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JONATHAN DA ROSA

Egg banks of ostracods (Crustacea) in temporary lakes of a tropical floodplain

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Dissertação 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 Mestre em Ecologia e Limnologia.
Área de concentração: Ecologia e Limnologia

Orientadora: Dr.ª Janet Higuti
Coorientador: Prof. Dr. Koen Martens

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"As nuvens mudam sempre de posição, mas são sempre nuvens no céu. Assim devemos ser todo dia, mutantes, porém leais com o que pensamos e sonhamos."
(PAULO BELEKI)

Banco de ovos de ostrácodes (Crustacea) em lagoas temporárias de uma planície de inundação tropical

RESUMO

Os ambientes aquáticos frequentemente passam por eventos estocásticos de seca, os quais forçam as comunidades a utilizar estratégias de sobrevivência. Durante períodos de seca prolongada, alguns organismos aquáticos produzem ovos de resistência em resposta a essas condições, e assim persistir no ambiente. Avaliou-se o banco de ovos de ostrácodes em lagoas temporárias na planície de inundação do alto rio Paraná, Brasil para analisar a sua contribuição com a diversidade e sua variação espacial. Amostras de sedimentos foram coletadas, durante a estação de seca em lagoas temporárias, secas em estufa, e depois re-hidratadas e mantidas em câmara de germinação, com temperatura e fotoperíodo controlado. Duas distintas abordagens foram usadas. A primeira abordagem compara as comunidades passiva (banco de ovos) e ativa de ostrácodes em uma lagoa temporária (Lagoa Osmar). A riqueza e abundância de ostrácodes foram maiores na comunidade passiva e a composição de espécies diferiu entre as comunidades passiva e ativa. Isso esta associado às séries de diferentes ciclos de seca/inundação e acumulação dos ovos de resistência de diferentes gerações. A comunidade passiva adicionou táxons não encontrados na comunidade ativa, assim contribuindo para a manutenção e enriquecimento da fauna local e regional. Os eventos de inundação, provavelmente, contribuem para a entrada de ostrácodes alóctones na lagoa Osmar pelo plêuston de macrófitas flutuantes. A segunda abordagem avalia a variação espacial dos ovos de resistência de ostrácodes entre a margem e o centro de cinco lagoas temporárias. Os resultados mostraram que a abundância e a composição de espécies são homogêneas entre as diferentes regiões (margem e centro) das lagoas. Os eventos de inundação podem ser responsáveis pela homogeneização do banco de ovos, devido a conexão das lagoas com o canal principal do rio Paraná. Durante a inundação, um grande volume de água invade as lagoas temporárias e pode redistribuir os sedimentos. Treze espécies de ostrácodes eclodiram dos ovos de resistência (ou do estágio "torpidity"). Esses táxons são resilientes a eventos extremos e tem potencial invasor, devido a combinação de reprodução assexuada (partenogênese) e a dispersão passiva pelos ovos de resistência a seca. Os ovos de resistência contribuíram para a diversidade e estruturação das comunidades de ostrácodes nessas lagoas temporárias. Deste modo, esses ambientes representam um relevante "pool" regional de espécies para os ambientes de planícies de inundação tropical.

Palavras-chave: Microcrustáceos. Ovos de resistência. Comunidade passiva. Seca extrema. Variação espacial.

Egg banks of ostracods (Crustacea) in temporary lakes of a tropical floodplain

ABSTRACT

Aquatic environments often undergo stochastic drought events, which force communities to use survival adaptive strategies. During prolonged drought periods, some aquatic organisms produce drought-resistant eggs in response to these conditions, to persist in the environment. The main aim of this study was to evaluate the egg banks of ostracods in temporary lakes in the Upper Paraná River floodplain, Brazil, analyze the contribution to diversity and spatial variation. Sediment samples were collected during the dry season in these temporary lakes, and sediments were oven-dried, then re-hydrated and kept in germinating chambers, with controlled temperature and photoperiod. Two distinct approaches were used. The first approach compared the passive (egg bank) and active communities of ostracods in a temporary lake (Osmar Lake). The richness and abundance of ostracods were higher in the passive community, and species composition differed between passive and active communities. This is associated with series of different drought/inundation cycles and the accumulation of the resting eggs from different generations. The passive community added species not found in the active community, thus contributing to the maintenance and enrichment of the local and regional fauna. Flooding events probably also contributed to the entrance of allochthonous ostracod species in the Osmar Lake by pleuston of floating macrophytes. The second approach evaluated the spatial variation of ostracod resting eggs between the edge and the centre of five temporary lakes. The results showed that the abundance and species composition are homogeneous between different regions (edge and centre) of the lakes. Flood events may be responsible for the homogenization of the egg banks, owing to the connection of lakes with the principal channel of the Paraná River. During flooding, water masses powerfully enter the temporary lakes and can redistribute the sediments. Thirteen ostracod species hatched from resting eggs (or torpidity stages). These species are resilient to extreme events and have invasive potential, owing to a combination of asexual reproduction (parthenogenesis) and of passive dispersal by drought resistant eggs. The resting eggs contributed to the species diversity and structure of the ostracod community in these temporary lakes. Thus, these environments represent a relevant regional pool of species for tropical floodplain environments.

Keywords: Microcrustaceans. Resting eggs. Passive community. Extreme drought. Spatial variation.

