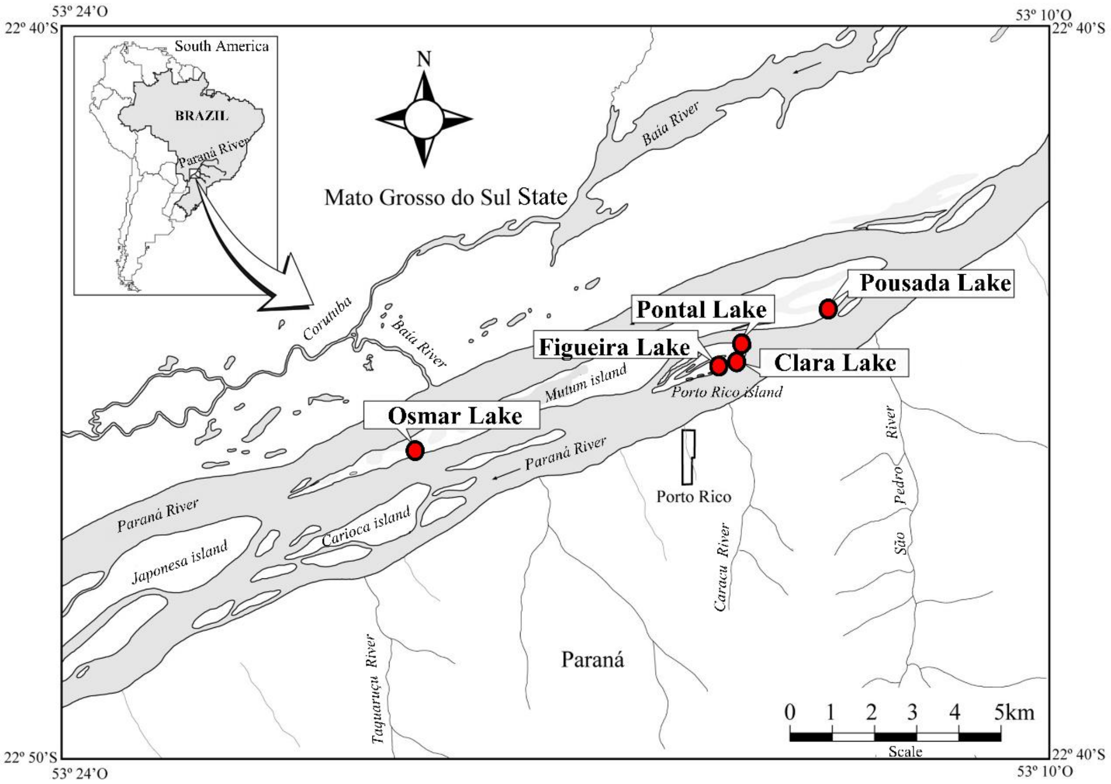

Figure 1. Location of the sampled temporary lakes in the Upper Paraná River floodplain (Paraná, Brazil).

2.2.1 Sampling and hatching procedures

Sediments were collected at the edge and the centre of the temporary lakes during a dry season (September 2017). Twelve samples were collected at the edge of each lake, while five samples were collected in the lakes' centre, with an acrylic core sampler (194.5 cm^3 volume). Each sample contained a layer of approximately 6 cm of moist/dry sediment. These sediments from each lake and region (edge and centre) were separately pooled to form two composite samples per lake, totalling ten samples from the five lakes (five samples from the edge and five samples from the centre). The sediment was stored in dark plastic bottles to reduce contact with light and was kept refrigerated at 4 °C for two months to avoid the break of dormancy, following the methods described by Maia-Barbosa et al. (2003).

The experiment was carried out in the Laboratory of Macroinvertebrate Ecology at the Centre of Research in Limnology, Ichthyology and Aquaculture (Nupélia) of the State University of Maringá (Brazil). For the hatching experiments, 300 g of sediment from each composite sample was individually oven-dried at 50 °C in plastic trays (1 L) for 24 hours to trigger the hatching (Rosa et al. 2021). Each dry sediment sample was subsequently inundated with 500 ml of distilled water (after cooling down to room temperature) and was kept in its microcosm at 25 °C (Rossi et al. 2004) for 98 days until a marked reduction in hatching was observed. The photoperiods were maintained at 12 hours light/12 hours dark (Rossi et al. 2012).

Samples were checked at weekly intervals to avoid that specimens would reach sexual maturation, which could lead to reproduction between individuals. Every seven days, the water from the artificial microcosm was filtered by a plankton net (68 µm) (Rosa et al. 2021). Afterwards, fresh distilled water was added to the microcosm. The filtered material, retained in the net, was sorted under a stereoscope microscope (Olympus SZX16). Hatched juveniles were kept individually in glass bottles with distilled water and were fed with fresh shredded spinach and reared to the adult stage in their separate chambers for identification and counting. The general survival rate of hatched juveniles was around 85%. The adult ostracods were identified down to species level, following illustrations in Higuti et al. (2013) and subsequent papers, and papers referenced in Martens & Behen (1994).

2.2.2 Ostracod traits

Ostracods were classified according to the following functional traits: body size, presence/absence of pointed/ rounded external structures, general body morphology and reproductive mode. The body size classification was established using measures of length

(L) and height (H) of the ostracod adult carapace. These measurements were obtained using scanning electron microscopy (SEM) images and/or stereoscope microscope. The species were categorized as small ($L \leq 0.54$ mm and/or $H \leq 0.32$ mm), medium ($0.55 \leq L \leq 1.32$ mm and/or $0.33 \leq H \leq 0.72$ mm) and large ($L > 1.32$ mm and/or $H > 0.72$ mm) (Matsuda et al. 2015). Pointed or rounded external structures were classified as "present" or "absent", and body morphology as "laterally flattened" (as "flat") and "rounded" in dorsal view, according to visual observations of the ostracod carapace in a stereoscope microscope and SEM. Finally, reproductive mode was differentiated in asexual, sexual, and mixed reproduction, according to Meisch (2000). All these traits were selected owing to their importance in life history characteristics (e.g., body size, Matsuda et al. 2015), dispersal limitation (e.g., body morphology, Matsuda et al. 2015; De Campos et al. 2018), defence against predators (e.g., presence of external structures) and dispersal and population establishment (e.g., reproductive mode, Horne et al. 1998) of ostracod species.

2.2.3 Data analyses

A principal coordinate analysis (PCoA) was used to summarise the species composition (taxonomic) and the functional traits of ostracods over the weeks of monitoring. Taxonomic dissimilarity was based on a matrix with data on the presence and absence of the ostracods, which had hatched weekly, using the Jaccard distance (Legendre & Legendre 1998). For functional composition, we first used the modified Gower distance on the species-traits matrix (Pavoine et al. 2009). Then, we calculated the functional dissimilarity based on equation 12 from Pavoine and Ricotta (2014). After running PCoA, we created three groups (or periods) according to the time of hatching. The first (early) group included the species hatched between the 1st and the 5th week (the early period of the experiment), the second (middle) group of the species which hatched between the 6th and 10th week (middle period of the experiment) and the third (late) group included the species hatched between the 11th and 14th week (late period of the experiment).

We calculated the community-weighted mean values (CWM) of each trait in each week to determine the dominant trait categories in each period (early, middle and late). The CWM values were assessed using the species trait and occurrence data. The absolute abundance of species was transformed into relative abundance and calculated using the "functcomp" function implemented in the FD R-package. The CWM values of communities may change according to the abundance of species (Ricotta & Morreti 2011). Therefore, we performed a non-parametric Kruskal-Wallis test to check the changes over the three periods.

A "Multivariate Permutational Variance Analysis" (PERMANOVA – using the function "adonis" from the vegan package; Anderson 2001) was performed to evaluate differences in the taxonomic and functional composition of the weekly hatched ostracod cohorts in the three groups. The test was based on a dissimilarity matrix using the Jaccard distance. A total of 999 permutations were performed to assess the significance of differences. We also performed a "Permutational Analysis of Multivariate Dispersion" (PERMDISP – using the "betadisper" function in the vegan package) to estimate the multivariate dispersion of taxonomic and functional composition of the five lakes in each of the weekly cohorts. Then, we performed Pearson's correlations between the mean distance to centroid values PERMDISP (i.e., amongst the five lakes in each of the week cohorts) and incubation time (weeks), separately for taxonomic and functional composition.

Finally, we tested the differences in hatching time amongst the ostracod species using a Generalized Linear Model (GLM) with Poisson distribution and log link function to account for overdispersion (Crawley 2007). After that, Tukey's post-hoc test was performed for pairwise comparisons of hatching phenologies between the ostracod species.

Statistical analyses were carried out with software R 3.2.4 (R Development Core Team 2013), using the lme4 (Bates et al. 2015), multicomp (Hothorn et al. 2008), vegan (Oksanen et al. 2018), picante (Kembel et al. 2010) and permute (Simpson 2018) R packages.

2.3 Results

A total of 12 species of ostracods hatched from the sediments of temporary lakes, represented by 11 species of Cyprididae and one of Candonidae (Table 1). No ostracods hatched in the 1st week of the experiment. The hatching of *Chlamydotheca colombiensis* Roessler, 1985 and *Chlamydotheca iheringi* (Sars, 1901) started in the 2nd week of incubation, and the highest numbers of individuals of these species were recorded in the 3rd and 5th weeks, respectively (Figure 2A). *Strandesia bicuspis* (Claus, 1982) and *Strandesia velhoi* Higuti & Martens, 2013 also hatched from the 2nd week onwards, whereas *Strandesia lansactohai* Higuti & Martens, 2013, *Strandesia nupelia* Higuti & Martens, 2013 and *Strandesia mutica* (Sars, 1901) started hatching from the 3rd week onwards. *Strandesia variegata* (Sars, 1901) only started hatching from the 10th week of incubation onwards. Considering the number of hatched specimens, we could clearly see two groups in the genus *Strandesia*: *S. bicuspis*, *S. lansactohai,* and *S. nupelia* had a higher abundance in the early and middle periods (between the 2nd and 6th weeks), while a higher number of hatchings of *S. mutica*, *S. variegata*, and *S. velhoi* were recorded in middle and late periods (between the 9th and 12th weeks). In general, hatching decreased over time with a sharp reduction of individuals in the 14th week (Figure 2B)

The 1st hatching of *Physocypria schubarti* Farkas, 1958, the only species of the Candonidae, was observed in the 2nd week, and the highest abundance occurred in the 7th week (Figure 2C). Only one specimen of *Bradleytriebella trispinosa* (Pinto & Purper, 1965), *Cypridopsis vidua* (O. F. Müller, 1776), and *Cypricercus alfredo* Almeida et al. 2021 each was recorded in the 5th, 9th, and the 12th week, respectively (Table 1).

Table 1. Ostracod species and functional traits recorded during the incubation time (14 weeks) in the present experiment.

Ostracod species	Body Size	External structures	Body morphology	Reproduction	Hatching (weeks)
Cyprididae					
Bradleytriebella trispinosa (Pinto & Purper, 1965)	medium	present	rounded	Assexual	5
Chlamydotheca iheringi (Sars, 1901)	Large	present	rounded	Mixed	2, 4, 5, 6, 7, 8
Chlamydotheca colombiensis Roessler, 1985	Large	absent	rounded	Assexual	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
Cypricercus alfredo Almeida et al. 2021	medium	present	rounded	Mixed	12
Cypridopsis vidua (O. F. Müller, 1776)	Small	absent	rounded	Asexual	9
Strandesia bicuspis (Claus, 1982)	Large	present	rounded	Assexual	2, 3, 4, 5, 6
Strandesia lansactohai Higuti & Martens, 2013	medium	absent	rounded	Assexual	3, 4, 6, 7, 8
Strandesia mutica (Sars, 1901)	Large	absent	rounded	Assexual	3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
Strandesia nupelia Higuti & Martens, 2013	medium	absent	rounded	Assexual	3, 4, 5
Strandesia variegata (Sars, 1901)	Large	absent	rounded	Assexual	10, 11, 12, 13, 14
Strandesia velhoi Higuti & Martens, 2013	medium	absent	rounded	Assexual	2, 5, 6, 8, 9, 10, 11, 12, 13, 14
Candonidae					
Physocypria schubarti Farkas, 1958	Small	absent	flat	Sexual	2, 4, 7, 8, 9, 10

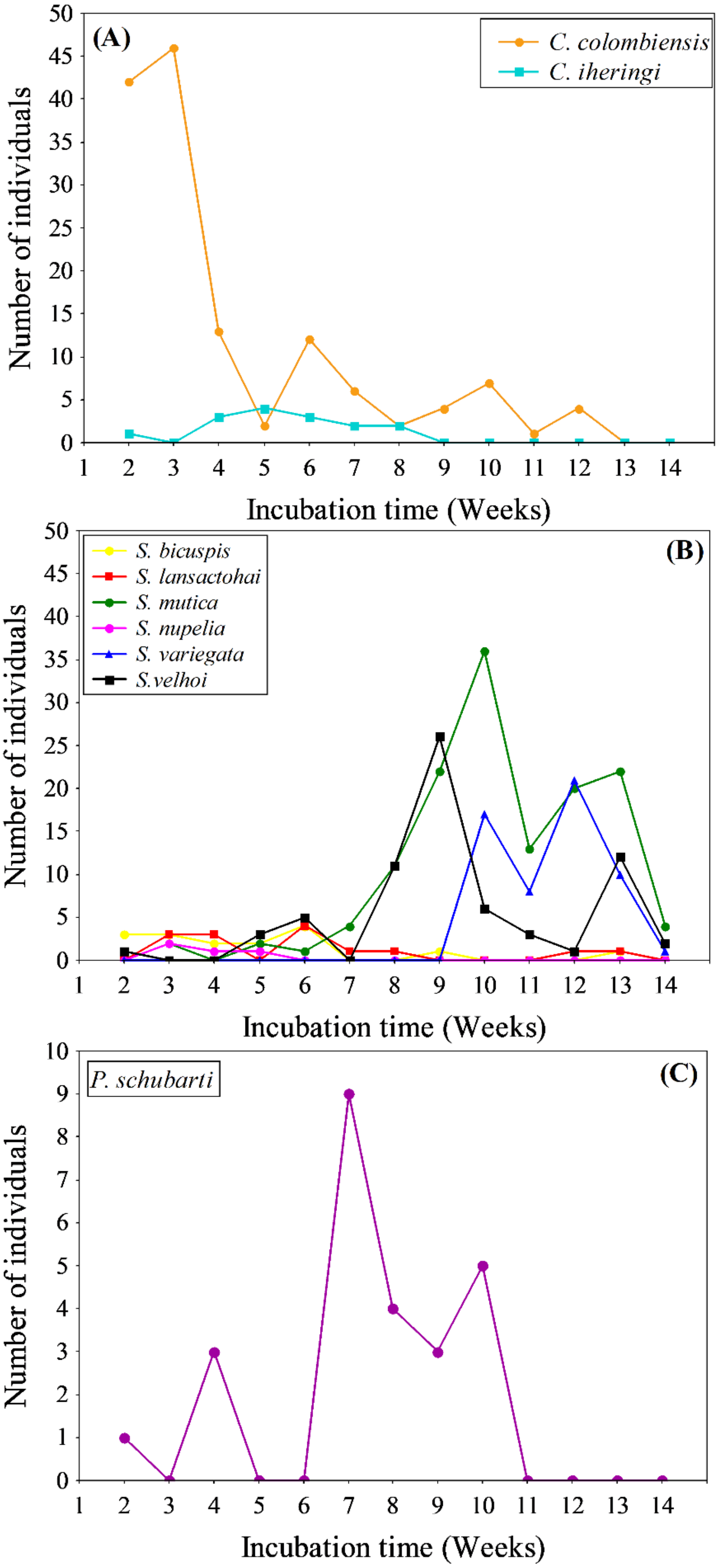

Figure 2. Number of eggs hatched of the ostracod species during the incubation time (14 weeks). (A) *Chlamydotheca* species; (B) *Strandesia* species; (C) *Physocypria schubarti.*

Three distinct periods (early, middle and late periods) were observed over time. The 1st peak occurred in the early period, mostly consisting of hatchlings of *C. colombiensis*. The 2nd peak was recorded at the end of the middle period and the beginning of the late period and mostly consisted of hatchlings of *S. velhoi* and *S. mutica*. The 3rd peak occurred in the late period and was mainly formed by *S. variegata* and *S. mutica* hatchlings (Figure 2A).

Considering the functional traits of the ostracod species, all types of traits in all categories (body sizes, presence or absence of external carapace structures, body shapes and reproduction modes) were already present during the 2nd week of incubation, i.e. at the beginning of the early period (Table 1). The species with medium and large body sizes were recorded from the 2nd to the 14th week of monitoring (i.e., virtually the duration of the entire incubation time of the experiment), while the small-sized species hatched up to the 10th week only. In the last two weeks of the incubation experiment (13th and 14th week), only ostracod species without external carapace structures hatched. Species with rounded body morphology were observed during the entire incubation time of the experiment, while the flat species only hatched until the 10th week of incubation. Finally, species with asexual (parthenogenetic) and mixed reproduction were recorded during the entire incubation time of the experiment, whereas the single species with sexual reproduction was recorded only until the 10th week. However, the latter also coincides with the fact that this is one of the small species (*Physocypria schubarti*).

We observed similar effects of most functional traits on the phenology of the different species, and these were to some degree also linked to the three hatching peaks described above (the early, middle and late periods). The highest CWM trait values of the pointed or rounded external carapace structures and mixed reproduction were observed in the early period, while the CWM values of medium and small size, flat carapaces and sexual reproduction were highest in the middle period. Lastly, the highest CWM trait values of large size, absence of the pointed or rounded external carapace structures, rounded carapaces and asexual reproduction were (mostly) recorded in the late period (Figure 3). The CWM trait values changed over the incubation time (p <0.05). However, significant differences were observed only for the presence of external carapace structures (H= 5.78; p = 0.04) and asexual reproduction (H = 6.75; p = 0.03), with a reduction in the abundance of species with external carapace structures and an increase in asexual reproduction over time.

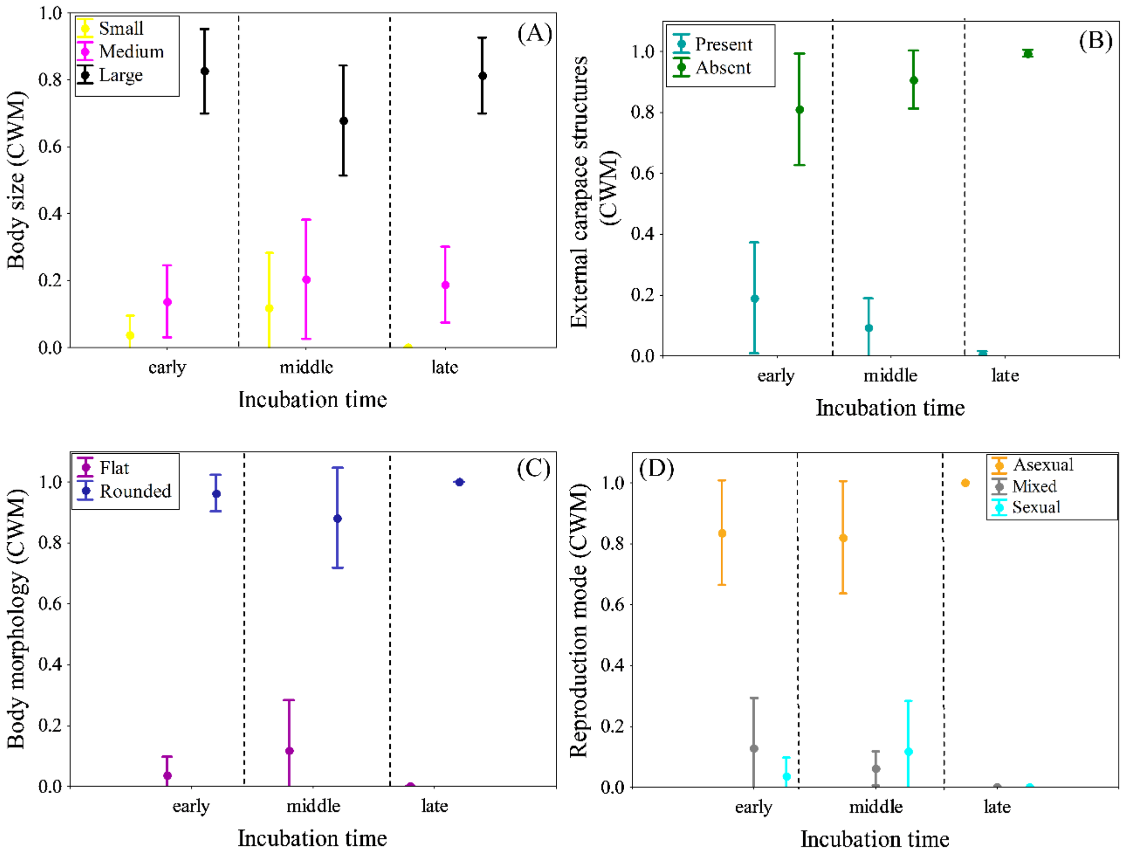

Figure 3. Community-weighted mean trait values (CWM) of the trait categories. (A) body size; (B) presence/absence of pointed or rounded external structures; (C) body morphology and (D) reproduction mode.

The results of the PCoA showed, in general, three distinct periods (early, middle and late periods) based on species compositions over time. The species composition changed significantly over the 14 weeks of incubation (PERMANOVA: $p<0.05$). The species composition of the early and middle periods differed significantly from that of the late period (Figure 4A; Table 2). Although less pronounced than the effects of the taxonomic hierarchy, the functional composition also significantly changed over these periods (PERMANOVA: $p<0.05$). The functional trait composition of the ostracod species was significantly different between the middle and the late periods of the experiment (Figure 4B; Table 2).

Table 2. Pairwise PERMANOVA of the taxonomic composition and functional traits of the hatched ostracod community during the hatching period. Bold p-values indicate significance at $p < 0.05$.

	Taxonomic	**Functional**
Early x Middle	0.412	0.647
Early x Late	**0.006**	0.203
Middle x Late	**0.046**	**0.048**

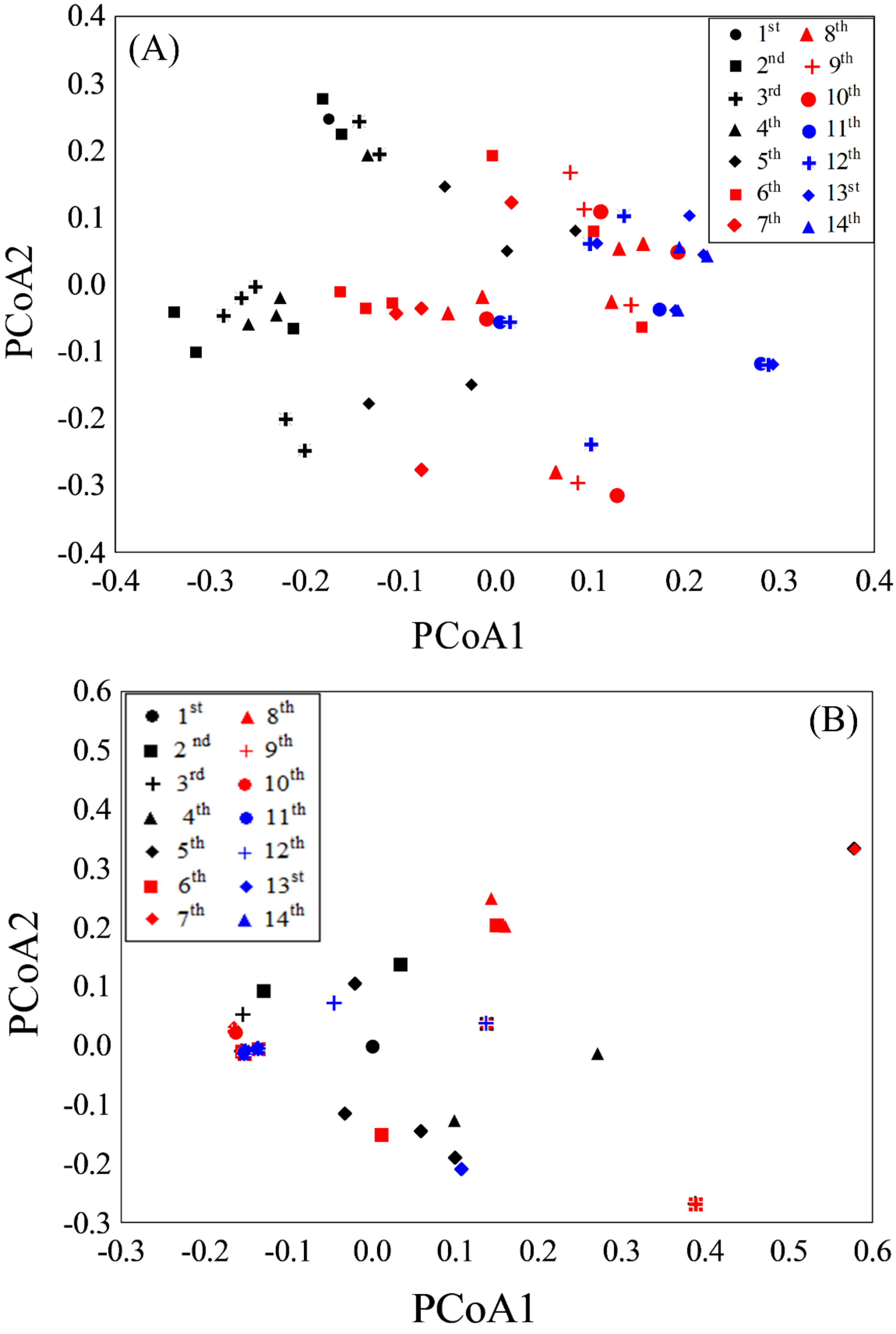

Figure 4. Principal coordinate analysis showing the ostracod species composition (A) and the functional traits composition of ostracod species (B) during the incubation time. The black symbols represent the early period (1st–5th week), the red symbols indicate the middle period (6th–10th week), and the blue symbols the late period (11th–14th).

Based on the results of PERMDISP, the variability of the species composition was constant during the three periods of the experiment (Figures 5A, B). Consequently, the variability values were not correlated with the incubation time (weeks) (r= 0.31; p= 0.29). On the other hand, lower variability of the functional traits was observed in the late period when compared to the early period (Figures 5C, D). In addition, the functional variability values were significantly correlated with the time of incubation, as measured in weeks (r= -0.71; p= <0.01).

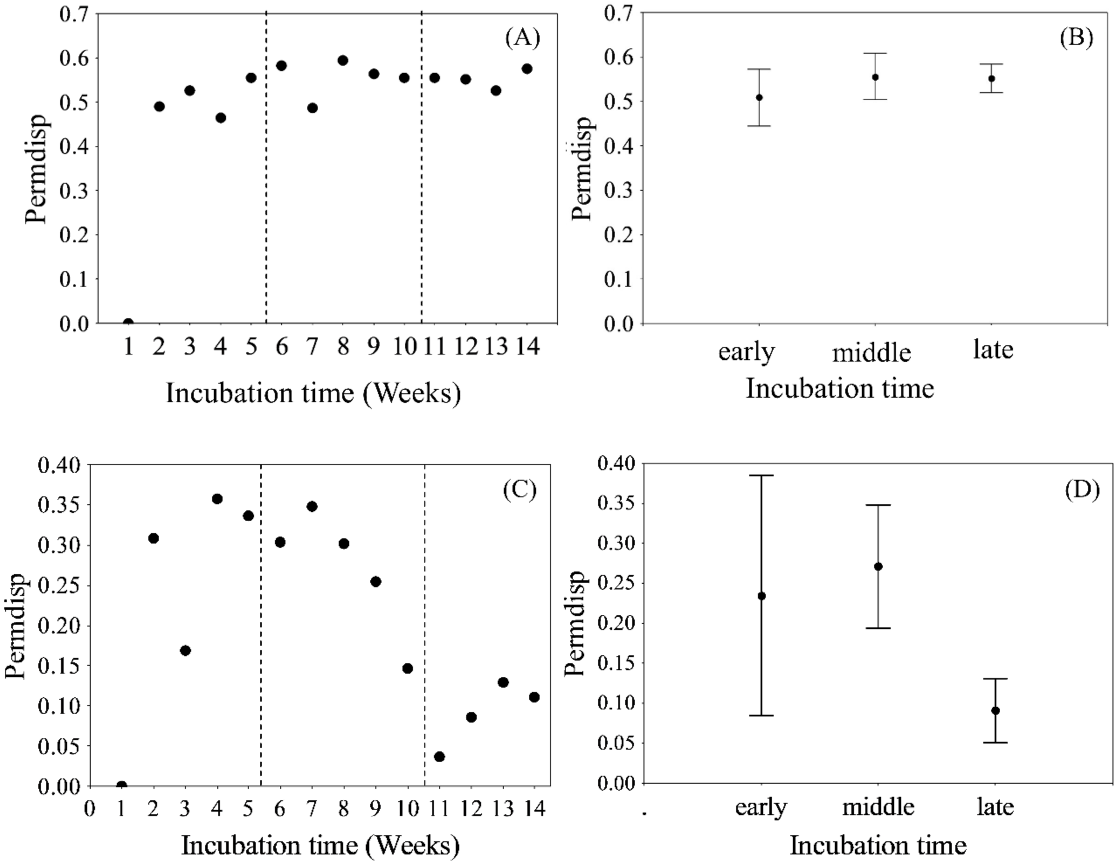

Figure 5. Mean distance of the sample units to the centroid evaluated through the dispersion homogeneity test (PERMDISP). (A) species composition in each week of incubation; (B) mean and standard error of the species composition in each of the three periods; (C) functional traits composition in each week of incubation; (D) mean and standard error of the functional trait composition in each of the three periods.

We summarize the phenology of species and functional traits of ostracods in a conceptual model (Figure 6). Most species hatched from the 2nd week onwards, with a lag period of three weeks for three species of *Strandesia* (*S. lansactohai*, *S. mutica* and *S. nupelia*) and ten weeks for *S. variegata*. The hatching of the congeneric *Chlamydotheca* species started from the 2nd week. However, the hatching time of *C. colombiensis* extended to 12 weeks, whereas that of *C. iheringi* already finished between the 8th and the 9th week. For the congeneric *Strandesia* species, the hatching time was mostly long, except for *S. variegata* and *S. nupelia*. *Physocypria schubarti* hatched over a nine-week period (Figure 6).

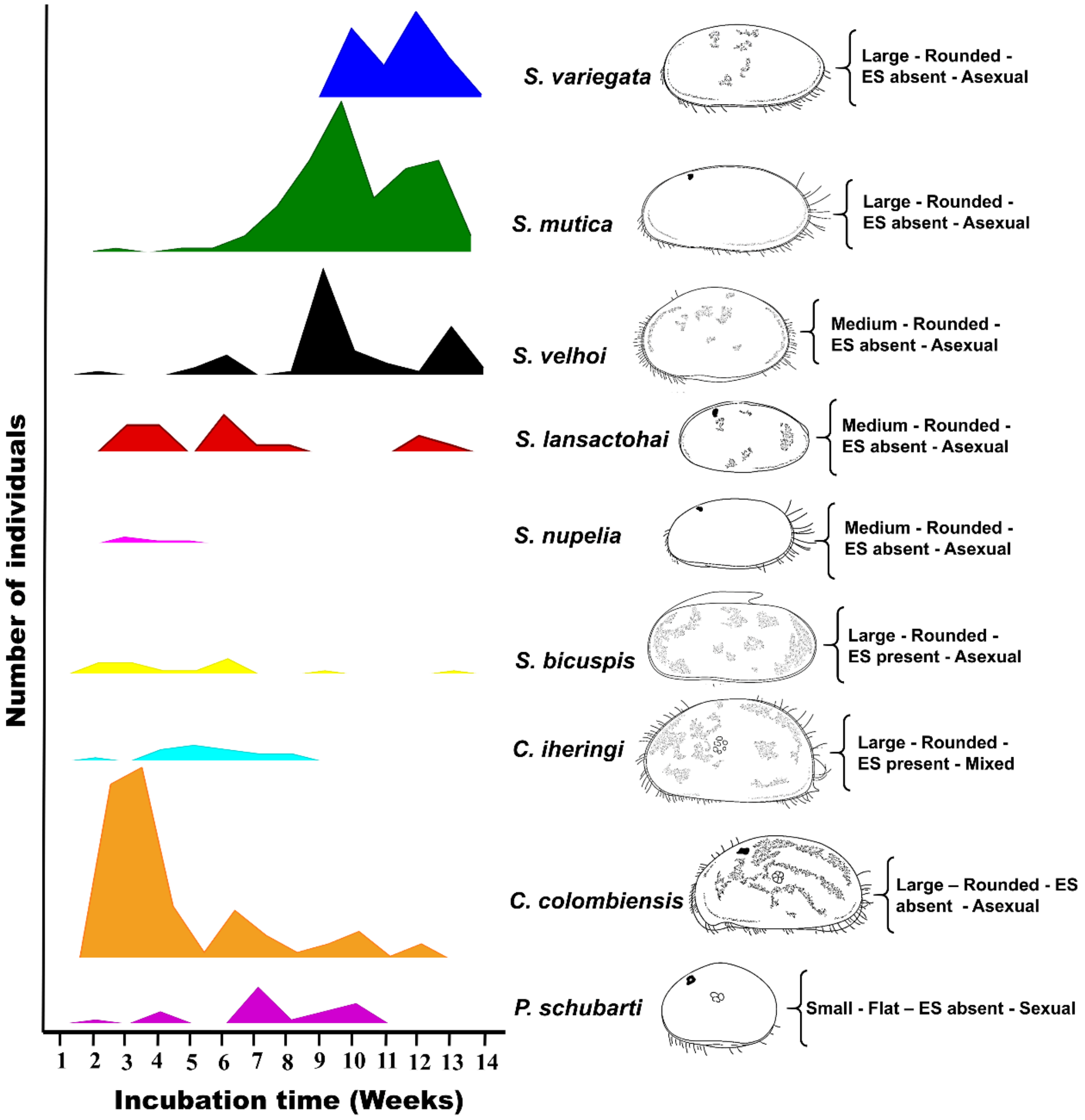

Figure 6. Model illustrating the hatching phenology of ostracod species found during the present experiment and their respective functional traits during the incubation time. ES = external structures.

2.3.1 Hatching time of congeneric versus non-congeneric species

The Generalized Linear Model results (p <0.05) on *Chlamydotheca* species showed that these congeneric species have similar phenology. Within the genus *Strandesia*, the pattern was different. *Strandesia mutica* and *S. variegata* had similar phenologies, but these were significantly different from *S. bicuspis*, *S. lansactohai* and *S.*

nupelia. In addition, the hatching time of *S. velhoi* was again different from those of *S. bicuspis*, *S. lansactohai*, *S. nupelia* and *S. variegata* (Table 3).

We also found significant differences between the non-congeneric species. The hatching time of both *Chlamydotheca* species was different from that of *Strandesia mutica*, *S. variegata* and *S. velhoi*. Finally, the hatching time of *Physocypria schubarti*, the only ostracod species belonging to the Candonidae, differed significantly from *C. colombiensis*, *S. lansactohai*, *S. mutica*, *S. variegata* and *S. velhoi* (Table 3).

Table 3. GLM, Tukey's post-hoc test between the hatching phenology of ostracod species during the present experiment. Bold p-values indicate significance at p <0.05.

Congeneric species	**p-value**	**Non-congeneric species**	**p-value**
C. colombiensis x *C. iheringi*	0.319	*C. colombiensis* x *S. bicuspis*	0.725
S. bicuspis x *S. lansactohai*	0.999	*C. colombiensis* x *S. lansactohai*	0.976
S. bicuspis x *S. mutica*	**< 0.001**	*C. colombiensis* x *S. mutica*	**< 0.001**
S. bicuspis x *S. nupelia*	0.970	*C. colombiensis* x *S. nupelia*	0.999
S. bicuspis x *S. variegata*	**< 0.001**	*C. colombiensis* x *S. variegata*	**< 0.001**
S. bicuspis x *S. velhoi*	**< 0.001**	*C. colombiensis* x *S. velhoi*	**< 0.001**
S. lansactohai x *S. mutica*	**< 0.001**	*C. colombiensis* x *P. schubarti*	**< 0.001**
S. lansactohai x *S. nupelia*	0.995	*C. iheringi* x *S. bicuspis*	0.999
S. lansactohai x *S. variegata*	**< 0.001**	*C. iheringi* x *S. lansactohai*	0.968
S. lansactohai x *S. velhoi*	**< 0.001**	*C. iheringi* x *S. mutica*	**< 0.001**
S. mutica x *S. nupelia*	**0.002**	*C. iheringi* x *S. nupelia*	0.896
S. mutica x *S. variegata*	0.386	*C. iheringi* x *S. variegata*	**< 0.001**
S. mutica x *S. velhoi*	0.466	*C. iheringi* x *S. velhoi*	**< 0.001**
S. nupelia x *S. variegata*	**< 0.001**	*C. iheringi* x *P. schubarti*	0.491
S. nupelia x *S. velhoi*	**< 0.001**	*P. schubarti* x *S. bicuspis*	0.124
S. variegata x *S. velhoi*	**0.008**	*P. schubarti* x *S. lansactohai*	**0.009**
		P. schubarti x *S. mutica*	**< 0.001**
		P. schubarti x *S. nupelia*	0.244
		P. schubarti x *S. variegata*	**< 0.001**
		P. schubarti x *S. velhoi*	**0.014**

2.4 Discussion

2.4.1 Hatching phenology of resting eggs

The taxonomic proximity at the genus level (congeneric species) can be a factor linked to the similarity of hatching time between *Chlamydotheca colombiensis* and *C. iheringi*. However, it seems that some species have specific phenologies, regardless of taxonomic proximity or environmental triggers, since several species belonging to the same genus *Strandesia* had different phenologies, which might be attributed to the distinct functional traits, which supports our first and second hypotheses (see discussion below on Functional Traits). Different hatching times amongst ostracod species of the same genus facilitates the coexistence in temporary lakes over time and can have an important effect on the structure and dynamics of populations, and hence also of ecosystems. Temporal segregations of life history can occur between taxonomically closely related species since there is a limit to the niche similarity between competitive species (MacArthur & Levins 1967).

Similarities between hatching patterns have previously been observed for ostracods. Most species hatch in the first weeks of incubation, specifically from the 2nd week onwards (Stenert et al. 2010; Ávila et al. 2015; Bandeira et al. 2020). For instance, the hatching of the *Chlamydotheca* species in our experiment (*C. colombiensis* and *C. iheringi*) as well as in rice fields (*Chlamydotheca incisa* (Claus, 1892)) from southern Brazil (Stenert et al. 2010), were observed in the 2nd week of incubation. While we found different hatching phenologies amongst *Strandesia* species in our experiment (see Figure 6), such changes in hatching time were also noted by Ávila et al. (2015) and Bandeira et al. (2020) for *Strandesia bicuspis*, *Strandesia* sp. and *Strandesia obtusata* (Sars, 1901). However, half of the *Strandesia* species which hatched in our experiment was only recently described by Higuti & Martens (in Higuti et al. 2013), and no information on the hatching phenology of these species can therefore be found in the literature.

Our results largely agree with those of Ávila et al. (2015), who also observed delays in the hatching of some ostracod species. Different hatching times of ostracod species can lead to different species compositions in communities over time. Our results support this since most of our species hatched in the 2nd week after incubation, while others hatched several weeks later. This supports our third hypothesis. In addition, the results indicate that the egg banks guarantee ecological buffers for future drought events or changes in environmental conditions. These changes in species composition over time can also occur in egg banks of other aquatic taxa (e.g. Anostraca, cladocerans and rotifers) since desynchronized hatching is common in such communities (Hall 1959; Vanoverbeke & De Meester 2009; Tarzona et al. 2017).