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1 INTRODUCTION

River-floodplain systems are characterized by a variety of aquatic environments, such as closed and open lakes, backwaters, temporary pools, connecting channels and main channels of the rivers and tributaries (THOMAZ; BINI; BOZELLI, 2007). The geomorphological and hydrological conditions of the floodplains promote flood pulses (JUNK; BAYLEY; SPARKS, 1989), which cause significant variability in fluctuations of the flow regime. These, in turn, structure the flooding and drought cycles (NEIFF, 1990). However, currently drought periods have been more frequent and longer owing to climate change and anthropogenic impacts, changing the structure and resilience of the aquatic communities (DERKSEN; BROWN, 2012; LOOY et al., 2019). It is worth noting that the dynamics of temporary lakes in floodplains are governed mainly by flood pulses, which may result from heavy precipitation a long distance upstream, unlike completely isolated temporary ponds, which depend (almost) exclusively on local precipitation (WILLIAMS, 2006; WILLIAMS et al., 2010).

Owing to the unpredictable cycle of drought and inundation events of their habitat, most temporary pool organisms have the capacity to produce drought-resistant stages, which allow persistence of the species and populations in the environment during periods of drought (WILLIAMS, 2006; STRACHAN; CHESTER; BELINDA, 2015). Thus, these aquatic communities have two distinct phases of temporary pool ecosystems, the active phase, in favourable conditions of inundation, and a passive phase, in adverse drought conditions (WALSH, 2013).

The drought resistant stages accumulate in the sediment, forming an egg bank community (BROCK et al., 2003). These structures can remain viable for decades until inundation stimulates hatching (HAIRSTON; BRUNT; KEARNS, 1995). Thus, the egg banks contribute to diversity in the active phase and increase the possibility for both dispersal and persistence of populations in the environment over time (HAIRSTON; BRUNT; KEARNS, 1995; BRENDONCK; PINCEEL; ORTELLS, 2017).

The production, incubation, hatching and viability of the drought resistant stages are influenced by abiotic environmental factors (e.g. temperature, light intensity and duration, hydrostatic pressure) and biotic factors (e.g. presence of absence or pheromones of potential predators such as fish) (BRENDONCK, 1996). The passive community (the drought-resistant stages) 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 and individual history, including maternal effects (BRENDONCK, 1996; SANTAGELO et al., 2014; BRENDONCK; PINCEEL; ORTELLS, 2017).

One evolutionary strategy often associated to living in unpredictable temporary conditions is called bet-hedging. Bet-hedging this context means that not all eggs laid by a single generation will hatch at the same time. Some eggs will hatch at the first subsequent inundation, others require several floodings before they will hatch. This way, not all eggs in the egg bank will die if the first subsequent inundation is insufficient for the new generation to complete the life cycle and produce a new batch of eggs (EVANS; DENNEHY, 2005).

Ostracods are commonly found in egg banks of temporary ponds and lakes (HAY; JEKINS; KINGSFORD, 2018). They are small crustaceans (size range in the Neotropical: 0.3-5 mm) inhabiting a diversity of aquatic environments, such as rivers, channels, temporary and permanent lakes (HIGUTI et al., 2017). Most non-marine ostracod groups can produce resting eggs (HORNE; MARTENS, 1998). Three main modes of reproduction in non-marine ostracod species are known: fully sexual reproduction, mixed reproduction (males, sexual and asexual females), or fully asexual by parthenogenesis. The ostracods develop from eggs pass through eight juvenile instars until they reach the (ninth) adult instar (SMITH et al., 2015).

There are approximately 11 non-marine Ostracoda families, but the Cyprididae and the Candonidae represent more than 70% of the total specific diversity (MEISCH; SMITH; MARTENS, 2019). Five families of freshwater ostracods (Cyprididae, Candonidae, Darwinulidae, Ilyocyprididae and Limnocytheridae) are presently known from Brazil, and the Cyprididae are again the most speciose and abundant ostracod family (HIGUTI et al., 2010; ; HIGUTI; MARTENS, 2016; HIGUTI; ROCHE; MARTENS 2017; HIGUTI et al., 2017; PEREIRA et al., 2017).

Most species in the Cyprididae can produce resting eggs, but not all of the other families of non-marine ostracods can do so (e.g. Darwinulidae) (Horne & Martens, 1998). However, there are other structures and strategies used by ostracods to avoid desiccation, such as the torpidity stage in Candonidae (HORNE; MARTENS, 1998). But in general, very few studies have thus far addressed the evolutionary ecology of resting eggs of ostracods.

The ostracod resting eggs is a double-walled spheroide of chitin and calcium carbonate. In the space between the two spheroids there is fluid. Thus, these two characteristics of the egg allow it to withstand desiccation (SMITH et al., 2015). The eggs are sticky and can adhere to one another when placed in solid substrates (McLAY, 1978). The colour, size and shape of eggs can be quite variable (McLAY, 1978; SMITH et al., 2015).

The present dissertation has two manuscripts on resting eggs of ostracods in temporary lakes of the tropical floodplain. In the first manuscript, we compare the passive (the egg bank) and the active (living) communities in a temporary lake. In the second manuscript, we analyse the spatial variation of ostracod resting eggs between edge and central regions in five temporary lakes in the tropical floodplain of the Upper Paraná River.