The time required for the hatching of resting eggs can be affected by several environmental triggers (Chaki et al. 2021a; 2021b), such as flooding, local limnological variables (i.e., temperature) (Brendonck & Riddoch 2001), lake characteristics (i.e. depth) (Chaki et al. 2021a) or biological (fish) kairomones (Lass et al. 2005). However, the exact mechanisms that control the diapause of ostracod resting eggs after drought periods remain poorly known (Özolug & Suludere 2016). It is important to highlight that temperature, light, and hydration protocols were controlled during our experiment to avoid the influence these factors can have on ostracod hatching times (Brendonck & De Meester 2003; Iglesias et al. 2016; Bandeira et al. 2020).

Maternal effects can also influence hatching times since the phenology of eggs can be influenced by factors experienced by the mother specimens (Rossi et al., 2016). The environment where the mothers lived, as well as maternal age, can determine, or at least influence, conditions under which resting eggs will hatch and can have an anticipatory effect on the hatching time. For example, the older the mothers were while depositing the eggs, the shorter the dormancy period (Rossi et al., 2016). Resting eggs in egg banks might thus belong to different generations in the temporary lakes, and maternal effects were therefore not controlled in our experiment. In addition, although we did not test the potential effect of bet-hedging in our experiment, this strategy can influence the ostracod species from the egg bank since some eggs may not have hatched at the first hydration.

2.4.2 Functional traits

The fact that congeneric species showing different hatching times can have different functional traits corroborates our second hypothesis to some extend. For instance, the congeneric species, *S. lansactohai* and *S. nupelia*, both have a medium body size and still showed differences in the phenology of resting eggs compared to *S. mutica* and *S. variegata* which have a large body size. Our results suggest that the hatching time of resting eggs can be related to functional traits of ostracod species but that the link between such traits and phenology remains poorly understood. The relationship between functional traits and hatching time has already been shown for two congeneric species of mantids (Mantidae), in which divergent hatching phenology was attributed to different body sizes and reproductive strategies of the species (Hurd 1988).

The highest CWM values for specimens with pointed or rounded external structures prevailed in the early and middle periods of the experiment, possibly as a strategy of defence/protection against predators (Barnhisel 1991). The bivalve carapace of ostracods protects against predation, although it can still occur without destroying the valves. Hayashi & Ohba (2018) showed that laterally flattened ostracod species and those without spines seem to be preferred by larvae of predatory diving beetles (see Hayashi & Ohba, 2018 - video in supplementary material). The presence of pointed or rounded external structures (helmets, spines) is common in ostracods and important for the identification of species (Higuti & Martens 2020). These structures can differ from instar to instar during the life cycle of organisms and are generally more evident in adults (Kesling 1954), although there are several exceptions in which juvenile stages can have more pronounced external valve ornamentation, for example, in species of the Australian genus *Bennelongia* De Deckker & McKenzie, 1981 (De Deckker & Martens, 2013). The variation in size and number of spines during the development stages (generally nine instars in Cyprididae) can also be a survival strategy of these microcrustaceans. Some ostracod species recorded in our study have spines since the juvenile stages (several small spines on the posterior parts of the valves) until the adult stage, which shows a single spine on the right posterior valve such as *C. iheringi*. *Cypricercus alfredo*, on the other hand, develops a single spine only in the adult stage. The absence of spines in the juvenile stages of *C. alfredo* can be a reason for its delayed hatching (12th week) since these organisms would be more vulnerable to predation during the juvenile stages.

According to Schmit et al. (2013), an increase of sexual populations of ostracods is recorded in environments with short and unpredictable hydroperiods, while the dominance of parthenogenetic populations is observed in environments with a longer and more predictable hydroperiod. This is partly corroborated by our findings since the number of parthenogenetic species increases after the stabilization of the flood (late incubation time).

The general absence of hatching of small-sized ostracods during the late period of the experiment may be because these microcrustaceans produce a lower number of smaller eggs (as was demonstrated in, for example, copepods - Hopkins 1977). In addition, smaller eggs can develop more rapidly. Thus, egg banks of small species tend to hatch quickly in the early period of the incubation. Bandeira et al. (2020) have supported a pattern that recorded a small ostracod species, *Cypridopsis vidua*, in the early period of the hydration time.

The change in functional traits composition over the three periods was observed experimentally in the laboratory and resulted from the succession of ostracod species over time. The higher variability of traits composition in the experiment's early and middle periods is due to the unique traits of *Physocypria schubarti*, which combines exceptional character states of three different traits: sexual reproduction, flat body morphology, and small body size. As resting eggs of this species stopped hatching in the 10th week of incubation, this significantly reduced the variability of the functional traits in the community as a whole. However, the size of the egg bank (number of eggs stored) can also influence the variability of the traits over the weeks, as the eggs of some species may have all hatched after several weeks of incubation (Rossi et al. 2016).

2.5 Conclusion

Our results have shown that taxonomic distance and functional traits can affect the hatching phenology of ostracod resting eggs from temporary floodplain lakes. Different hatching phenologies of resting eggs between congeneric species was presumed to be linked to the presence of different functional traits. The different hatching times of species alter the ostracod composition of the community, which can reduce competition between species with similar niches. This can contribute to the maintenance of higher overall specific biodiversity levels in a temporary habitat.

The grouping of organisms based on functional traits allows for higher predictability of the structure of communities when compared to the taxonomic distance approach. Therefore, future studies addressing the hatching phenology of ostracod resting eggs (as well as other invertebrates) should also include functional traits.

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3. DRIED AQUATIC MACROPHYTES ARE FLOATING EGG BANKS AND POTENTIAL DISPERSAL VECTORS OF OSTRACODS (CRUSTACEA) FROM PLEUSTON COMMUNITIES

Abstract: In aquatic ecosystems, such as Neotropical floodplains, it is common to find dried aquatic macrophytes along the margins of various environments (e.g. lakes and rivers) during the dry season. *Eichhornia crassipes* (Mart.) Solms is one of the most abundant floating plants in the Upper Paraná River floodplain (Brazil) and harbours a high diversity of ostracods in the pleuston of its root system. We evaluate the potential of dried *Eichhornia crassipes,* as a dispersal vector of ostracod resting eggs, by assessing the abundance, richness and beta diversity of the dormant associated fauna. Dried *E. crassipes* from floodplain lakes were hydrated with distilled water. The microcosms were kept in germinating chambers with controlled temperature and photoperiod during 147 days. A total of 397 ostracods representing seven species hatched from the resting eggs attached to dried macrophyte roots. An increase in richness and a decrease in abundance were observed over the weeks, although these trends were not significant. However, the beta diversity increased significantly over the incubation time. Our results show that the complex root systems of *E. crassipes* have the potential for storage and transport of ostracod resting eggs. The increase of ostracod diversity from hatching of resting eggs can contribute to the recolonization of the floodplain environments and to wide dispersal after drought periods. In addition, the dispersal of ostracod resting eggs by dried roots can facilitate the process of invasion, since this plant can transport dormancy structures of exotic species.

Keywords: Microcrustacean, Egg banks, Diversity, Exotic species, Floodplain.

3.1 Introduction

The complex root system of *Eichhornia crassipes* (Mart.) Solms shelters a high diversity of aquatic invertebrates, and the free-floating life form facilitates the dispersal of invertebrates associated with the roots (hypopleuston) through the flow of watercourses (Higuti et al., 2007; Villamagna & Murphy, 2010). *Eichhornia crassipes* is native to South America, but is considered an invasive species in over 50 countries (Villamagna & Murphy, 2010). This macrophyte species may have negative impacts on the environment, acting itself as vector of invasive species (Villamagna & Murphy, 2010), although in other cases it acts as successful substrate for native species (Higuti & Martens, 2016).

Aquatic macrophytes are important for ostracods, which use these substrates for reproduction, feeding and protection against predators (Matsuda et al., 2015; Higuti et al., 2017; De Campos et al., 2021). These small crustaceans produce resting eggs to survive drought-events (Rosa et al., 2020; Rosa et al., 2021a; 2021b), forming an egg bank that remains viable for many years. As such, they provide a mechanism for the long-term maintenance of populations in aquatic habitats and contribute to the diversity maintenance and restoration of ecosystems after disturbance (Brock et al., 2003). Ostracod resting eggs are double-walled spheroids of chitin and calcium carbonate, separated by a fluid, which allows it to withstand desiccation (Smith et al., 2015). Such characteristics of resting eggs, also those produced by several other crustacean groups, allow high passive dispersal over long-distance by several vectors, such as wind (Moreno et al., 2016), flowing water (Rosa et al., 2020), water birds (Morais Junior et al., 2019), aquatic macrophytes (Battauz et al., 2017), mammals (Waterkeyn et al., 2010) and human activities (Valls et al., 2016).

Dispersal of aquatic invertebrates is an important process in the structuring of communities, species distribution in general and ecosystem functioning (Bilton et al., 2001). The dispersal potential of organisms also influences regional beta diversity since dispersal limitations can restrict species occurrence at different localities (Padial et al., 2014). Diversity can be measured at various levels of organization. One component of diversity proposed by Whittaker (1972) is beta diversity (β), defined as the variation in species composition amongst at least two sites. For example, species composition (beta diversity) of egg banks will change spatially amongst and within habitats, owing to different environmental characteristics, such as vegetation type (Freiry et al., 2020). In addition, beta diversity can also change temporally, because hatching time is both a species-specific factor and is also induced by environmental and biotic triggers, such as temperature, light intensity and functional traits (Bandeira et al., 2020; Rosa et al., 2021b).

In river-floodplain systems, the dispersal of ostracods increases during the flooding period owing to higher connectivity between the environments, e.g. between connected lakes and rivers (Sabattini & Lallana, 2007; Higuti et al., 2010; Pereira et al., 2017). For instance, several macrophytes (especially the free-floating life forms and their biota) from adjacent environments are introduced into the main river course by the water flow when water levels are decreasing, thus promoting the displacement and redistribution of macrophytes between the environments (Sabattini & Lallana, 2007). On the other hand, during the drought period, the environments will have lower connectivity as they are more isolated. Water levels will decrease and so some macrophytes (and their biota) will accumulate at the edge of the water bodies and will dry out. During flooding in the next rain season, these dried macrophytes might be rehydrated and be carried elsewhere by water flow. The drought resistant structures attached to the roots of the plants (e.g. eggs and seeds) can hatch in the same or in different environments. By means of this dispersal of macrophytes, organisms might (re-) colonize the (new) environments, thus contributing to both the maintenance and resilience of local biodiversity, and the increased diversity of different environments (Pereira et al., 2017). In addition, aquatic macrophytes will play an important role in the conservation, the viability and the richness of egg banks. When the dried macrophytes are covering the sediment, they protect these eggs from agents such as ultraviolet radiation (Eskinazi-Sant'Anna & Pace, 2018).

Here, we evaluated the potential of *E. crassipes* as dispersal vector of ostracod resting eggs by assessing the structure of the dormant assemblages (species richness, abundance and beta diversity) associated to dried macrophytes. We test the hypotheses (1) that the roots of *E. crassipes* shelter and disperse the dormant assemblages of ostracod (resting eggs), considering that ostracods in the pleuston use these roots as substrate to attach eggs (Matsuda et al., 2015) and (2) that the abundance, richness and beta diversity of dormant assemblages will increase over the incubation time after re-hydration, owing to different specific hatching times of ostracod species (Rosa et al., 2021b). Thus, such floating egg banks will increase dispersal over time and will contribute to the colonization of new environments after extreme drought periods.

3.2 Material and methods

3.2.1 Study area

The study was conducted in the Upper Paraná River floodplain (South Brazil), which encompasses an environmental protection area: "Area de Proteção Ambiental das Ilhas de Várzea do Rio Paraná" (Agostinho et al., 2004). It comprises a variety of environments, such as channels, rivers, connected and isolated lakes, and temporary lakes and ponds. The climate of the region is tropical-subtropical with distinct rainy and dry seasons (Cfa) (Eletrosul, 1986). The pluvial regime is characterized by a rainy season (November to March) and a dry season (April to October) (Agostinho et al., 2004).

We sampled in two lakes, Patos Lake (22°49’07.8”S/ 53°33’12.3”W) connected to the Ivinhema River and Guaraná Lake (22°43’18.1”S/ 53°18’08.5”W) connected to the Baía River in the Upper Paraná River floodplain (Figure 1A). During the collection, both lakes were shallow with depth of approximately 1.5 meters. The surrounding vegetation of these lakes is composed of mainly by Poaceae, *Polygonum* spp., *Eichhornia crassipes* and *Eichhornia azurea* (Schwartz) Kunth.

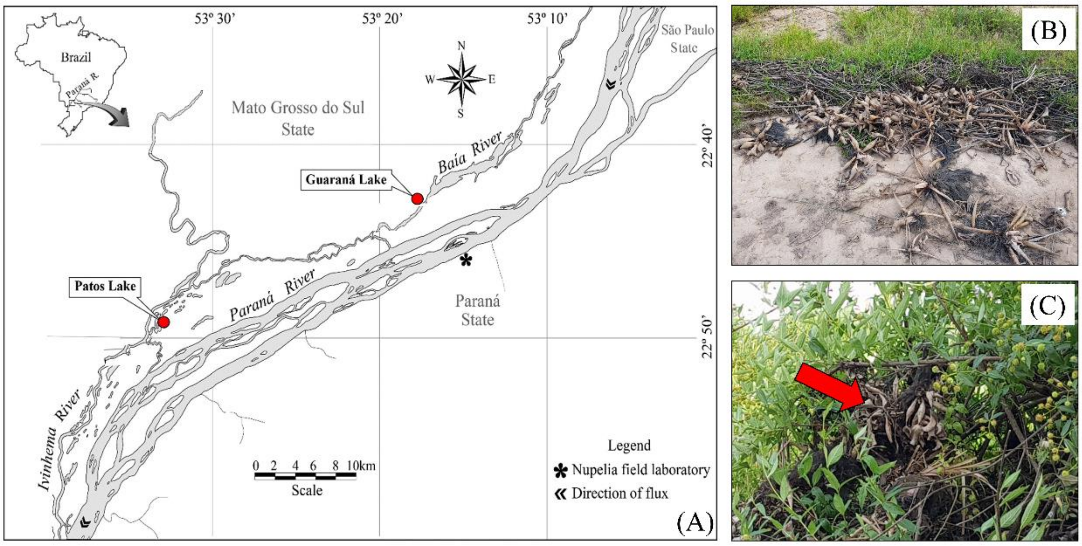

Figure 1. (A) Location of the Patos Lake and Guaraná Lake in the Upper Paraná River floodplain. (B) Dried *Eichhornia crassipes* on the sediment and (C) Dried *E. crassipes* hanging on the riparian vegetation, sometimes several m high. The arrow shows the dried macrophyte.

3.2.2 Sampling and hatching procedures

Dried roots of aquatic macrophytes were found only in two lakes from the Upper Paraná River floodplain. Dried *E. crassipes* were collected from the edge of both lakes, either from the sediment (Figure 1B) or hanging on the riparian vegetation (e.g. shrubs) (Figure 1C), at a distance c. 15 meters from the water body, in the dry season (September 2017).

We used only *E. crassipes* because the abundance and species composition of ostracod can differ among different macrophytes, and due the dominance of dried roots of this plant in both environments (Higuti et al., 2010; Matsuda et al., 2015). The leaves of E. *crassipes* have been discarded and a total of 500 g of roots were collected manually from the edge of each lake. The roots were stored in plastic bags in darkness to avoid the break of dormancy, following the methods described by Battauz et al. (2017). The experiment was conducted in November 2017.

For the hatching procedure in the laboratory, the roots from each lake (Patos and Guaraná lakes) were placed in individual plastic trays (two artificial microcosms). One sample of dried roots (500 g) from each lake was hydrated with 500 mL of distilled water and was maintained in the microcosm at 25°C (Rosa et al., 2020) for 147 days (21 weeks) in a germination chamber (Model SOLAB, SL.225) under a 12-h light-dark regimen.

The experiment described below followed the protocol of Rosa et al. (2020). Every seven days, the water from the microcosm was filtered using a plankton net (68 µm) and the ostracods were sorted under a stereomicroscope. The average life cycle of freshwater ostracods is c. three weeks (Meisch, 2000), so the seven-day interval is acceptable because ostracods do not reach sexual maturity for reproduction in that time. After the filtering and sorting, fresh distilled water and the material retained in the plankton net were added in the microcosms, in order to avoid the loss of eggs, since the eggs has a size >90 µm in diameter (Smith et al., 2015). was added in the microcosms. Hatched juveniles were separately kept in glass bottles with distilled water, fed with fresh shredded spinach until reared to the adult stage for identification and counting. The ostracod species were identified following Martens & Behen (1994), Higuti et al. (2010), Ferreira et al. (2020) and Higuti & Martens (2020).

3.2.3 Data analysis

We used the data from both lakes for the temporal analyses. A generalized linear mixed model (GLMM; Logan, 2010) was performed to determine the relationship between the incubation time (weeks) and the number of individuals (abundance) and species (richness), using a Poisson and Gaussian distribution, respectively. The GLMM was carried out using the abundance and richness as the response variables, and the incubation time (weeks) as the explanatory variable (fixed effect). We removed the spatial effect by adding the lakes as random variable (random effect). The data were checked for normality and homogeneity of variance to meet the conditions of multiple regression.

We also constructed rarefaction curves to determine the efficacy of sampling on the species richness during the incubation time, at comparable levels of numbers of individuals (Gotelli & Colwell, 2001). We used the Hill numbers through the function iNEXT (iNEXT package, Hsieh et al., 2016). The iNEXT package used Chao 2 (for incidence data or "reference sample") to estimate the number of undetected species. We used $q = 0$ to estimate species richness and the maximum extrapolated size was up to 100% of the reference sample size. According to Heck et al. (1975), establishing between 50% - 75% of the estimated richness might be satisfactory.

Finally, we calculated the beta diversity of the dormant assemblages of ostracod by the dissimilarities of species composition between the lakes, in each week over the 21-week period, using a Jaccard dissimilarity based on presence-absence. Beta diversity was calculated using the R-function vegdist, which allows for the use of a low number of samples. In addition, we evaluated the relationship between beta diversity and incubation time using a beta regression, because this is the most appropriate model for response variables ranging from 0 to 1. In the beta regression, the response variable was the beta diversity values, and the predictor variable was the incubation time (weeks).

All statistical analyses were performed with the environment R version 3.3.1 (R Development Core Team, 2013), using the “vegdist” function on “vegan” package for Jaccard dissimilarity, “*nlme*” packages for GLM analyses, and “*betareg*” package for the beta regression (Cribari-Neto & Zeileis, 2010).

3.3 Results

A total of 397 ostracods, from 42 samples (2 microcosms x 21 weeks), including the juveniles that died before the reached adulthood, hatched from the resting eggs attached to dried macrophyte roots. 224 individuals from Guaraná Lake and 173 individuals from Patos Lake. Seven ostracod species were recorded, all species belonging to the family Cyprididae. *Strandesia lansactohai* Higuti & Martens, 2013 was the most abundant species, representing 64% (254 individuals) of all ostracods which hatched in the microcosms (Table 1).

Table 1. Abundance of ostracod resting eggs hatched from dried roots of *Eichhornia crassipes* during the incubation time.

Family/ Subfamily	**Species**	**Guaraná Lake**	**Patos Lake**	**Total abundance**
Cyprididae				
Cypridinae	*Chlamydotheca iheringi* (Sars, 1901)		1	1
Cypridopsinae	*Brasilodopsis baiabonita* Almeida et al., 2021	10	6	16
Cypricercinae	*Diaphanocypris meridana* (Furtos, 1936)		8	8
	Strandesia lansactohai Higuti & Martens, 2013	104	149	253
	Strandesia mutica (Sars, 1901)	31	4	35
	Strandesia nakatanii Ferreira et al., 2020	1		1
	Strandesia variegata (Sars, 1901)	24		24
	Juveniles (died before reaching adult)	54	5	59

The first hatchings were recorded after 4th week of incubation. The higher abundance was observed in the 5th week, however the abundance decreased over the weeks (Figure 2A). The number of ostracod species increased over incubation time (Figure 2B). We did not observe a significant relationship between abundance and time (weeks) (GLMM: Z = 2.184, p = 0.075), nor between richness and time (GLMM: Z = 1.896, p = 0.058). However, the richness values of GLMM, shows a trend in the increase of richness over the weeks (Table 2).

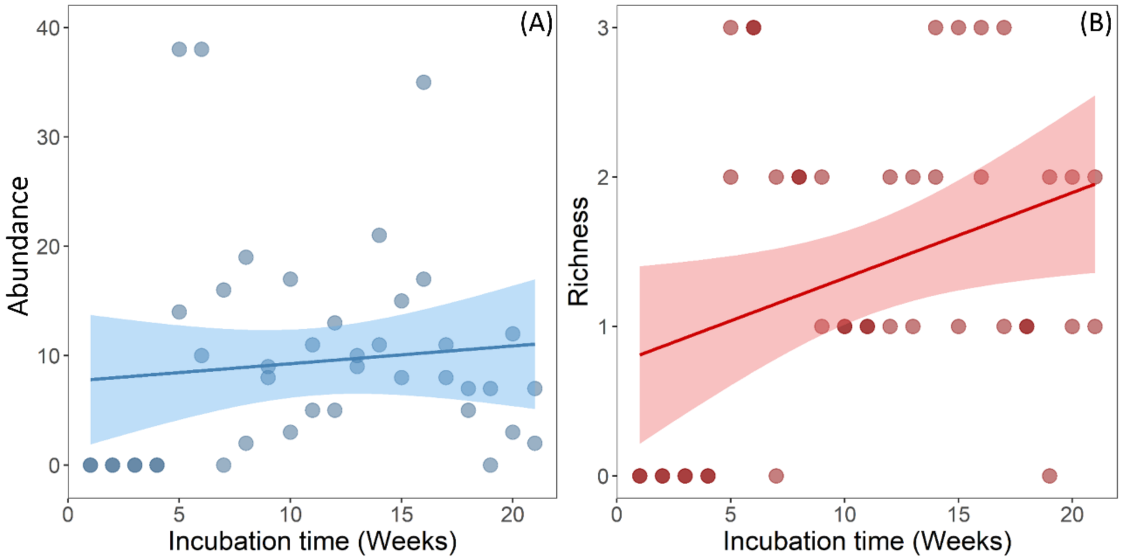

Figure 2. Linear models of (A) ostracod abundance and (B) species richness hatched from the dried roots of *Eichhornia crassipes* during the incubation time. The shaded bands represent the standard errors.

Table 2. Generalized linear mixed model (GLMM) results for the relationship between the ostracod abundance and richness from the dried roots and the incubation time (weeks).

Response variable	**Effects**	**Estimate**	**Standard error**	**df**	**Z-value**	**P-value**
Abundance	Intercept	0.984	0.787	39	2.263	0.984
	Abundance * Time	0.075	0.781	39	2.184	0.075
Richness	Intercept	-0.170	0.306	39	-0.556	0.578
	Richness * Time	0.041	0.022	39	1.896	0.058

Strandesia lansactohai, *Strandesia mutica* (Sars, 1901), *Diaphanocypris meridana* (Furtos, 1936) and *Brasilodopsis baiabonita* Almeida et al., 2021 hatched from the 5th and 6th week onwards, whereas *Strandesia variegata* (Sars, 1901), *Chlamydotheca iheringi* (Sars, 1901) and *Strandesia nakatanii* Ferreira et al., 2020 hatched from the 15th week onwards.

The rarefaction curve was close to asymptote value for ostracod richness. The observed richness represented 88.6% of the estimated richness. This indicates that the incubation time was adequate to represent the dormant assemblages of ostracod attached to dried macrophytes roots (Figure 3).

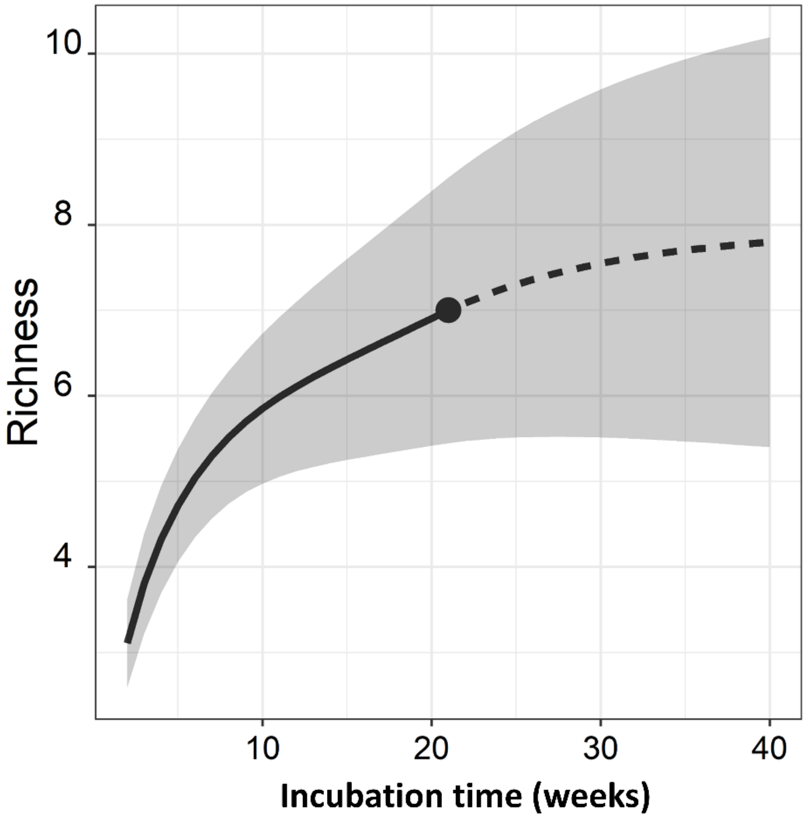

Figure 3. Sample-size-based rarefaction (solid line) and extrapolation (dashed lines) for Hill numbers (q = 0, species richness) for ostracod species of the dormant assemblages (resting eggs) from lakes of the Upper Paraná River floodplain. The 95% confidence intervals were obtained by a bootstrap method.

The beta diversity values increased significantly over the weeks (incubation time) (Pseudo r = 0.485; *z* value = 3.924; p <0.001) (Figure 4).

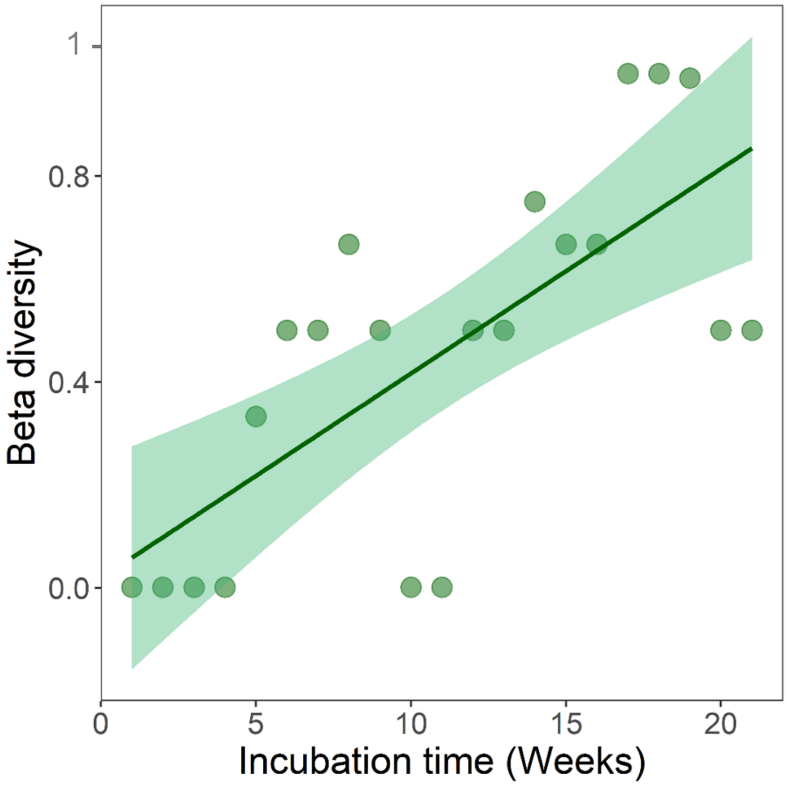

Figure 4. Linear model of beta diversity values of the microcosms during the incubation time (weeks). The shaded bands represent standard errors.

Finally, figure 5 represents and summarises the dispersal of resting eggs attached to macrophytes roots in floodplain. During or at the end of rainy periods, ostracods produce resting eggs, which can attach to the roots of aquatic macrophytes, as observed in the present study (Figure 5A). During drought periods, these plants are either on the sediment or attached to riparian terrestrial vegetation, such as trees. However, the eggs attached to the roots remain viable and can be dispersed by biotic and abiotic vectors (Figure 5B). During the rainy period, the dried roots of *E. crassipes*, and consequently the attached resting eggs, can be moved by the water flow, and will be induced to hatch, contributing to the increase of abundance, richness and diversity of the active community over time (Figure 5C).

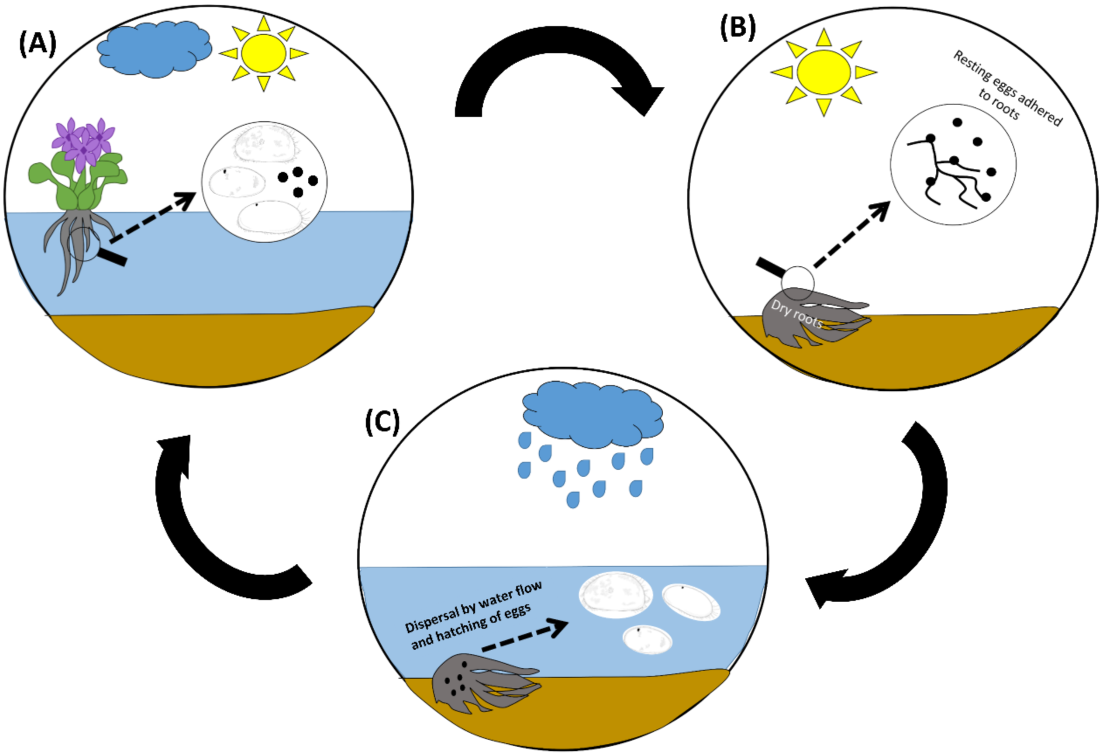

Figure 5. Illustrative model of the dispersal of ostracod resting eggs attached to macrophytes roots in floodplains. (A) Active community of ostracods in *E. crassipes* during flood periods; (B) Resting eggs attached to macrophytes roots during the drought period; (C) Hatching and dispersal of resting eggs attached to macrophytes roots during the flood period.

3.4 Discussion

3.4.1 Ostracods hatching from dried macrophyte roots

The fact that seven ostracod species hatched from eggs attached to dried macrophytes corroborates our first hypothesis. The high abundance and richness of the dormant assemblages of ostracod in the dried macrophyte roots are probably related to the adhesive substance around the ostracod resting eggs (McLay, 1978), which facilitates the adhesion to different solid substrates (e.g. grass stems, twigs, leaves and sediment grains). In addition, some studies have shown an association between the active ostracod communities and the structural complexity of the *E. crassipes* root systems in floodplain lakes, since the high root complexity of this macrophyte species provides ample substrate for feeding and reproduction (Higuti et al., 2010; Matsuda et al., 2015). Such factors can all contribute to the high richness and abundance of ostracod resting eggs in dried roots of *E. crassipes*. Battauz et al. (2017) recorded dormant eggs of zooplankton (rotifers, cladocerans and copepods) in different living aquatic macrophytes species in floodplain lakes of Argentina. Thus, both living and dried forms of macrophytes have the potential to harbour and disperse the dormant eggs of invertebrates.

One of the reasons for the absence of significant differences in the number of hatchings over the incubation time can be related to the constant number of hatchings over the weeks, which can provide a long-term hatching of the organisms in the environment, even under favourable conditions. These results are in agreement with the bet-hedging strategy of ostracods (Pinto et al., 2007). This delayed hatching strategy can influence the phenology (hatching time) of resting eggs, since some eggs may remain in dormancy, even though environmental conditions are appropriate (Pinceel et al, 2017). This delayed hatching will increase the probability of a population persisting in stochastic events, for example early rains that are insufficient to carry hatchlings to reproductive age (Evans & Denneyi, 2005).

The hatched ostracod assemblages were represented only by species of Cyprididae, a family known to produce resting eggs (Horne & Martens, 1998; Rosa et al., 2020; 2021a). Not surprisingly, most of the species that hatched from the ostracod resting eggs attached to dried roots, as well as others species of Cyprididae, have been found associated with different species of aquatic macrophytes in the Upper Paraná River floodplain, including the lakes studied here (Higuti et al., 2010; 2017; 2020; Matsuda et al., 2015; De Campos et al., 2021). In addition, this is the first record of specimens of *Brasilodopsis baiabonita* and *Strandesia nakatanii* hatched from resting eggs in the Upper Paraná River floodplain.

3.4.2 Beta diversity

The increase of beta diversity over time corroborates our second hypothesis, which is furthermore in agreement to the results of Wisnoski et al. (2019). These authors reported that dormancy is a life-history trait that influences the community structure, dispersal and diversity at both temporal and spatial scales, owing to the specific hatching time of the organism. Each ostracod species has a specific hatching time, largely determining its general phenology (Rosa et al., 2021b). Differences in hatching time of invertebrates may thus affect community species richness and diversity over time (Florencio et al., 2020; Rosa et al., 2021b). Therefore, beta diversity tends to increase over time, owing to the hatching of new species contributing to the colonization and resilience of the environments. This pattern of increasing beta diversity over time was also observed for the active communities of ostracods (Higuti et al., 2020) and zooplankton in the same floodplain of the present study (Soares et al., 2015).

3.4.3 Alien invasive ostracod species

Studies about the dispersal of organisms by biotic and abiotic vectors are important to understand ecological aspects and risks of species invasions, since the dispersal of exotic organisms associated to plants has increased owing to human activities (Ruiz & Carlton, 2003). The potential dispersal of ostracod resting eggs by dried plants observed in the present study was also reported for other types of vectors, for example, by humans (e.g. waterproof shoes) and birds (Valls et al., 2016; Morais-Junior et al., 2019). Dispersal by vectors increases the distribution of such groups and this is a natural process increasing the fitness and long-term survival of such species. However, this is also a warning about the biological invasion process, since human activities have increased transport of otherwise natural vectors (plants and animals) and their associated fauna between continents (Lenda et al., 2014). This is so, because these aquatic plants are used for ornamentation and they are easily obtaining through purchases over the internet (Havel et al., 2015).

A known example of an exotic ostracod species from South America is *Strandesia bicuspis* (Claus, 1892), a common species in temporary lakes of the Upper Paraná River floodplain and abundant in the resident egg banks (Rosa et al., 2021a). This species was introduced in Europe by transport of ornamental aquatic plants (Matzke-Karasz et al., 2014). Rossi et al. (2003) reported invasive ostracod species in rice fields in Italy, where human activities introduced exotic species belonging to the genera *Chlamydotheca* and *Strandesia*. We highlight that these two genera were recorded in the dried roots of *E. crassipes*, especially *Strandesia* which had the highest number of species hatching in our study.

3.4.4 Effects of flood pulses

In floodplains, the dispersal of *E. crassipes* may intensify during the rainy season, with the increase of water levels and consequently, higher current velocities, allowing greater exchange of floating plant material between the lakes and the main channel (Thomaz et al., 2007; Pereira et al., 2017). Therefore, so-called flood pulses can facilitate the dispersal of ostracod resting eggs attached to macrophyte roots and increase both the recruitment and the homogenization of the dormant and active communities all over the floodplain (Thomaz et al., 2007; Pereira et al., 2017; Rosa et al., 2020).

3.5 Conclusion

The high abundance and richness indicates that root systems of *Eichhornia crassipes* have a high capacity to shelter and transport the ostracod resting eggs. In addition, the increase of beta diversity over time will act as a diversity boost and will contribute to the recolonization and resilience of the floodplain lake ecosystems after drought events. Dried roots of aquatic plants can therefore be ecological reservoirs of eggs, or floating egg banks.

We also highlight that this type of dispersal can facilitate the process of alien species invasion, since the floating roots can act as an egg bank for both native and invasive species and can serve as a vector for dispersal of the resting eggs to a new environment. It is thus important to consider the risk of invasion by the dormant eggs in programs of the conservation of aquatic biodiversity.

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4. THE PROLIFERATION OF FUNGI IN LAKE SEDIMENT CAN INFLUENCE THE COMMUNITY STRUCTURE OF DORMANT AQUATIC MICROFAUNA

Abstract: Most aquatic microfauna produces dormancy structures under adverse conditions which accumulate in the sediment of aquatic environments. In studies on dormant communities, however, fungi may develop in sediments during storage. Here, we evaluate if the presence of fungi in stored sediments influences the structure (richness and species composition) of dormant aquatic microfauna communities (ciliates, rotifers, ostracods and cladocerans) of a temporary floodplain lake. Microcosms were prepared with lake sediment, with the presence (treatment) and absence (control) of fungi. The samples were hydrated with distilled water, kept in the germination chamber with temperatures stable at 25°C and controlled photoperiod. A total of 99 taxa of aquatic microfauna hatched from the sediment: 82 ciliate, eight rotifer and nine microcrustacean species. The highest species richness of hatched microfauna was recorded in the fungi treatment. Significant differences were observed in species composition between control and fungi treatments for all microfaunal groups analysed. Thus, the presence of fungi seems to have a positive indirect influence on the richness and composition of the dormant microfauna, probably because the fungi can contribute to the reduction of organic matter and nutrient availability to microfauna. Our results show that fungi do not affect the viability of the dormant structures.

Keywords: microfaunal egg banks, ciliates, rotifers, microcrustaceans, decomposition.

4.1 Introduction

Aquatic communities often experience adverse conditions such as extreme drought events, which are becoming more frequent owing to the climate change (Humphries & Baldwin, 2003; De Campos et al., 2019; Williams, 2006). For instance, the intensification and duration of the drought has caused a drastic reduction in water levels in permanent lakes and longer duration of completely dry temporary environments. Therefore, only organisms with strategies or structures to avoid desiccation can survive and remain in these environments (Strachan et al., 2015). Cladocerans, rotifers and ostracods are amongst the organisms that produce dormant eggs, while the ciliates produce cysts (Rosa et al., 2021; Weisse, 2017). These structures contribute to increased local species and genetic diversity, a higher potential for passive dispersal and augmented resilience of communities after disturbance events (Brendonck & De Meester, 2003; Cochak et al., 2021; Rosa et al., 2020; 2022).