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2 RESTING EGGS OF OSTRACODS (CRUSTACEA) IN A TROPICAL TEMPORARY LAKE: A COMPARATIVE APPROACH BETWEEN ACTIVE AND PASSIVE COMMUNITIES

ABSTRACT

Some microcrustaceans living in temporary water bodies produce drought-resistant stages under adverse conditions. These structures accumulate in the sediment, forming a bank of propagules that can remain viable for many years. Here, we evaluate the contribution of ostracod resting eggs to the structure of the active ostracod community in a tropical temporary lake. We test the hypotheses (1) that the passive community (the egg bank) is more species rich and has higher abundances than the active community, owing to the potential accumulation of eggs from several generations and seasons, and (2) that species of the active community can also be of allochthonous origin. In addition, we assess the species composition between passive and active communities. Sediment samples were collected monthly during the dry season in a temporary lake located in the Upper Paraná River floodplain, Brazil. Sediments were oven-dried, then re-hydrated with filtered lake water and kept in hatching chambers with controlled temperature and photoperiod. A total of 405 ostracods eggs hatched from the sediment, comprising eight species of Cyprididae. In the active community of this lake, only six ostracod species were recorded, only two of which also hatched from the resting eggs. Four species of the active community can be of allochthonous origin and were most likely introduced during the flood pulse in the floodplain or dispersal by other biotic vectors. Six species found in the passive community were not found in the active community. Egg banks thus clearly contribute to the maintenance and enrichment of both local and regional faunas.

Keywords: Microcrustaceans; resilience; dormancy; potential invasive; extreme weather events; floodplain.

2.1 INTRODUCTION

Temporary waters are habitats with recurrent cycles of wet and dry periods. Temporary pool or lake animals either survive the dry periods in the same locality as drought-resistant stages, or they recolonize the freshly inundated habitat from elsewhere (amphibians, flying insects) (Willians 2006; Sim et al. 2013; Radzikowski 2013). Thus these aquatic communities present two distinct life phases: the active phase, present in wet conditions, and the passive or dormant phase, characterized by the presence of drought-resistant or dormant stage (Radzikowski 2013; Walsh 2013).

Ostracods are one of the most common organisms found in temporary environments (Williams 1997; Hay et al. 2018), as many of these small freshwater crustaceans (0.3-5 mm in the Neotropical), can produce resting eggs, especially species of the family Cyprididae (Horne & Martens 1998). Ostracod resting eggs accumulate in the sediment over several generations and seasons forming the egg banks, and can remain viable for several dozens of years (Angell & Hancock 1989; Martens 1994).

The egg banks allow for long-term coexistence of organisms and contribute to the resilience of aquatic communities, i.e., with the ability of the communities to return to their equilibrium after disturbance (Pimm 1984; Brendonck & De Meester 2003).

The recruitment of the active community by hatching of resting eggs occurs when conditions of the environment become favourable (e.g. presence of sufficient water with favourable abiotic conditions) (Radzikowski 2013, Hay et al. 2018). However, some eggs may remain in dormancy, even though environmental conditions are appropriate to survive. This evolutionary strategy fits in to concept "bet-hedging" and serves as a protection against potential future catastrophic events in a habitat, such as drying of the habitat before the organisms could complete their life cycle and produce new resting eggs (Evans & Denneyi 2005).

The hatching of resting eggs can be triggered by environmental (abiotic and biological) stimuli (Gylströn & Hansson 2004). For instance, water level and hence hydrostatic pressure has been shown to be an important factor for the hatching of resting eggs, (Brendonck 1996; Williams 2006). Also temperature, daylight length and other factors can stimulate hatching (Paes et al. 2016). On the other hand, the presence of large predators (fish, salamandra) or even just their kairomones can inhibit hatching (Blaustein 1997). Finally, hatching success and timing can also be influence by (epi-) genetic factors, such as maternal effects (De Meester & Jager 1993).

Resting eggs play an important role in the structuring and the dynamics of populations and communities since they act as a mechanism of passive dispersal by both biotic and abiotic vectors. They can thus increase the genetic and taxonomic diversity of both the passive and the active community (Ellner & Hairston 1994; Brendonck et al. 2017).

Studies on passive communities are important to understand the functioning and dynamics of aquatic communities over time, because egg banks often harbour greater richness of species, when compared to the active community (Crispim & Watanabe 2001). Some studies have demonstrated that the zooplankton egg banks contribute to the taxonomic richness of the active community (Hairston 1996; Crispim & Watanabe 2001). For example, Palazzo et al. (2008a) recorded a cladoceran species for the first time in the Paraná River floodplain by hatching it from the egg bank from a temporary lake.

Here, we evaluate the contribution of ostracod resting eggs to the diversity and community structure in a tropical temporary lake. We test the hypotheses (1) that the passive community (the egg bank) is more species rich and has higher abundances than the active community, owing to the potential accumulation of eggs from several generations and seasons, and (2) that species of the active community can also be of allochthonous origin. In addition, we assess the species composition between passive and active communities.

2.2 MATERIAL AND METHODS

2.2.1 *Study area*

The study area is located in the Upper Paraná River floodplain, which belongs to Environmental Protection Area of the "Várzea" Islands of the Paraná River, Brazil. This floodplain is composed of a wide variety of environments, including rivers and tributaries, secondary channels, isolated and permanently connected lakes, backwaters and temporary lakes and ponds (Thomaz et al. 2004).

Osmar Lake (22º46'27.53 "S and 53º19'57.95"W) is an elongated, isolated and temporary lake located on Mutum Island, on the right bank of the Paraná River. It is about 60 meters long and 15 meters wide, with mean depth of 1.1 m when fully flooded (Fig. 1) (Roberto et al. 2009; Mormul et al. 2012). This lake has recurrent periods of drought. The vegetation around the lake shelters remnants of forest and aquatic macrophytes (e.g. *Oxycaryum cubense* (Poepp. & Kunth) Palla; *Polygonum punctatum* Elliot and *Polygonum ferrugineum* Wedd) (Thomaz et al. 2009; Ferreira et al. 2016). Here, we briefly characterize the Osmar Lake according to some environmental variables measured *in situ* during the