Dormant structures produced by aquatic microfauna are accumulated on and in the sediment of the environments forming banks of dormant propagules, also called "egg banks", which remain viable for many years and might hatch when environmental conditions become favourable again (Brendonck & De Meester, 2003; Hairston, 1996). In this way, limnological and sediment variables, such as temperature, luminosity and amount of organic matter, can influence the hatching and viability of these structures (Rosa et al., 2020; Rossi et al., 2012; Silva Bandeira et al., 2020). The degree of hatching of dormant structures is directly related with the quality and storage of the sediment (Kearns et al., 1996; Rosa et al., 2020). For example, some pollutants (e.g. pesticides) of the sediment of lakes can inhibit hatching of dormancy structures, thus negatively affecting the dynamics of dormant communities (Patterson et al., 2021). In addition, important biological processes, such as decomposition of organic matter by bacteria and fungi, can occur in sediments (Booth et al., 2019), favouring or inhibiting the hatching of resting eggs and cysts.

To address the importance of egg banks in the evaluation of biological and ecological aspects of aquatic communities, the method of hydration of dry sediment for the hatching of dormant structures has been commonly used (Bhusnale et al., 2016; Freiry et al., 2020; 2021; Rosa et al., 2020; 2021). To study the dormancy structures of aquatic organisms it is necessary to keep the sediment samples under refrigerated conditions or in stable temperatures, depending on the methodology used (Maia-Barbosa et al., 2003). However, a potential problem during sample storage is the deterioration in the sediment quality and in the viability of dormancy structures, since long-term storage of moist sediment can lead to the development of fungi.

The development of fungi in sediment samples for experimental purposes can easily occur since some fungal species are resistant to cold temperatures and dry conditions (Komagata et al., 2022; Vries et al., 2018). However, when fungal development occurs in the sediment samples, this is usually discarded, although there is no evidence of the influence (or absence thereof) of fungi on dormant communities. In contrast, a higher diversity of aquatic and terrestrial fungi has been recorded in lake sediments and these organisms can play an important role in the functioning of aquatic ecosystems owing to their function in the decomposition of organic matter, especially lignocellulose (Lepère et al., 2019; Shearer et al., 2017). The fungi, especially the zoospores, are also important in cycling nutrients in aquatic food webs by liberating particulate organic matter that than becomes available to other organisms (e.g. microcrustaceans) (Maier et al., 2016). Thus, understanding the biological factors (i.e. fungi presence) that influence the structure of the dormant communities and the viability of the dormant structures may facilitate the methodological steps of sediment storage and experimental execution of future studies.

The aim of the present study was to evaluate if the presence of fungi in sediment influences the structure (richness and species composition) of the dormant aquatic microfauna community (ciliates, rotifers and microcrustaceans) of a tropical temporary lake in Brazil. Considering that fungi reduce the amount of organic matter in the sediment, which generally in high concentrations (organic matter) has a negative effect on egg bank hatching due the hypoxia conditions (Rosa et al., 2020), as well as increase bioavailability of particulate organic matter to other micro-organisms (e.g. ciliates) during the decomposition process, we test the hypotheses (1) that the sediment samples with fungi would present a higher species richness of microfauna, and (2) dissimilarities in species composition when compared to the control treatment.

4.2 Material and methods

4.2.1 Study area

The Upper Paraná River floodplain (South Brazil) is located in an environmental protected area: “National Environmental Protection Area of the Paraná River” (Agostinho et al., 2004). It is composed of a variety of environments, such as channels, rivers, connected and isolated lakes, and temporary lakes. The climate of the region is tropical-subtropical with rainy and dry seasons (Cfa) (Eletrosul, 1986).

The sediment was collected from a temporary lake (Pontal Lake, 22°45 '05.7" S/ 053°15'23.6" W) located on the Porto Rico island in the Upper Paraná River floodplain. It is an isolated and elongated lake of about 50 m long and 15 m wide, and a mean depth of 1.5 m, which has recurrent drought periods. The riparian vegetation of the lake shelters remnants of terrestrial and aquatic macrophytes, for example, *Eichhornia azurea* Kunth.

In addition, the sediment of the lake has approximately 20% of organic matter concentration derived from the riparian (terrestrial) vegetation. In general, Pontal Lake has oxygenated waters and a slightly acidic pH (Rosa et al., 2020).

4.2.2 Sampling and hatching procedures

Samples of sediment, containing approximately 100 grams each, were collected in the centre of the lake during the dry season (July 2018) (Figure 1A). The sediment was collected down to a sediment depth of c 5 cm using a trowel and was stored in plastic vials in darkness at c. 4°C, according to the method of Maia-Barbosa et al. (2003) to prevent premature hatching (Figure 1B). After two years of storage of the sediment samples, some samples had septate filamentous fungi (phylum Ascomycota; family Orbiliaceae) (treatment with fungi) and others samples were in natural conditions, without fungus development (control).

For the hatching procedure in the laboratory, the control treatment (n=3 samples) and fungi treatment (n=3 samples) were homogenized separately and 300 grams of sediment from each treatment were taken and placed separately in two plastic trays. The sediment was oven-dried at 25°C for 120 hours to trigger the hatching.

Subsequently, three microcosms/replicates (600 ml glass jars; 10 cm x 6 cm) were prepared with 100 grams of dry sediment for each treatment (control and fungi), totalling six microcosms (Figure 1C). Then, the samples were hydrated with 300 ml distilled water and kept in the germination chamber at 25°C, with 12-hour light/dark photoperiod (Rossi et al., 2012) for 56 days (Brochet et al., 2010).

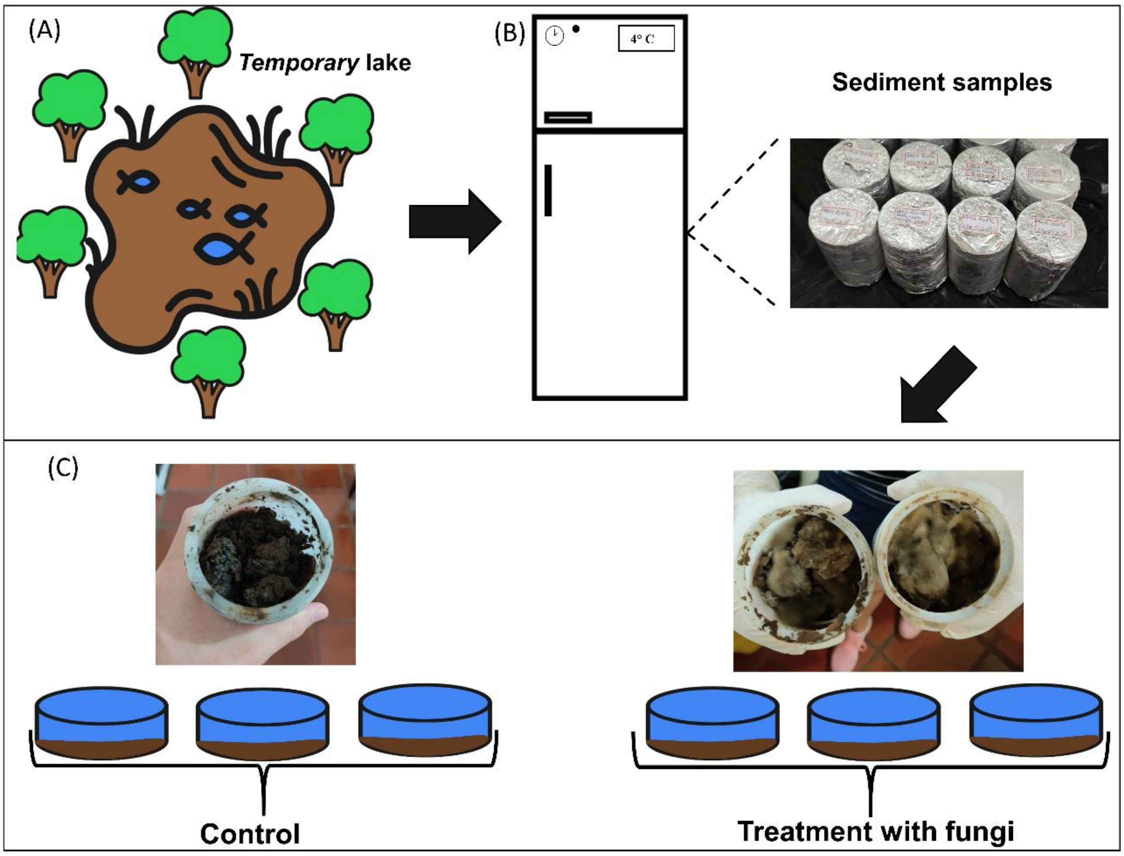

Figure 1. Schematic model of the sampling and hatching procedures. (A) Representation of the sediment sampling site; (B) Storage of the sediment samples in refrigeration (4°C) and dark conditions during two years; (C) Preparation of the microcosms of the control group (n=3) and the microcosms of the fungi treatment (n=3).

4.2.3 Microfauna analysis

We analysed different communities of the aquatic microfauna, ciliates, rotifers and microcrustaceans (cladocerans and ostracods). For this purpose, specific procedures were applied to facilitate the sorting of the different biological groups owing to their different body sizes. The microcosms were monitored weekly during the incubation period (8 weeks).

4.2.3.1 Ostracods

The water from the microcosm was filtered using a plankton net (68 µm). After the filtering, fresh distilled water was added to the microcosms. Ostracods were sorted from the material retained in the plankton net under a stereomicroscope following the protocol of Rosa et al. (2020). Hatched juveniles were kept separately in glass vials with distilled water, fed with fresh spinach until reared to the adult stage for identification and counting. The ostracod species were identified following Higuti et al. (2013).

4.2.3.2 Ciliates

The ciliate identification and counting was performed with both materials retained in the plankton net (after sorting the ostracods) and the filtered water of each microcosm. Water samples were concentrated in 100 mL using a 10 µm mesh. Quantitative counting was performed using 10 aliquots (slides) of 100µL of sample (1mL in total) under an optical microscope at 100 and 400x magnification. In order to record rare ciliate species not found during quantitative counting, a qualitative analysis was also performed in Sedgewick Rafter chamber using 1 mL of sample. Ciliates were quantified and identified in *vivo* to the lowest possible taxonomic level using specialized bibliography (Foissner & Berger, 1996).

4.2.3.3 Rotifers and cladocerans

After the counting of ciliates, this water was filtered again in a plankton net (68 µm) and the retained material was fixed in 70% alcohol for subsequent sorting of rotifers and cladocerans. These organisms were fixed because the monitoring time every 7 days is sufficient for them to reach the adulthood stage. Finally, all samples were fully counted and the individuals identified to species level (whenever possible) under optical microscope. No copepods hatched from the microcosms. The identification was carried out with specialized literature for cladocerans (Elmoor-Loureiro, 1997; Smirnov, 1992) and rotifers (Joko, 2011; Koste & Voigt, 1978).

4.2.4 Data analyses

The analyses were first performed for the microfauna (all groups), then separately for the distinct communities, ciliates, rotifers and microcrustaceans (Cladocera and Ostracoda). To analyse the richness differences between the control and fungi treatment, we performed an analysis of variance (One-way ANOVA) to test a significance level set at $p < 0.05$. The assumptions of normality and homoscedasticity were checked for all groups.

We performed a principal coordinate analysis (PCoA) to visualize the (dis)similarities in the species composition between the control and fungi treatment, based on a matrix with data on the presence/absence of the aquatic microfauna, which had hatched weekly, using the Jaccard distance (Legendre & Legendre, 1998). A Multivariate Permutational Analysis of Variance (PERMANOVA - using the "adonis" function of the vegan package (Anderson, 2001; Oksanen et al., 2020) was performed to evaluate statistical differences in the species composition between the treatments. This test was also based on a presence and absence matrix using the Jaccard distance. A total of 999 permutations were performed to assess significant differences (Anderson, 2006). This analysis cannot be applied separately for the microcrustacean group due to the low number of samples with hatchings.

All analyses were performed in R software (R Development Core Team, 2019). PCoA and PERMANOVA analyses were performed according to the "ADONIS" function using the Vegan (Oksanen et al., 2020) and Permute (Simpson, 2022) packages.

4.3 Results

A total of 98 taxa of aquatic microfauna hatched from the 48 samples (6 microcosms x 8 weeks): 81 species of ciliates, eight of rotifers and nine species of microcrustaceans (eight cladocerans and one ostracod) (Table 1).

Table 1. List of dormant microfauna species hatched from the sediment of the control and fungi treatment. NI= non identified.

SPECIES	TREATMENTS	
	Control	**Fungi**
PROTIST CILIATES		
Acineria incurvata Dujardin, 1841		X
Acineria uncinata Tucolesco, 1962	X	
Anteholosticha multistilata (Kahl, 1932)		X
Aspidisca turrita (Ehrenberg, 1831)		X
Aspidisca sp. 1		X
Chaenea stricta (Dujardin, 1841)	X	
Chilodonella uncinata (Ehrenberg, 1838)	X	
Colpidium colpoda (Losana, 1829)	X	
Colpoda steinii Maupas, 1883		X
Cyrtolophosis mucicola Stokes, 1885		X
Cyrtolophosis sp. 1		X
Dexiostoma campylum (Stokes, 1886)		X
Dexiotrichides centralis (Stokes, 1885)		X
Discomorphella pectinata (Levander, 1894)		X
Disematostoma buetschlii Lauterborn, 1894		X
Dysteria fluviatilis (Stein, 1859)	X	
Enchelys gasterosteus Kahl, 1926	X	
Epistylis coronata Nusch, 1970		X
Epistylis galea Ehrenberg, 1831	X	X
Euplotes aediculatus Pierson, 1943		X
Euplotopsis affinis (Dujardin), 1841	X	X
Euplotes moebiusi Kahl, 1932		X
Euplotes sp. 1		X
Gastrostyla steinii Engelmann, 1862		X
Halteria grandinella (Müller, 1773)	X	X
Halteria sp. 1	X	X
Halteria sp. 2	X	X
Hastatella radians Erlanger, 1890		X

Histriculus vorax (Stokes, 1891)	X	X
Holophrya sp. 1		X
Holosticha sp. 1		X
Kahlilembus attenuatus (Smith, 1897)	X	
Kerona pediculus (Müller, 1773)		X
Lagynophrya acuminata Kahl, 1935	X	
Limnostrombidium viride (Stein, 1867)	X	X
Limnostrombidium sp. 1		X
Metopus spp. *sensu lato*	X	X
Monodinium balbianii Fabre-Domergue, 1888	X	
Oxytricha chlorelligera Kahl, 1932		X
Oxytricha sp. 1		X
Paraurostyla viridis (Stein, 1859)		X
Paraurostyla sp. 1		X
Rimostrombidium humile (Pénard, 1922)	X	X
Rimostrombidium lacustris (Foissner, Skogstad & Pratt, 1988)		X
Rubrioxytricha ferruginea (Stein, 1859)	X	X
Stichotricha secunda Perty, 1849	X	
Stylonychia pustulata (Müller, 1786)		X
Stylonychia vorax Stokes, 1885		X
Stylonychia sp. 1	X	X
Uroleptus gallina (Müller, 1786)		X
Uroleptus musculus (Kahl, 1932)		X
Uroleptus sp. 1		X
Urotricha farcta Claparède & Lachmann, 1859	X	X
Urotricha sp. 1	X	X
Vorticella aquadulcis-complex Stokes, 1887	X	
Vorticella campanula Ehrenberg, 1831	X	
Vorticella convallaria-complex Linnaeus, 1758	X	
Vorticella octava-complex Stokes, 1885	X	
Vorticella sp. 1	X	
Hypotrichia (NI) 01		X
Hypotrichia (NI) 02		X
Hypotrichia (NI) 03		X
Hypotrichia (NI) 04		X
Hypotrichia (NI) 05		X
Hypotrichia (NI) 06		X
Hypotrichia (NI) 07		X
Hypotrichia (NI) 08		X
Hypotrichia (NI) 09	X	
Hypotrichia (NI) 10	X	
Hypotrichia (NI) 11	X	
Odontostomatida (NI) 1		X
Odontostomatida (NI) 2		X

Oligotrichida (NI) 1		X
Oligotrichida (NI) 2		X
Oligotrichida (NI) 3		X
Oligotrichida (NI) 4	X	
Scuticociliatia (NI)		X
Ciliate sp. 1		X
Ciliate sp. 2		X
Ciliate sp. 3	X	
Ciliate sp. 4	X	
ROTIFERS		
Bdelloidea	X	X
Beauchampiella eudactylota (Gosse, 1886)	X	
Cephalodella sterea (Gosse, 1887)		X
Cephalodella sp. 1		X
Collotheca ornata (Ehrenberg, 1832)	X	
Lecane furcata (Murray, 1913)	X	
Lecane sp. 1	X	
Proales syltensis Tzschaschel, 1979	X	
CLADOCERANS		
Alona cf *guttata* Sars, 1862		X
Alonella dadayi Birge, 1910		X
Ceriodaphnia cornuta Sars, 1885		X
Ceriodaphnia reticulata (Jurine, 1820)		X
Ceriodaphnia richardi Sars, 1901		X
Ceriodaphnia silvestrii Daday, 1902		X
Chydorus pubescens Sars, 1901	X	
Euryalona brasiliensis Brehm & Thomsen, 1936		X
OSTRACODS		
Strandesia nupelia Higuti & Martens, 2013		X

The highest species richness of microfauna (all groups combined) was recorded in the fungi treatment compared to the control treatment, showing a significant difference (ANOVA, F = 8.99, p = 0.04; Figure 2A). For the ciliate community, significant differences were also observed, with a higher species richness in the treatment with fungi presence (ANOVA, F = 10.45, p = 0.03; Figure 2B). In contrast, higher rotifer richness was found in the control treatment, however these differences were not statistically significant (ANOVA, F = 1.8, p = 0.25; Figure 2C). Finally, the microcrustacean communities (Cladocera and Ostracoda) also had the same pattern of the general microfauna and the ciliates, with a higher species richness in the treatment with fungi presence (ANOVA, F = 13, p = 0.02; Figure 2D).