sampling of the active community (March and November 2004, March and September 2017). The mean value of water temperature was 26.3°C +/- 1.97. The pH of the lake was slightly acidic, ranging from 5.5 to 6.78. The range of variation of electrical conductivity was from 44.6 to 96.4 $\mu S.cm^{-1}$ and low values of dissolved oxygen were observed in the lake, ranging from 0.6 to 4.2 $mg.L^{-1}$.
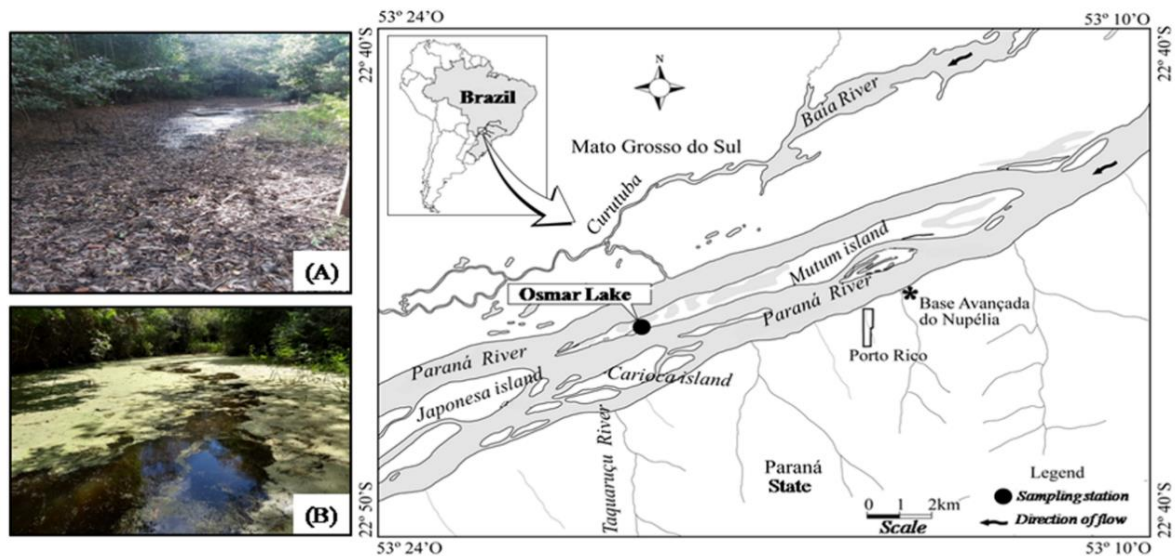
Fig. 1 – Location of the Osmar Lake in the Upper Paraná River floodplain. (A) drought period; (B) flooding period.

2.2.2 Water level variation of the Paraná River and precipitation

During the period between 2004 and 2017, measurements of the water level were taken twice a day through fixed rulers located on the left bank of the Paraná River and daily values of precipitation were obtained at the meteorological station, in the field station of Porto Rico from the State University of Maringá Brazil.

2.2.3 The passive community

The sediment (5 cm depth) was collected monthly during the drought period (May to September 2015), in two random sites of the central region of the Osmar Lake, using a core sampler. The sediment samples (approximately 500 grams) were stored in plastic bottles and kept under refrigeration at 4° C (Maia-Barbosa et al. 2003), until the hatching test in March 2017. Ten artificial microcosms (plastic trays) were prepared for hatching of resting eggs of ostracods, from five drought months and two sites of the lake.

The following protocol was used for hatching of resting eggs. First, the samples were placed in an oven at 50ºC during 24 hours for complete drying of the sediment (Nielsen et al. 2015). Then the samples were hydrated with the water of the lake (500 mL), filtered through a plankton net (10 μm mesh size), and kept in the germination chamber at 25° C, and with photoperiod 12 hours/light and 12 hours/dark during 149 days.

The incubation period was monitored in three phases: the first period was performed in intervals of every two days (14 days), the second period was weekly (105 days), and the third period was every 15 days (30 days). At each monitoring, all the water from the microcosm was filtered with a plankton net (15 μm mesh size), then the trays were re-filled with filtered lake water using a net of 10 μm mesh size. This protocol had previously been used for zooplankton (Fialek 2018). All specimens of ostracods were separated from other organism and detritus in a stereomicroscope.

Juveniles of ostracod were stored separately alive in petri dishes and were kept until they reached the adult stage (ninth instar) for species identification.

2.2.4 The active community

The ostracods were collected in March and November 2004 and March and September 2017 in the Osmar Lake. Samplings were performed by hauling a rectangular net (28 x 14 cm, mesh size 160 μm) close to the water-sediment interface in the littoral region of the lake and amongst macrophytes. The samples were preserved in ethanol (70º) and subsequently sorted under stereomicroscope.

Cytheridella ilosvayi Daday, 1905 was the most abundant species of the active community in the Osmar Lake. Thus, we performed a procedure to test the production of resting eggs and juvenile stages of dormancy (torpid stages) of this species. Samples were collected in June 2018 in a lake of the Ingá Park (23°25'29.00 "S and 51°55'46.80" W), located in Maringá, Brazil. The ostracods associated to the aquatic macrophyte *Ceratoptereris thalectroides* (Linné) Brongniart were collected removing the plants from the water by hand and placed immediately in a plastic bucket (Campos et al. 2017). The plants were washed in the bucket to release the organisms and the water was filtered in a 160 μm mesh net. The material was stored in plastic bottles.

In the laboratory, ostracods were sorted alive under stereomicroscope. Three artificial microcosms (glass vials) were prepared with 60 ml of distilled water. In each microcosm 30 females of *C. ilosvayi* with eggs were added and they were kept at room temperature until complete drying of the water during 14 days, for the possibility of producing resting eggs.

The microcosms were remained dry for 14 days, after that they were rehydrated again with distilled water. The material was monitored daily with a stereomicroscope during 30 days, in order to observe juvenile or hatching resting eggs.

2.2.5 Identification

The ostracods of passive and active communities were identified at specific level using Martens & Behen (1994) and papers included, Higuti & Martens (2012; 2014), and Higuti et al. (2013).

2.2.6 Data analysis

We compared the number of species (richness) and number of individuals (abundance) of the passive and active ostracod communities. The samples from the two central stations from the passive community were grouped for analysis of the data.

A non-parametric analysis of variance (Kruskal-Wallis) was performed to test significant differences of richness and abundance between the passive and active communities.

Principal coordinate analysis (PCoA) was used in order to summarise the variability of species composition between passive and active communities (Legendre & Legendre 1998). A Multivariate Permutational Variance Analysis (PERMANOVA) was performed to evaluate differences in ostracod species composition between passive and active communities (Anderson 2005). The test was based on a dissimilarity matrix using the Jaccard distance. A total of 999 permutations were performed to assess significance.

The dispersion homogeneity test (PERMDISP) was performed to test the variability in the ostracod species composition (beta diversity) between passive and active communities. This analysis is based on the distances between the sample units for the centroid of the group, using a dissimilarity measure (Jaccard), within a dimensional space calculated by PCoA. A higher mean of the Jaccard distance corresponds to a higher variability in species composition. The significance level ($p < 0.05$) of the variability in ostracod species composition between active and passive communities was tested using a permutation test (ANOVA).

The rarefaction curves were performed to compare species richness between both passive and active communities at comparable levels of individuals (Gotelli & Colwell 2001).

Statistical analyses were carried out in software R 3.2.4 (R Development Core Team 2018), using the vegan (Oksanen et al. 2016) and permute (Simpson, 2016) packages for

PCoA and PERMDISP, and according to the function "ADONIS" of the vegan package (Oksanen et al. 2016) for PERMANOVA. Rarefaction analysis was performed using BioDiversity Pro version 2 software (McAleece et al. 1997).

2.3 RESULTS

2.3.1 Water level variation of the Paraná River and precipitation

The results of the water level dynamics of the Paraná River over time showed several floods between 2004 and 2017 (see Fig. 2A, in which 350 cm is considered a reference value for overflow of the Upper Paraná River floodplain (Souza Filho 2009)). However, during the collection of the passive (2015) and active communities (2004 and 2017), low water levels of the Paraná River were observed (Figs 2B, C, D). A prolonged drought period with the lowest water levels occurred in 2015 (Fig. 2A, C), especially in July and August, during the collection of the passive community.
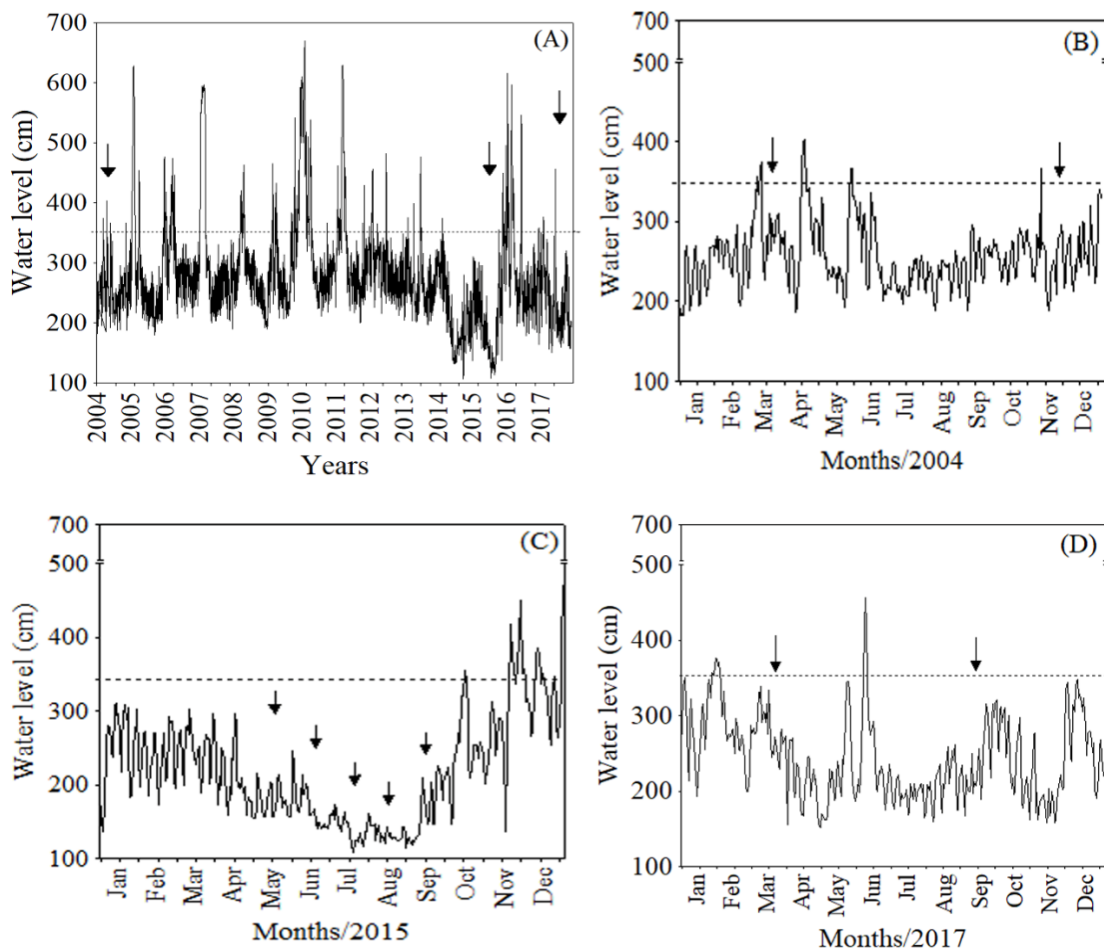
Fig. 2 – Water level variation of Paraná River from (A) 2004 to 2017, (B) in 2004, (C) in 2015 and (D) in 2017. The arrows indicate the times of sampling.