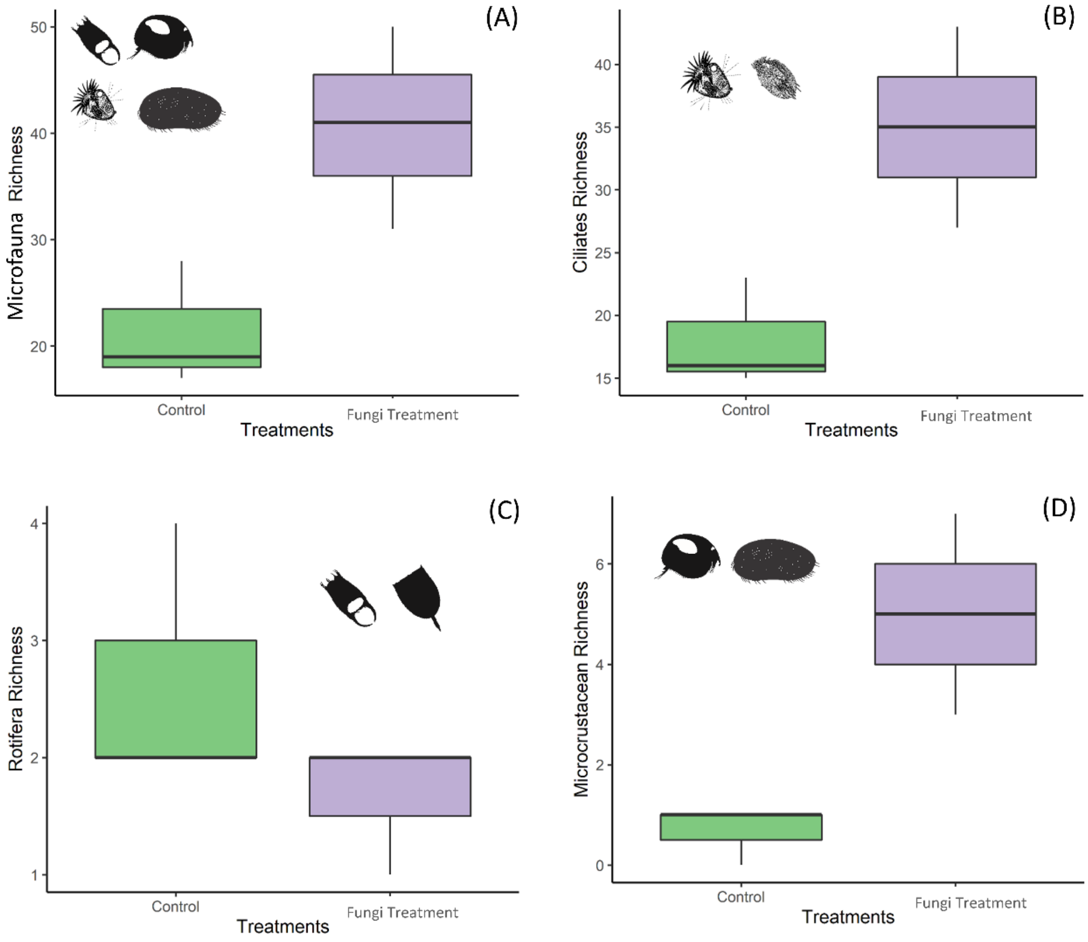

Figure 2. Mean richness of (A) microfauna, (B) ciliates; (C) rotifers and (D) microcrustaceans in the control and fungi treatment. Bars represent standard error.

PCoA and PERMANOVA showed significant differences in species composition between control and fungi treatment for the microfauna (Pseudo-F = 4.63, p = 0.001; Figure 3A), ciliates (Pseudo-F. = 3.89, p = 0.001; Figure 3B) and rotifers (Pseudo-F. = 6.19, p = 0.002; Figure 3C). However, it was not possible to perform the composition analyses for the microcrustacean community due to the low sample number with hatchings in the control (n = 2).

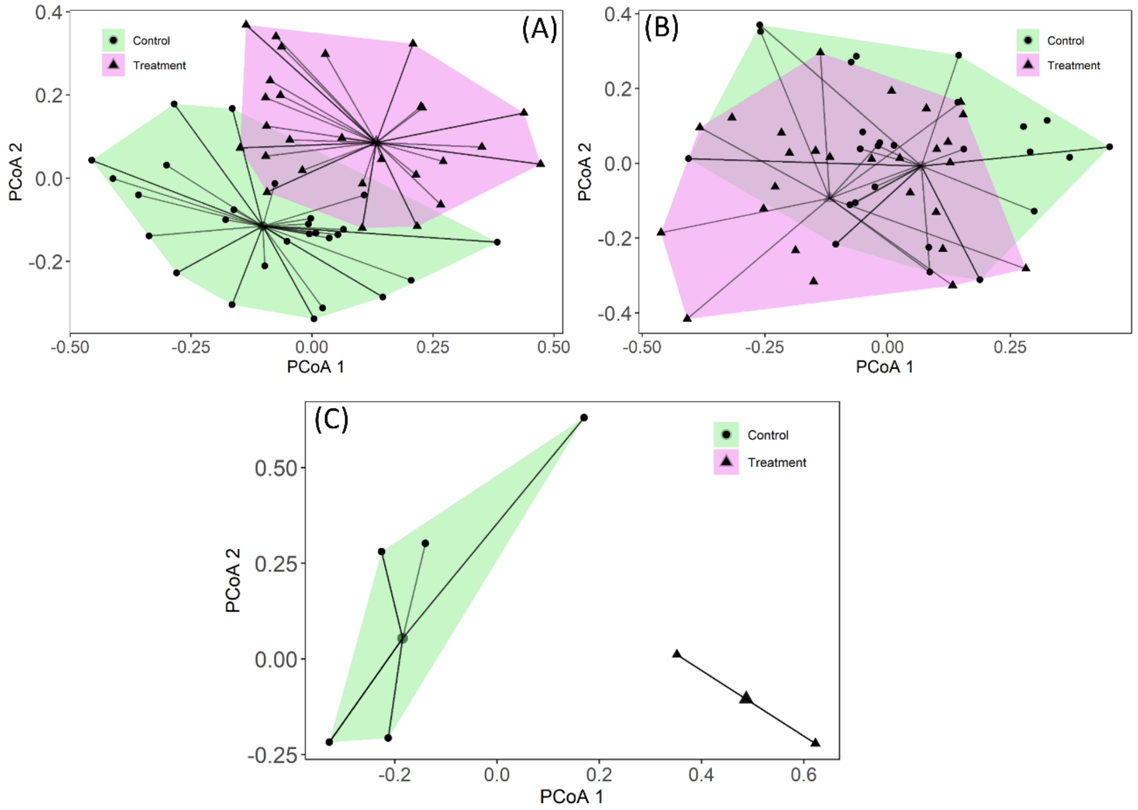

Figure 3. Principal coordinate analysis showing the variability in the species composition in the control and fungi treatment. (A) microfauna; (B) ciliates and (C) rotifers.

4.4 Discussion

A higher species richness of the microfauna (except rotifers) was recorded in the fungi treatment and it may be related to the decomposer niche of the fungi, corroborating our first hypothesis. The fungi present in the sediment contribute to the decomposition of dead organic matter, mainly of vegetal origin, such as lignocellulose (De Boer et al., 2005; Newell, 1996). However, Rosa et al. (2020) observed a negative correlation between high concentrations of organic matter in the sediment and hatching success of ostracod resting eggs, because high amounts of organic matter can cause anoxic conditions in the sediment and subsequent burial of the egg bank (Gleason et al., 2003; Watkins et al., 2011). As fungi contribute to the decomposition process, reducing the amount of organic matter in the sediment and make minerals and nutrients bioavailable, they can have a positive effect on hatching of dormancy structures. This positive indirect influence of fungi on the hatching of dormant structures can explain the higher species richness found in the treatments with fungi present in our study.

Amongst the microfauna communities, the ciliate community showed the highest species richness in the treatment with fungi, probably caused by the food availability trigger. Although there is also competition between fungi and bacteria in relation to organic matter decomposition processes, studies on the interactions of these two communities on substrates rich in lignocellulose revealed the importance of fungi in providing services to prokaryotic communities (De Boer et al., 2005). Some of these services are the lignocellulose degradation, fungal pathways that allow bacteria to move along hyphae and water/nutrients supply (Kohlmeier et al., 2005, Worrich et al., 2017). Most ciliates consume bacteria, and their spatial and seasonal distribution generally follows the fluctuations of bacteria production (Santoferrara & Alder, 2009). In this way, the high ciliate richness in the fungi treatment may be owing to an indirect influence of the fungi, as they provide improved conditions for the growth of bacterial communities which are food for the ciliates. These conditions might be a trigger to hatching of bacterivorous/omnivorous ciliate species.

Another possible reason for the higher ciliate richness in the fungi treatment is that the fungi decompose the coarse organic matter into particulate organic matter (Gulis et al., 2019), which creates more an available food resource for the omnivorous ciliates (Posch & Arndt, 1996). Thus, the large amount of particulate organic matter (food) for the omnivorous ciliates (Posch & Arndt, 1996), can have favoured the hatching of the cysts. According to Minkoff et al. (1983) and Gilbert (2010) food availability is an important factor responsible for the hatching of dormancy structures.

The richness of the dormant microcrustacean community represented by cladocerans and ostracods also showed a positive effect with fungi treatment, probably related to the influence of fungal activity that provides food for these groups. Some studies show the importance of aquatic fungi in the food web of these aquatic environments (e.g. Farthing et al., 2021; Kagami et al., 2014; Maier et al., 2016). For example, zoospore fungi have a decomposition role similar to the studied fungi, and they can play an important role in nutrient and organic matter cycling in aquatic food webs by release of particulate organic matter that becomes accessible to small organisms (e.g. microcrustaceans) (Maier et al., 2016). In addition, the zoospore fungi are a source of high nutritional quality food for zooplankton (including cladocerans), providing a new pathway, referred to as “micoloop”, that acts in the formation and stability of aquatic ecosystems (Kagami et al., 2014). Finally, based on our results we can also assume that there can be an indirect relationship between fungi and microcrustaceans, because the fungi trigger higher ciliate abundance, and this is important food for juvenile microcrustaceans.

Conversely, for the rotifer community, no effects of treatment were observed. A factor that may have contributed to the similarity in richness between treatments in rotifers is the bet-hedging strategy observed for these organisms in previous studies (García-Roger et al., 2014; Tarazona et al., 2017). This strategy can influence the hatching time of the organisms, since some resting eggs remain dormant even under favourable environmental conditions for hatching, in order to avoid extinction of the population in stochastic events (Evans & Denneyi, 2005; Simons, 2011). Thus, as part of the rotifer eggs could not have hatched during this first hydratation (flood) due to their bet-hedging strategy, it was not possible to detect the real effect of the fungi on the rotifer dormant community structure. We do not exclude the possibility that this strategy may also have occurred in other groups (e.g. Cladocera and Ostracoda), especially for the ostracods for which a single species was recorded (Pinto et al., 2007). In addition, the resting eggs of rotifers can hatch in minimal concentrations of oxygen and light, which can buffer the effects of the organic matter reduction caused by fungi (Gilbert, 2017).

The differences in species composition found for aquatic microfauna, and which corroborates our second hypothesis, can be owing to the same reasons discussed above regarding the differences in species richness. Species composition and richness are amongst the most important ecological attributes in structuring communities. The high species richness recorded in the experiment is probably owing to the ability of the egg bank to harbour species from several generations (Brendonck & De Meester, 2003). The set of species in the egg bank that inhabited the environment in the past, can influence the present or future communities by differential hatching of dormancy structures, and this process is called ecological memory (Padisak, 2009; Rosa et al., 2021).

Studies with resting eggs are a new and promising field in South America (Iglesias et al., 2016). For such studies, it is often necessary to store the sediments for certain periods of time. One of the best methods of storage of the sediment samples is under refrigeration and in dark conditions (the methodology applied in this study), since Chittapun et al. (2005), found lower abundance and species richness hatched from sediments that were stored at room temperature and in the dark. However, the long-term storage of moist sediment in the fridge to study the dormancy structures of aquatic organisms can lead to the proliferation of fungi. The development of fungi is influenced by various environmental conditions, such as the amount of organic matter. The studied lake has a dense riparian (terrestrial) vegetation which is responsible for the input of organic matter of vegetal origin (e.g. leaves, branches, fruits; Kita & De Souza, 2003) that can serve as food for the fungi. In addition, fungi may interact with other environmental variables to determine the overall structure of the microbial community (Booth et al., 2019).

4.5 Conclusion

In conclusion, the presence of fungi seems to have a positive indirect influence on the richness and species composition of most of the dormant aquatic microfaunal communities, since fungi can contribute to the reduction of organic matter in sediment and at the same time increase the nutrient availability to microfauna. Based on the above, we suggest that sediment samples that have the presence of these fungi can be preferentially used in inventories studies of the aquatic microfauna species because they do not affect the viability of the dormant structures and can suppress the problems related to low sample numbers owing to the discarding of mouldy samples.

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5. THE EFFECT OF THE INCREASE OF TEMPERATURE ON THE STRUCTURE OF DORMANT AQUATIC MICROFAUNA: PERSPECTIVES ON DIFFERENT CLIMATE TEMPERATURE SCENARIOS

Abstract

1. Dormant structures of aquatic microorganisms are ecologically important in aquatic environments since they are responsible for restoring the ecosystem after extreme climatic events. Here, we evaluate the effect of the increase of temperature on the structure of the aquatic dormant microfauna (ciliates, rotifers, cladocerans, copepods and ostracods).

2. Sediment samples were collected in a temporary lake of the Upper Paraná River floodplain, Brazil. The material was dried in temperatures of 25ºC, 30ºC, 40ºC, 50ºC and 60ºC, hydrated, and kept at 25ºC and a 12-hour light photoperiod during eight weeks.

3. The increasing temperature significantly reduced the richness of dormant aquatic microfauna and led to differences in species composition between temperature treatments.

4. Temperatures above 50ºC (not uncommon for sediments exposed to direct sun in tropical areas) can negatively affect the dormant microfauna, promoting the loss of biodiversity and biotic homogenization of the egg banks in temporary aquatic ecosystems.

5. Thus, climate change, such as global warming, is a threat to aquatic dormant microfauna, highlighting the importance of mitigation measures for the conservation of the egg bank.

KEYWORDS

egg bank, passive community, biodiversity, global warming.

5.1 Introduction

Climate change, such as global warming, is a threat to aquatic and terrestrial biodiversity (Hader & Barnes, 2019). Global warming has altered the duration and frequency of extreme events, including droughts and heatwaves (Weiskopf et al., 2020). These events have significant impacts on ecosystems by reducing their ecological resilience and affecting the structure of the biological communities (Derksen & Brown, 2012; IPCC, 2014). High temperatures provoke behavioural responses (e.g. distributional changes), morphological (e.g. faster growth rates and smaller body size) and phenological (e.g. changes in reproduction time) adaptations in populations and even in individual species (Hader & Barnes, 2019; Weiskopf et al., 2020). This is worrying because there is a clear trend of increasing temperature, owing the climate change, with an increase up to 4.8°C in global surface temperature predicted over the next 100 years (IPCC, 2014; Masson-Delmotte et al., 2021). Modelled climatic scenarios suggest that parts of Australia will experience temperature increases of as much as 58 °C by 2070 in regions that are situated more than 200 km from the coast (Suppiah et al., 2007; Hughes, 2011). Owing to this fact, the dried sediment of the temporary aquatic environments can also become very hot, easily exceeding 50°C because of heat transfer (Fang & Stefan, 1996; Jacobi et al., 2007).

In aquatic ecosystems, the biotic communities present different responses and adaptations to changes in environmental conditions (Lake, 2011). Increase temperatures and reduction in water level affect the metabolism of some aquatic invertebrates, promoting the production of resistant structures to survive and persist in the environment (Radzikowski, 2013; Hay et al., 2018). For instance, copepods, rotifers, ciliates, cladocerans and ostracods produce dormant structures (e.g. resting eggs and cysts) (Alekseev & Pinel-Alloul, 2019; Rosa et al., 2021a), which accumulate in the sediment of aquatic environments, forming egg banks (Brendonck & De Meester, 2003). These egg banks play important roles in the structure and dynamics of populations and communities because they contribute to passive dispersal and increase the genetic and taxonomic diversity of the active community as individuals of different generations hatch (Ellner & Hairston, 1994; Rosa et al., 2022). In addition, they are responsible for recolonizing the environment after extreme drought events (Brendonck et al., 2017; Rosa et al., 2021a). However, with prolonged drought events, the dormant structures in the dry sediment of temporary aquatic environments are exposed to high temperatures for a longer period, which can affect the viability of these structures and reduce the hatching rates, consequently influencing the resilience of the ecosystems (Nielsen et al., 2015).

The occurrence of extreme climatic events can also lead to aquatic biotic homogenization, a process responsible for increasing the similarity of species composition and reducing taxonomic, functional and genetic diversity of communities (Rahel & Olden, 2008; Petsch, 2016). For example, extreme drought events in temporary floodplain lakes can lead to biotic homogenization of benthic invertebrates, because only the drought resistant stages of tolerant and adapted species survive during dry conditions (Bertoncin et al., 2019). In addition, biotic homogenization as a result of global warming can also cause a decrease in the resilience and resistance of aquatic communities (Petsch, 2016).

Here, we evaluated the effect of the increase of temperature on the structure (richness and species composition) of the aquatic dormant microfauna (ciliates, rotifers and microcrustaceans). We test the hypothesis that the high temperatures affect the viability and hatchability of dormant microfaunal structures, causing a reduction of species richness and biotic homogenization of the egg banks (dormant).

5.2 Material and methods

5.2.1 Study area

Sediment was sampled from a habitat in the Upper Paraná River floodplain, South Brazil, in an environmental protected area: “National Environmental Protection Area of the Paraná River” (Agostinho et al., 2004). This area is composed by a variety of environments, such as channels, rivers, permanent and temporary lakes. The climate of the region is tropical-subtropical with distinct rainy and dry seasons (Cfa) (Eletrosul, 1986).

We studied the dormant microfauna communities of a temporary lake located on the Porto Rico Island (Pontal Lake, 22°45 '05.7" S/ 053°15'23.6" W) in the Upper Paraná River floodplain. Pontal Lake is an isolated waterbody, about 50 m long and 15 m wide, with a mean depth of 1.5 m. The lake experiences recurrent drought periods. The riparian vegetation of the lake shelters remnants of forest and aquatic macrophytes, for example *Eichhornia azurea* Kunth. When inundated, it is an oxygenated and slightly acidic environment (Rosa et al., 2020).

5.2.2 Sampling and hatching procedures

A total of 12 sediment samples were collected in the centre of the lake during the dry season (July 2018). Each sediment sample, approximately 100 grams, was collected at a depth of *ca.* 5 cm using a trowel. Sediment was stored in plastic vials under darkness and refrigeration at 4°C, according to the method of Maia-Barbosa et al. (2003) to avoid the break of dormancy of the structures.

The sediment samples were homogenized and fractionated in a total of 25 microcosms (600 ml glass jars; 10 cm x 6 cm) with approximately 180 grams of sediment each. The sediments were oven dried at different temperatures: 25º C (control), 30º C, 40º C, 50º C and 60º C. Five replicas for each temperature were prepared (Figure 1).

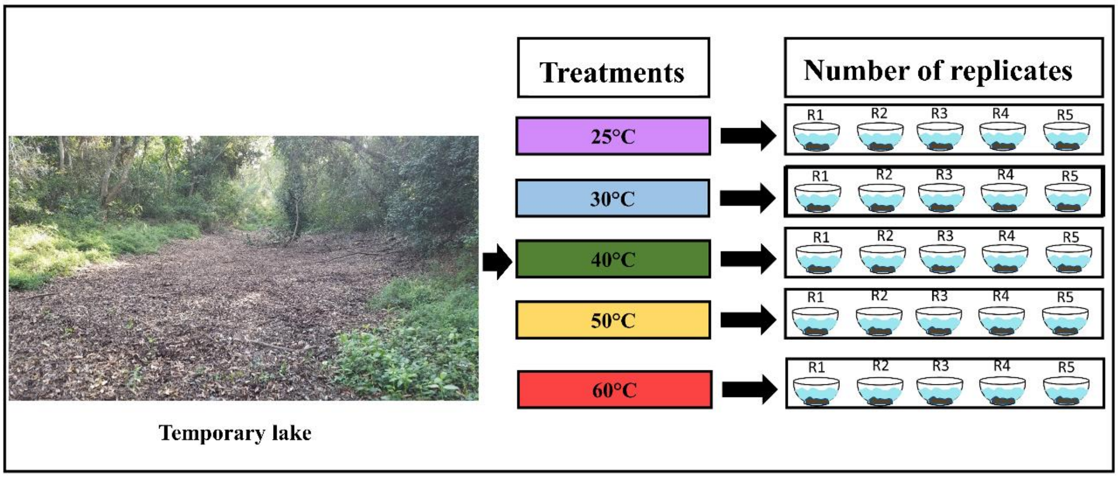

Figure 1. Representative scheme of the experimental design. R1 - R5 are the replicates of the samples (microcosms) dried at each temperature.

The microcosms were hydrated with 300 ml distilled water until they reached a depth of three centimetres. The microcosms were kept in a germination chamber at 25° C, with a 12h:12h photoperiod (Rosa et al., 2020). The microcosms were monitored weekly for a period of eight weeks.