A large amplitude of precipitation occurred during 2004 and 2017 (Fig. 3A). However, at the actual times of collecting the passive (2015) and active (2004 and 2017) communities there was less precipitation (Figs. 3B, C, D).
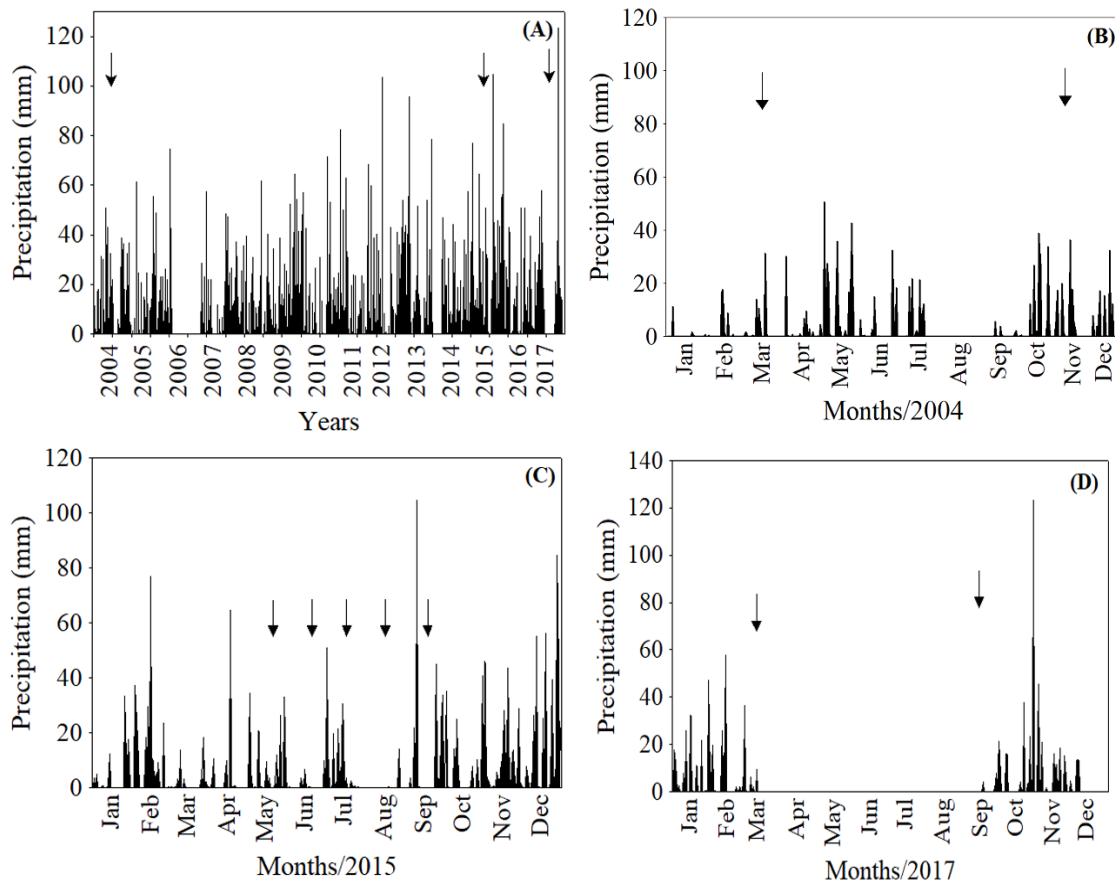
Fig. 3 – Precipitation values (A) from 2004 to 2017, (B) in 2004, (C) in 2015 and (D) in 2017 at the meteorological station at Porto Rico. Precipitation data is missing from April to August 2017, due to technical problems at the station. The arrows indicate the days of sampling.

2.3.2 The passive community

405 individuals and eight ostracods species hatched from the egg bank over the 145 days of incubation. All species belong to the family Cyprididae (Table 1, Fig. 4). The highest numbers of specimens and species were recorded in August (61%) (Fig. 4).

Table 1 – Ostracod species recorded in the passive community in 2015 and in the active community in 2004 and 2017 of the Osmar Lake in the Upper Paraná River floodplain, Brazil.

| | ACTIVE COMMUNITY | | | | PASSIVE COMMUNITY | | | | |
| | 2004 | | 2017 | | 2015 | | | | |
	Mar	Nov	Mar	Sep	May	Jun	Jul	Aug	Sep
Family Cyprididae (Baird, 1845)									
Chlamydotheca iheringi (Sars, 1901)						X		X	
Cypricercus centrurus (Klie, 1940)						X	X		
Cypridopsis cf. *vidua* sp.2	X	X				X	X		
Diaphanocypris meridana (Furtos, 1936)	X							X	
Strandesia bicuspis (Claus, 1982)					X	X	X	X	
Strandesia lansactohai Higuti & Martens, 2013						X			
Strandesia nupelia Higuti & Martens, 2013						X	X	X	
Strandesia velhoi Higuti & Martens, 2013					X	X		X	
Family Candonidae (Kaufmann, 1900)									
Candobrasilopsis brasiliensis (Sars, 1901)			X						
Candobrasilopsis rochai Higuti & Martens, 2012		X							
Pseudocandona agostinhoi Higuti & Martens, 2014	X								
Family Limnocytheridae (Kile, 1938)									
Cytheridella ilosvayi Daday, 1905	X	X	X						

     Strandesia bicuspis (Claus, 1982) was the most abundant and frequent species from the hatching of resting eggs, representing 34% of the total number of individuals. The second most abundant ostracod was *Strandesia nupelia* Higuti & Martens, 2013 in June, July and August 2015, represented by 23% of the total number of individuals. *Chlamydotheca iheringi* (Sars, 1901) was abundant in August 2015. *Diaphanocypris meridana* (Furtos, 1936) was found exclusively in August and *Strandesia lansactohai* Higuti & Martens, 2013 in June 2015 (Fig. 4).
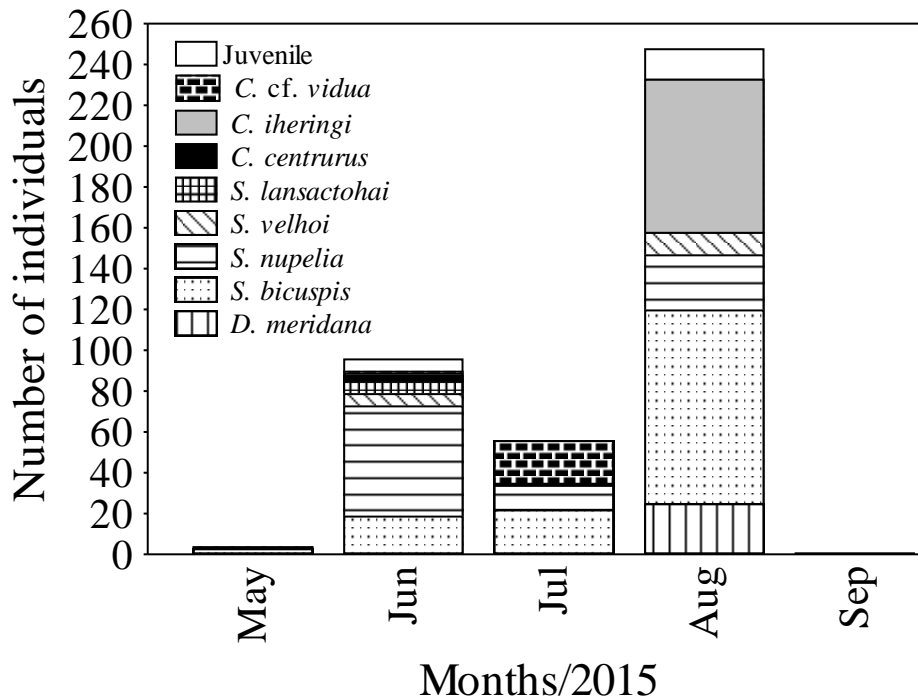
Fig. 4 – Number of individuals and species in the passive community, from May to September 2015, of the Osmar Lake in the Upper Paraná River floodplain.

2.3.3 The active community

In the active community, we recorded six species of ostracods, represented by two Cyprididae, three Candonidae and one Limnocytheridae (Table 1). Higher taxonomic richness as observed on March 2004 and higher abundance of ostracods in November 2004 (Fig.5).

Cytheridella ilosvayi Daday, 1905 was the most abundant species and represented 79% of the individuals collected (Fig. 5). The results of hatching experiment of *C. ilosvayi* evidenced no individuals after the addition of water in the microscosms, during 30 days of monitoring. However, juveniles were observed during water level reduction leading up to fully dry conditions in the tanks. *Cytheridella ilosvayi* did not produce neither resting eggs nor torpid stages in this experiment.

Three species of Candonidae were recorded only in the active community, *Pseudocandona agostinhoi* Higuti & Martens, 2014 and *Candobrasilopsis rochai* Higuti & Martens, 2012 were observed in 2004, and *C. brasiliensis* Higuti & Martens, 2012 in 2017 (Table 1, Fig. 5).
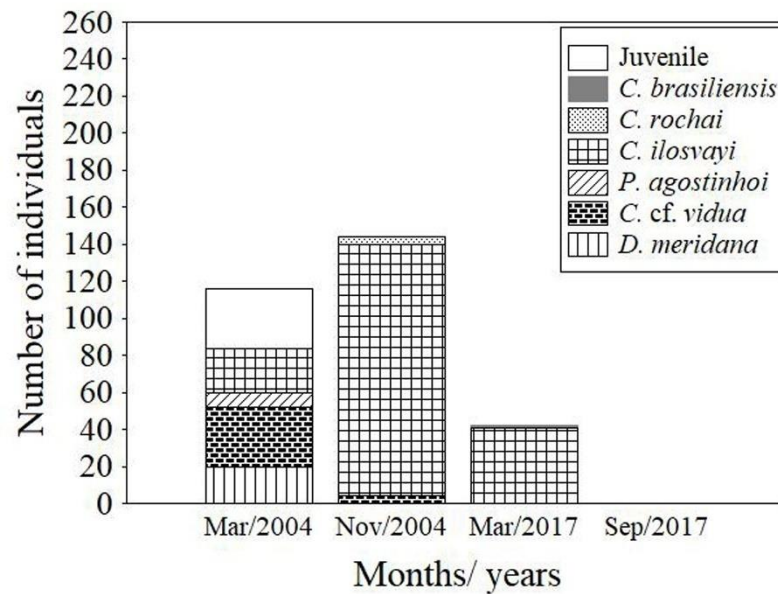
Fig. 5 – Number of individuals and species in the active community in 2004 and 2017 of the Osmar Lake in the Upper Paraná River floodplain.