5.2.3 Microfauna analysis

We analysed different communities of the aquatic microfauna, namely ciliates, rotifers and three groups of microcrustaceans (cladocerans, copepods and ostracods). Different procedures to sort the different biological groups were used, because of their different body sizes. First, we sorted living organisms (ostracods and ciliates), then we fixed the samples to sort rotifers, cladocerans and copepods.

5.2.3.1 Ostracods

The water from the microcosm was filtered using a plankton net (68 µm). After the filtering, fresh distilled water was added in the microcosms. Ostracods was sorted from the material retained in the plankton net under a stereomicroscope following the protocol of Rosa et al. (2020). Hatched juveniles were separately kept in glass vials with distilled water, fed with fresh spinach until reared to the adult stage for identification and counting. The ostracod species were identified following Higuti et al. (2013) and Almeida et al. (2023).

5.2.3.2 Ciliates

The ciliate identification and counting were performed with both material, retained in the plankton net (after sorting the ostracods) and the filtered water of each microcosm. Water samples were concentrated in 100 mL using a net with 10 µm mesh size. Quantitative counting was performed using 10 aliquots on a microscopy slide from 100µL of sample (1mL in total) under an optical microscope. In order to record rare ciliate species not found during quantitative counting, a qualitative analysis was also performed in Sedgewick Rafter chamber using 1 mL of sample. Ciliates were quantified and identified in *vivo* to the lowest possible taxonomic level using specialized bibliography (e.g. Foissner & Berger, 1996). Ciliate communities from 60°C treatment were not sampled owing to their sensitivity to high temperatures (Lu et al., 2021).

5.2.3.3 Rotifers, cladocerans and copepods

After the counting of ciliates in *vivo*, the same water was filtered again in a plankton net (68 µm) and the retained material was fixed in 70% alcohol for subsequent zooplankton sorting. Finally, all samples were fully counted, and zooplankton identified to species level (whenever possible) under optical microscope. The identification was carried out with specialized literature for rotifers (Koste & Voigt, 1978; Joko, 2011), cladocerans (Smirnov, 1971; Elmoor-Loureiro, 1997) and copepods.

5.2.4 Data analyses

All analyses were performed in R software 3.3.1 (R Development Core Team, 2013). The analyses were performed separately for the distinct communities, ciliates, rotifers and microcrustaceans. The microcrustaceans (cladocerans, copepods and ostracods) were grouped together because of the low number of samples that hatched eggs.

To analyse the differences in richness between the temperature treatments, we performed an analysis of variance (One-way ANOVA; “aov” function) or, when the assumptions for ANOVA (normality, homoscedasticity) were not reached, we applied a non-parametric analysis of variance (Kruskal Wallis; “kruskal.test” function) with a significance level set at $p < 0.05$. Afterwards, the Tukey (“TukeyHSD” function) or Dunn (dunn.test function for the package “PMCMR”) post-hoc tests were performed.

We performed a principal coordinate analysis (PCoA) to visualize the (dis)similarities in the species composition between different temperature treatments. We used a presence/absence matrix of the aquatic microfauna, which had hatched weekly, applying the Jaccard distance (Legendre & Legendre, 1998). A Multivariate Permutational Analysis of Variance (PERMANOVA - using the "adonis" function of the vegan package (Anderson, 2001)) was performed to evaluate statistical differences in the species composition between the treatments. This test was also based on a presence/absence matrix using the Jaccard distance. A total of 999 permutations were performed to assess significant differences. To test the hypothesis of biotic homogenization we also performed a "Permutational Multivariate Dispersion Analysis" (PERMDISP using the "betadisper" function in the vegan package) to estimate the multivariate dispersion of species composition in each temperature treatments.

5.3 Results

A total of 80 taxa of aquatic microfauna hatched from the 200 samples (25 microcosms x 8 weeks): 60 species of ciliates, 11 species of rotifers and nine species of microcrustaceans (six cladocerans, two ostracods and one copepod) (Table 1). Considering the overall richness of the microfauna in each temperature treatment, the highest richness values were recorded at 30°C (44 species) and 25°C (40 species) treatments. The same trend was observed for the number of unique specie: 17 species at the 25°C treatment and 14 species at the 30°C treatment (Figure 2). On the other hand, the lowest values of richness and unique species were recorded at the highest temperatures, 50°C and 60°C (Figure 2). At the 60°C treatment, only one microcrustacean (cladoceran) species was found (Figure 2).

Table 1. List of dormant aquatic microfauna species hatched from the sediment, dried at different temperatures.

	TEMPERATURES				
SPECIES	**25°C**	**30°C**	**40°C**	**50°C**	**60°C**
PROTIST CILIATES					
Acineria incurvata Dujardin, 1841			X		
Acineria uncinata Tucolesco, 1962	X		X		
Chaenea stricta (Dujardin, 1841)	X				
Chilodonella uncinata (Ehrenberg, 1838)	X		X		
Coleps hirtus (Müller, 1786)	X				
Colpidium colpoda (Losana, 1829)	X				
Colpoda steinii Maupas, 1883		X	X	X	
Dexiostoma campylum (Stokes, 1886)			X		
Dexiotricha granulosa Stokes, 1885		X			

Didinium nasutum (Muller, 1786)		X	X	
Discomorphella pectinata (Levander, 1894)			X	
Dysteria fluviatilis (Stein, 1859)	X			
Enchelys gasterosteus Kahl, 1926	X	X	X	
Epistylis galea Ehrenberg, 1831	X	X	X	
Epistylis sp.			X	
Euplotes affinis (Dujardin, 1841)	X	X		
Euplotes moebiusi Kahl, 1932		X		
Gymnostomatida				X
Halteria grandinella (Müller, 1773)	X	X	X	X
Halteria sp.1	X	X	X	X
Halteria sp.2	X	X	X	
Histriculus vorax (Stokes, 1891)	X			
Holophrya sp.		X		
Hymenostomatida			X	
Hypotrichia sp.1	X			
Hypotrichia sp.2	X			
Hypotrichia sp.3		X		
Hypotrichia sp.4		X		
Hypotrichia sp.5		X		
Hypotrichia sp.6	X			
Kahlilembus attenuatus (Smith, 1897)	X	X		
Lagynophrya acuminata Kahl, 1935	X	X		X
Limnostrombidium viride (Stein, 1867)		X	X	
Limnostrombidium sp.1	X	X	X	X
Litonotus sp.		X		
Metopus spp.sensu lato	X		X	
Monodinium balbianii Fabre-Domergue, 1888	X	X		
Oligotrichida sp1	X			
Oxytricha chlorelligera Kahl, 1932	X	X		X
Paraurostyla viridis (Stein, 1859)		X		
Rimostrombidium humile (Pénard, 1922)	X	X		
Rimostrombidium lacustres (Foissner, Skogstad & Pratt, 1988)		X		X
Scutico sp.1		X	X	
Stichotricha secunda Perty, 1849	X			
Stylonychia vorax Stokes, 1885	X	X	X	X
Stylonychia sp.1		X	X	
Thuricola sp.		X		
Trachelophyllum apiculatum Claparède & Lachmann, 1859		X		
Uronema nigricans Florentin, 1901		X		
Urotricha farcta Claparède & Lachmann, 1859	X	X	X	X
Urotricha sp.1	X	X	X	X

Vorticella aquadulcis-complex Stokes, 1887	X				
Vorticella campanula Ehrenberg, 1831	X				
Vorticella convallaria-complex Linnaeus, 1758	X				
Vorticella octava-complex Stokes, 1885	X				
Vorticella sp.1	X				
Ciliate sp. 1	X				
Ciliate sp. 2	X				
Ciliate sp. 3			X		
Ciliate sp. 4		X			
ROTIFERS					
Bdelloidea		X	X		
Beauchampiella eudactylota (Gosse, 1886)		X	X	X	
Brachionus falcatus Zacharias, 1898			X		
Cephalodella sterea (Gosse, 1887)			X		
Collotheca ornata (Ehrenberg, 1832)				X	
Collotheca sp. 1			X	X	
Lecane bula (Gosse 1851)			X		
Lecane furcata (Murray, 1913)	X	X			
Proales syltenensis Tzschaschel, 1979	X	X			
Ptygura pilula (Cubitt, 1872)		X			
Pitigura sp.1		X			
CLADOCERANS					
Alonella dadayi Birge, 1910			X	X	
Ceriodaphnia cornuta Sars, 1885			X		
Ceriodaphnia richardi Sars, 1901		X			
Chydorus pubescens Sars, 1901	X	X	X	X	X
Euryalona brasiliensis Brehm & Thomsen, 1936		X		X	
Kurzia polyspina Hudec, 2000				X	
COPEPODS					
Cyclopoida		X	X		
OSTRACODS					
Brasilocypria ricardopintoi Almeida *et al.* 2023	X	X			
Strandesia lansactohai Higuti & Martens, 2013	X			X	

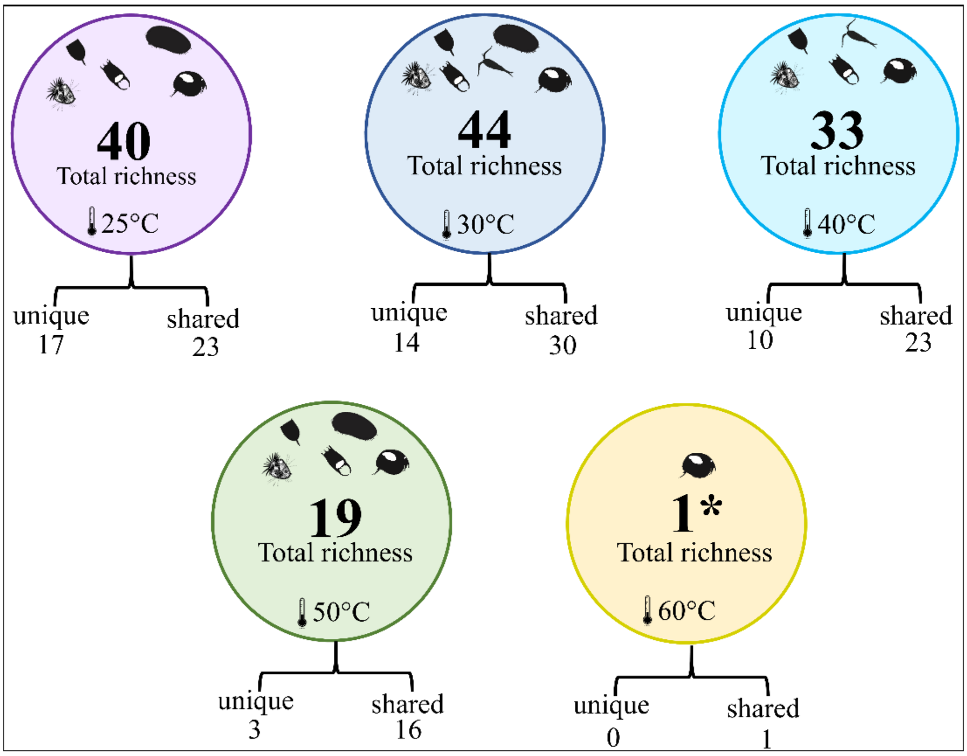

Figure 2. Schematic model with values of total richness, unique and shared species of dormant aquatic microfauna in different temperature treatments. * Ciliates were not sampled in the 60°C treatment.

All communities showed the same trend of reduction in the number of species at higher temperature treatments (Figure 3). The highest values of ciliate species richness were observed at 25°C treatment, and the lowest richness at 50°C (Figure 3A). For the rotifers, the highest richness was recorded at the 25°C, 30°C and 40°C treatments, while the lowest richness values were observed at the 50°C and 60°C treatments, with no hatchings at all at the 60°C treatment (Figure 3B). Finally, the highest values of microcrustacean richness were recorded at the 30°C and 50°C treatments, and the lowest richness at the 60°C treatment (Figure 3C).

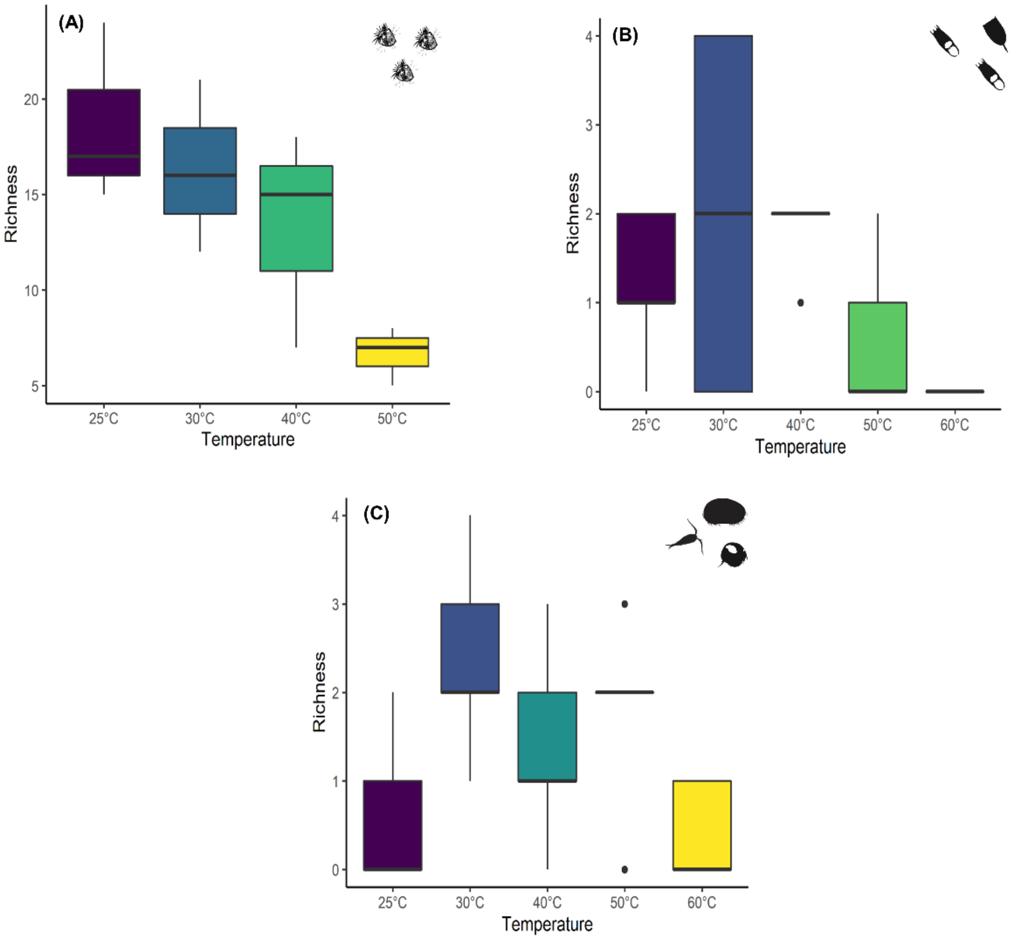

Figure 3. Mean richness of microfauna in different temperature treatments. Bars represent standard errors. (A) Ciliates; (B) Rotifers and (C) Microcrustaceans.

All analyses of variance showed significant differences in the species richness between the different temperature treatments for all communities (ciliates: ANOVA, p= 0.04; rotifers: Kruskall Wallis, p= 0.03; microcrustaceans: ANOVA, p=0.02). The post hoc test showed significant different between the 25°C and 50°C treatments for ciliates, between the 40°C and 60°C for rotifers, and between 30°C and 60°C for microcrustaceans (Table 2).

Table 2. Results of post hoc tests for species richness of aquatic dormant microfauna in different temperature treatments. The Tukey post-hoc test was performed for ciliates and microcrustaceans, and the Dunn post-hoc test was performed for rotifers. Significant values in bold ($p<0.05$). * = ciliates were not sampled in the 60°C treatment.

Temperature	**Ciliates p value**	**Rotifers p value**	**Microcrustaceans p value**
25°C x 30°C	0.91	0.99	0.06
25°C x 40°C	0.48	0.97	0.70
25°C x 50°C	**0.04**	0.90	0.34
25°C X 60°C	*	0.71	0.99
30°C x 40°C	0.83	0.96	0.51
30°C x 50°C	0.10	0.98	0.87
30°C x 60°C	*	0.30	**0.03**
40°C x 50°C	0.31	0.81	0.96
40°C x 60°C	*	**0.04**	0.51
50°C x 60°C	*	0.99	0.20

The result of the Principal Coordinate Analysis (PCoA) combined with the Permutational Multivariate Analysis of Variance (PERMANOVA) showed significant differences in the species composition of all communities (ciliates, rotifers and microcrustaceans) at the different temperature treatments ($p< 0.05$; Figure 4).

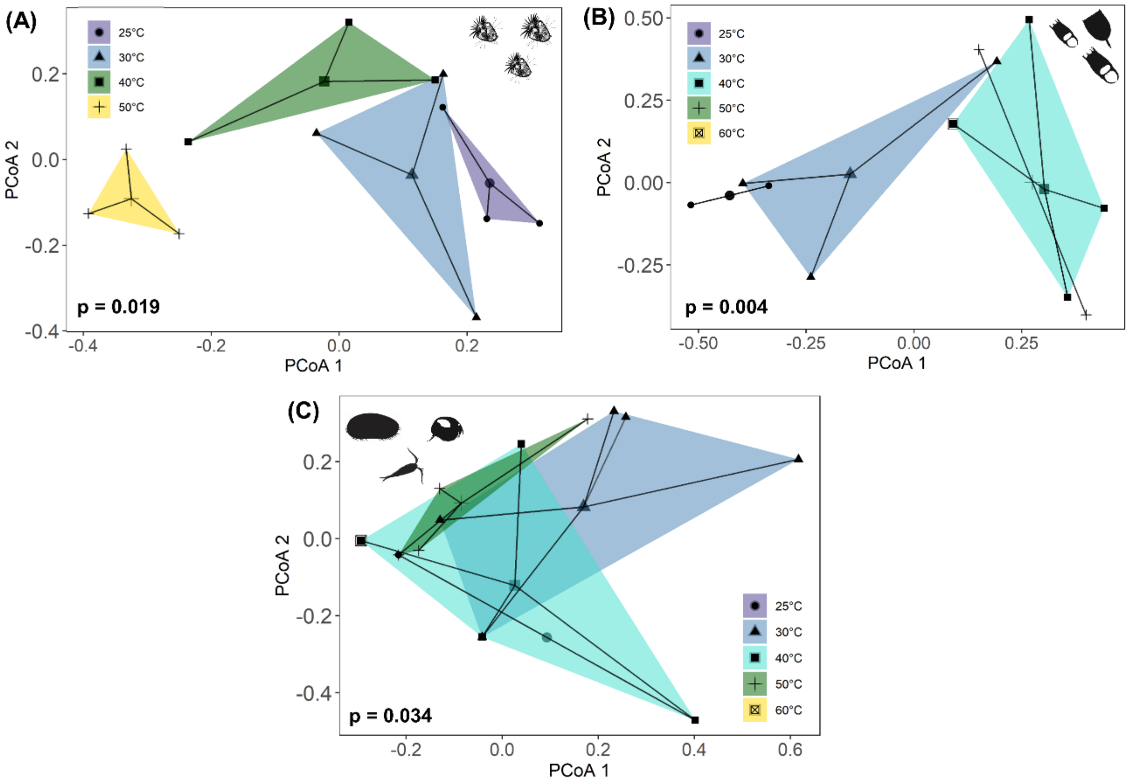

Figure 4. Principal Coordinate Analysis (PCoA) of the species composition of dormant aquatic microfauna in the different temperature treatments. (A) Ciliates; (B) Rotifers and (C) Microcrustaceans.

The species composition variability values (PERMDISP) reduced significantly with increasing temperatures for the ciliate and microcrustacean communities. In contrast, for the rotifers, no significant differences were observed in the composition variability values between the different temperature treatments (Figure 5; Table 3).

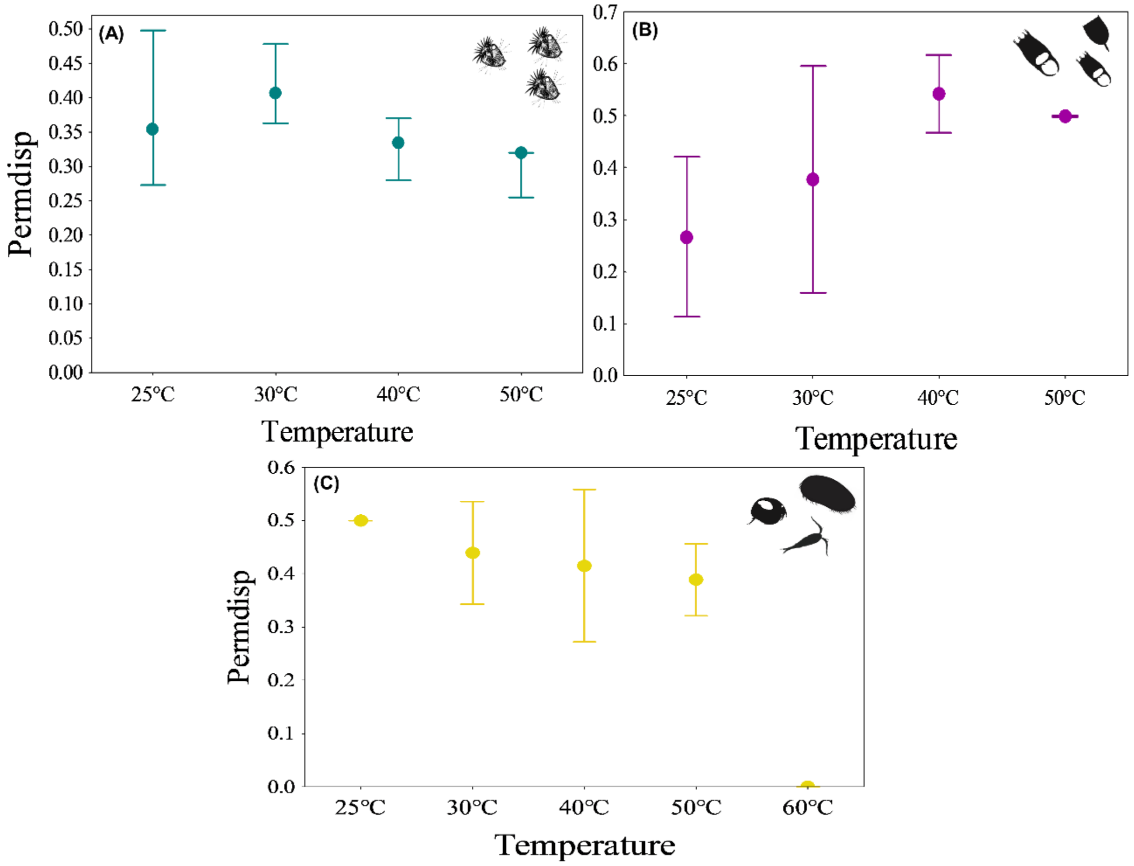

Figure 5. Mean distance of sample units to the centroid assessed by the test of homogeneity of dispersion (PERMDISP). (A) Ciliates; (B) Rotifers and (C) Microcrustaceans.

Table 3. Results of post hoc tests derived from PERMDISP values of aquatic dormant microfauna in different temperature treatments. Significant values in bold (p<0.05). * = ciliates were not analysed in the 60°C treatment; nd = no rotifers hatched in the 60°C treatment.

Temperature	Ciliates p value	Rotifers p value	Microcrustaceans p value
25°C x 30°C	0.60	0.69	0.75
25°C x 40°C	0.54	0.15	0.72
25°C x 50°C	0.34	0.41	0.35
25°C x 60°C	*	nd	**0.00**
30°C x 40°C	0.11	0.41	0.87
30°C x 50°C	**0.02**	0.72	0.67
30°C x 60°C	*	nd	**0.05**
40°C x 50°C	0.41	0.78	0.85
40°C x 60°C	*	X	0.11
50°C x 60°C	*	X	**0.02**

5.4 Discussion

5.4.1 Effects of higher temperatures on species richness

The increase of temperature led to a reduction in the species richness of aquatic microfauna, corroborating our hypothesis, which can be attributed to the fact that high temperatures can affect the viability and/or hatchability of dormant structures. Nielsen et al. (2015) also observed a reduction in the aquatic biota richness from sediment dried at high temperatures (above 50°C). An increase in temperatures is one of the recognised triggers for breaking dormancy (Chaki et al., 2021), but when these temperatures become excessively high, the viability of the dormant structures can be negatively affected (Nielsen et al., 2015). Most of the dormancy structures are formed by chitin layers that prevent desiccation during drought events (Lubzens et al., 2010; Sargin et al., 2019). However, the efficiency of protection by these chitin layers seems to be affected when drought conditions are combined with high temperatures.

Most dormant structures viable for hatching are found in the first few centimetres of sediment in temporary aquatic environments (Gleason et al., 2003). However, this surface sediment is strongly influenced by high air temperature (Ooi et al., 2009), negatively affecting the viability or hatchability of the dormant structures (Nielsen et al., 2015). The present trend of globally increasing temperatures, owing to human activities, also make that extreme heat events are more frequent and more prolonged (Masson-Delmotte et al., 2021). Recently, several countries have recorded heat waves exceeding 40°C (Kew et al., 2019), which will cause sediment temperatures to be even higher, thus negatively affecting the dormant faunal and floral structures.

The decrease in the number of viable or hatchable dormant species in the egg banks at high temperatures is problematic, as these structures are responsible for the recolonization of freshly inundated aquatic environments, especially temporary ponds and lakes, after extreme events such as extended drought periods (Chittapun et al., 2005; Cunillera-Montcusí et al., 2021; Rosa et al., 2020; 2021a). In addition, egg banks have accumulated dormant structures of species from different generations, reflecting the ecological memory of environments, and thus contribute to the dispersal processes and resulting diversity (Padisák, et al., 1992; Rosa et al., 2021a; 2022). However, at high temperatures, the recolonization, diversity and dispersal abilities are compromised, owing to the reduction of the richness of the egg banks.

5.4.2 Thermal limits of different taxonomic groups

The dormant structures of the organisms show different thermic tolerances for survival and hatching ability, depending on the taxonomic group. Some groups (such as cladocerans and ostracods) showed a higher tolerance to high temperatures, while others (such as ciliates) were more sensitive to increases in temperature. These differences can be related to chemical components of dormant structures, their physiology and the specific environmental triggers required for hatching (Chaki et al., 2021). For example, some recent laboratory experiments have shown a high hatching success of microcrustaceans, especially ostracods, in sediment dried at 50°C (Rosa et al., 2020; 2021a; 2021b).

Resting eggs of ostracods have a double-wall of chitin impregnated with calcium carbonate, and separated by a fluid, which allows it to withstand drought events (Smith et al., 2015). In addition, these microcrustaceans require higher temperatures for the breaking of dormancy, when compared to other groups. Some studies report that microcrustacean hatching increases at higher temperatures (Jones & Gilbert, 2016; Bandeira et al., 2021). Chaki et al. (2021) observed that increasing temperatures cause an increase in the hatchings of microcrustacean (cladocerans, copepods and ostracods) resting eggs. This is congruent with the present results, since higher values of microcrustacean richness were found at 30°C and 50°C. However, it is important to highlight that temperatures above 50°C are excessively high and influence hatching of microfauna negatively. In contrast, the fact that ciliates are more sensitive to temperature increase can be related to the type of dormant structures of ciliates. Indeed, cysts are more fragile when compared to the resting eggs of microcrustaceans. For example, the oligotrichids ciliates have fragile cyst walls composed of chemical components such as proteins (Kim et al., 2008), which are less robust than chitin and may thus be less efficient in protecting cyst viability.

The sensitivity/specificity of each group towards different temperatures may be the factor responsible for variations in species composition over the temperature gradient. The reduction of richness at high temperatures can also contribute to variation in species composition. Chaki et al. (2021) observed a significant effect of temperature on the species composition of the hatched microcrustaceans in their experiments. In addition, the higher number of unique species of ciliates (e.g. *Vorticella campanula*), rotifers (e.g. *Ptygura pilula*) and microcrustaceans (e.g. cladoceran: *Ceriodaphinia richardi*) in the lower temperature treatments probably contributed to the changes in species composition along the temperature gradient.

5.4.3 Biotic homogenization

Based on our results we observed that the increase in temperature led to biotic homogenization of the dormant aquatic microfauna, consequently leading to loss of species in the egg bank. In addition, biotic homogenization was also intensified by the loss of species at high temperatures. For the ciliates it was evident the decrease in the richness with the increase of temperature, remaining only the tolerant species, for example, *Halteria grandinella* e *Urotricha farcta* that contributed to the similarity between the treatments. The biotic homogenization has been reported for the active community of aquatic microfauna (Petsch, 2016) as a result of the temperature increase, however this information for the dormant communities is novel. This is worrying because the biotic homogenization in the egg bank may affect the ecosystem structure and functioning promoting the decrease of resilience (Rosa et al., 2021a).

5.5 Conclusion

High temperatures in the dried sediment, above 50°C, affect the viability of dormant structures, thus decreasing species richness in dormant communities, and consequently also the resilience of living aquatic communities in temporary aquatic environments. This is especially so for ciliate communities. In addition, temperature increases also lead to homogenization and the replacement of the dormant community species. This is alarming because extreme heat events are expected to become more frequent and prolonged. Thus, climate change, such as global warming, are pervasive and growing threats to aquatic dormant microfauna. Understanding the direction and magnitude of ecological responses of the dormant microfauna under high temperature conditions will allow human populations to prepare for these changes and try to mitigate the negative effects on biodiversity and environmental resilience.

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6 CONCLUDING REMARKS

The aquatic dormant microfauna (ciliates, rotifers, cladocerans, copepods and ostracods) analysed here showed a high species richness in the egg banks of the floodplain lakes. We analyse different ecological aspects (species phenology, dispersal, abiotic and biotic factors) on dormant communities. However, in Neotropical environments, there are still gaps in methodological studies addressing on the hatching of dormant structures, specifically focusing on different group of aquatic microfauna, as well as lack of studies on the biological interactions that occur in the egg bank.

For the ostracod community, drying the sediment at 50°C has been an optimal trigger to break of dormancy of the eggs. The hatching time of resting eggs has been influenced by their functional traits and taxonomic distance, which congeneric species have a similar hatching time. In addition, the hatching time of ostracod resting eggs has delayed compared to other aquatic microorganisms. The dried roots of *Eichhornia crassipes* shelter and transport ostracod resting eggs. This dispersal can facilitate the process of invasion by aquatic invertebrates. These findings can occur in other aquatic communities (e.g. cladocerans).

The aquatic microfauna (ciliates, rotifers and microcrustaceans) has been also evaluated, and has been observed that the quality of the sediment can influence the viability and structure (richness and composition) of these dormant communities. The presence of fungi in the sediment showed a positive effect on the structure of the aquatic dormant microfauna, i.e., fungi do not affect the viability of the dormant structures. It is relevant because sediments with fungi can be used for microfauna monitoring, since these samples are usually discarded. Finally, using experimental approaches, it was found that high temperatures, above 50°C, reduce the viability of dormant structures of dormant aquatic microfauna leading to biotic homogenisation of the egg bank in temporary environments. This is worrying because climate change, increases the temperature, will negatively affect the dormant structures, highlighting the importance of mitigation measures for the conservation of the egg bank.

COMPLEMENTARY INFORMATIONAL NOTE – List of activities performed and published papers during the thesis.

EDUCATION

1) Experiências do doutorado no exterior. Palestra proferida ao Programa de Pós graduação em Ecologia de Ambientes Aquáticos Continentais – Universidade Estadual de Maringá, Maringá, PR. 2022.

2) Ecologia de macrófitas. Palestra proferida na 5ª semana de extensão em ecossistemas límnicos: métodos aplicados a estudos de ecossistemas aquáticos – Universidade Estadual do Norte do Paraná, Jacarézinho, PR. 2022.

3) Aplicação do Curso Teórico-prático de ecologia de comunidades aquáticas. UNINGÁ, Maringá, Paraná. 2022.

4) Participação como membro titular da banca examinadora de defesa de Trabalho de Conclusão de Curso da pós-graduanda Lilian dos Santos. Título: “Percepção da sociedade em relação à ecotoxicologia”. Curso de curso de especialização em Conservação da Biodiversidade em Ecossistemas Alterados, Universidade Estadual do Paraná (UNESPAR), Campus União da Vitória, PR. 2022.

5) Participação como membro titular da banca examinadora de defesa de Trabalho de Conclusão de Curso da aluna Lara Hoffmeister Luz. Título: “ Macrofauna associada às macrófitas nativa *Egeria najas* Planch. e invasora *Hydrilla verticillata* (L.f.) Royle na planície de inundação do alto rio Paraná”. Curso de Graduação em Ciências Biológicas, Universidade Estadual de Maringá (UEM), PR. 2022.

6) Orientação da aluna Lara Hoffmeister Luz no Programa Institucional de Bolsas de Iniciação Científica - PIBIC/CNPq. Projeto intitulado: "Efeito da temperatura sobre a eclosão dos ovos de resistência de ostrácodes (Crustacea, Ostracoda): uma abordagem experimental baseada em mudanças climáticas". Data: 01/09/2020 a 31/08/2021.

7) Orientação da aluna Rayka Silva Santos no Projeto de Alfabetização Científica e os problemas ambientais locais: alunos pesquisadores na escola da Universidade Estadual de Maringá e do Programa Ciência na escola. Projeto intitulado: "Sensibilização sobre os efeitos das queimadas em regiões urbanas: impactos no meio ambiente e saúde humana". Data: 02/2020 – 12/2021.

8) Aplicação do Minicurso: "Análise de dados ecotoxicológicos utilizando o software R" ministrado no projeto de extensão Diálogos sobre a Ecotox!. Universidade Estadual do Paraná (UNESPAR) Campus de União da Vitória – PR. 2020.

9) Participação como membro titular da banca examinadora de defesa de Trabalho de Conclusão de Curso da aluna Mayomie Bados Dos Santos. Título: "Comparação de efeitos ecotoxicológicos em peixes expostos a herbicida convencional e a herbicida natural". Curso de Graduação em Ciências Biológicas, Universidade Estadual do Paraná (UNESPAR), Campus de União da Vitória, PR. 2020.

10) Aplicação do Minicurso: "Introdução às ferramentas de análise de dados ecológicos" ministrado na II Jornada de Biologia: O Papel do Biólogo do Desenvolvimento da Pesquisa Científica. Universidade Estadual do Paraná (UNESPAR) Campus Paranavaí – PR. 2020.

11) Participação no curso de Extensão: Modelos Regressivos e Análises Multivariadas. 2020.

12) Participação no curso de Extensão "Manipulação e visualização de dados ecológicos no R". 2019.

13) Participação nos Ciclos de debates de ecologia de água doce – Universidade Estadual de Maringá, PR. 2019.

PUBLISHED PAPERS

1) *FERREIRA, V. G.; ***ROSA, J**.; *ALMEIDA, N.M.; PEREIRA, J.S.; VENDRAMIN, D.; ZHU, H.; MARTENS, K.; HIGUTI, J. A comparison of three main scientific literature databases using a search in aquatic ecology. **Hydrobiologia**, p. 1-10, 2022.

*These authors share the position as first authors.

2) LECHINOVSKI, L.; BADOS, M.; **ROSA, J**.; MODA, D.B.; BUENO KRAWCZYK, A.C.D. Ecotoxicological effects of conventional herbicides and a natural herbicide on freshwater fish (*Danio rerio*). **Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes**, v. 57, p. 1-9, 2022.

3) **ROSA, J**.; MARTENS, K.; HIGUTI, J. Dried aquatic macrophytes are floating egg banks and potential dispersal vectors of ostracods (Crustacea) from pleuston communities. **Hydrobiologia**, p. 1-11, 2022.

4) **ROSA, J**.; OLIVEIRA, F.R.; PEREIRA, L.F.; MELO SILVA, M.; BUENO-KRAWCZYK, A.C.D. Temporal variation in Oligochaeta species composition in an anthropized stretch of a Neotropical urban river. **International Journal of Limnology**, v. 58, p. 1-10, 2022.

5) CAMPOS, R.; **ROSA, J**.; FERREIRA, V.G.; CONCEIÇÃO, E. O.; MARTENS, K.; HIGUTI, J. Macrophyte life forms influence the effects of environmental and spatial factors on the beta-diversity of associated ostracod communities (Crustacea). **Aquatic Sciences**, v. 83, p. 1-15, 2021.

6) CAMPOS, R.; **ROSA, J**.; HIGUTI, J.; BUGGENHAGEN, T.C.; BUENO-KRAWCZYK, A.C.D. Diel variation of aquatic insect drift in streams of Southern Brazil. **Acta Scientiarum Biological Sciences**, v. 43, p. e54931-e54931, 2021.

7) **ROSA, J**.; GOLEC, C.; BONECKER, C.C.; MARTENS, K.; HIGUTI, J. A comparison between active and passive communities of Ostracoda (Crustacea) in a tropical temporary lake. **Studies on Neotropical Fauna and Environment**, v. 56, p. 112-123, 2021.

8) **ROSA, J**.; PETSCH, D. K.; MARTENS, K.; HIGUTI, J. SPECIES' traits and taxonomic distance can predict the ostracod resting eggs from tropical floodplain lakes. International Review of **Hydrobiology**, v. 106, p. 226-238, 2021.

9) MANTOVANO, T.; DINIZ, L.P.; CONCEIÇÃO, E.O.; **ROSA, J**.; BONECKER, C.C.; BAILLY, D.; FERREIRA, J.H.D.; RANGEL, T.F.; LANSAC-TÔHA, F.A. Ecological niche models predict the potential distribution of the exotic rotifer *Kellicotia bostoniensis* (Rousselet, 1908) across the globe. **Hydrobiologia**, v. 848, p. 299-309, 2021.

10) HIGUTI, J.; **ROSA, J**.; FERREIRA, V. G.; ALMEIDA, N. M.; CAMPOS, R.; CONCEIÇÃO, E. O.; MARTENS, K. Inter-annual variation of ostracod (Crustacea) communities in the Upper Paraná River floodplain, Brazil. **Oecologia Australis**, v. 24, p. 474-478, 2020.

11) **ROSA, J**.; CAMPOS, R.; MARTENS, K.; HIGUTI, J. Spatial variation of ostracod (Crustacea, Ostracoda) egg banks in temporary lakes of a tropical flood plain. **Marine and Freshwater Research**, v.72, p. 26-34, 2020.

12) De Camargo, N.S.J.; Lehun, A.L.; ROSA, J.; Bueno-Krawczyk, A.C.D. Colonization of benthic invertebrates on artificial and natural substrate in a Neotropical lotic environment in Southern Brazil. **Acta Scientiarum. Biological Sciences**, v. 41, p. e45872-e45872, 2019.

EXTENSION

1) Organização da 5ª semana de extensão em ecossistemas límnicos: métodos aplicados a estudos de ecossistemas aquáticos – Universidade Estadual do Norte do Paraná, Jacarézinho, PR. 2022.

2) UEM na Expoingá – mostra científica de diferentes grupos biológicos durante a Expoingá, Pró-Reitoria de Extensão e Cultura da UEM, Maringá, PR. 2019 e 2022.

3) Mostra científica promovida pelo PELD/CNPq – UEM – Nupélia – PEA na sede do Parque Estadual das Várzeas do Rio Ivinhema e na Base Avançada do Nupélia, Porto Rico. 2019 e 2022.

4) Projeto de Pesquisa: “A planície de inundação do alto rio Paraná – PELD” 2017 – 2022.

5) Projeto de Ensino, Pesquisa, Extensão: “Alfabetização Científica e os problemas ambientais locais: alunos pesquisadores na escola Universidade Estadual de Maringá, PR. 2020-2021.

6) Participação no projeto de extensão: Se todos camPEArem (Capacitação e Ação Motivada à Preservação, Educação Ambiental e Recuperação de Ecossistemas aquáticos), a conservação acontece - SOS Riachos de Maringá, PR. 2019-2020.