2.3.4 The passive and the active communities

The rarefaction curve showed that ostracod richness reached an asymptote in both communities, which had a higher number of species in the passive community for the same number of individuals (Fig. 6). However, we did not observe significant differences in the richness (H = 0.30, p = 0.57) and abundance (H = 0.27, p = 0.57) between the passive and active communities.
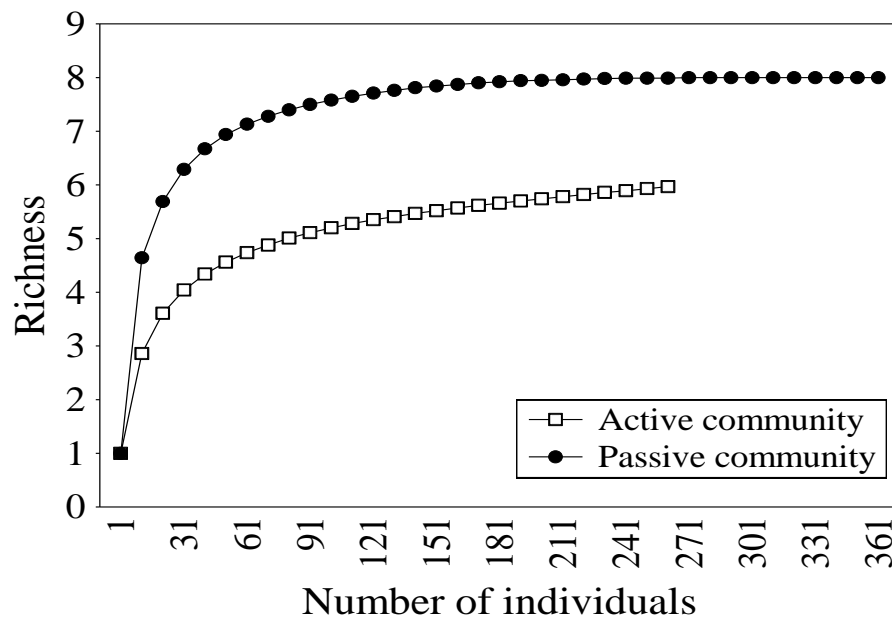
Fig. 6 – Rarefaction curves of the active and passive communities of ostracods of the Osmar Lake in the Upper Paraná River floodplain.

The PCoA ordered the variability of ostracod species composition between active and passive communities (Fig. 7). The species composition between active and passive communities was significantly different according to the results of PERMANOVA (F (1.8) = 3.99, p = 0.007).
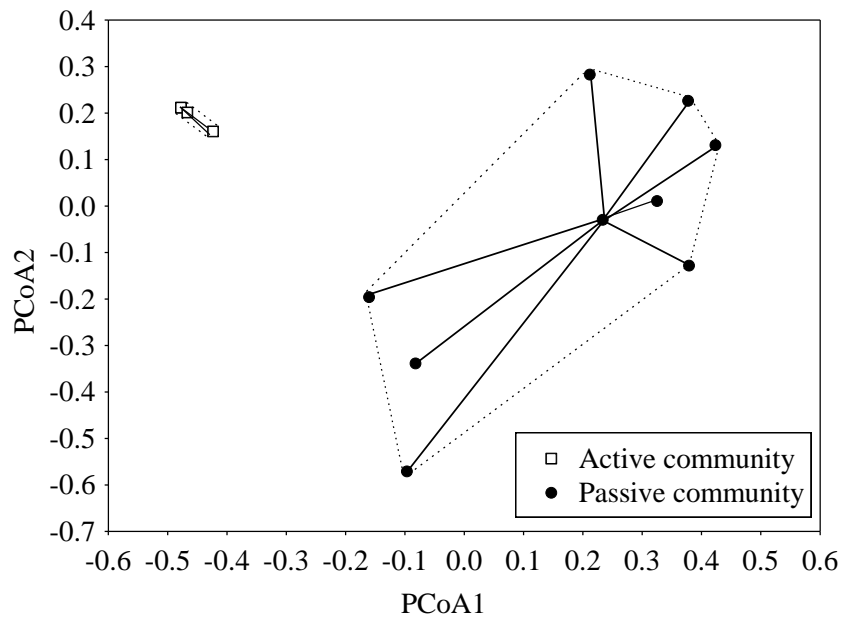
Fig. 7 – Principal coordinate analysis showing the variability in the ostracod species composition in both active and passive communities of the Osmar Lake in the Upper Paraná River floodplain.

The dispersion homogeneity test (PERMDISP) showed no significant differences (F = 1.63, p = 0.23) in the variability of ostracod species composition between active and passive communities, even though the average distance to the centroid was higher in the passive community (0.5323) compared to the active community (0.3800).

Only two species, *Cypridopsis* cf. *vidua* (O. F. Müller, 1776) and *D. meridana*, were common in both communities (Table 1).

2.4 DISCUSSION

2.4.1 The passive community

The eight ostracod species that hatched from the egg banks all belong to the family Cyprididae. The most species of Cyprididae can produce drought resistant eggs (Horne & Martens 1998), so this is not a real surprise. This strategy to produce drought resistant stages,

together with the ability to reproduce parthenogenetically and the ability to swim, are largely the causes of the evolutionary success of the group (Horne & Martens 1998). The production of drought resistant eggs at once allows survival in temporary habitats and facilitates the passive dispersion of these organisms by biotic and abiotic vectors, especially in combination with parthenogenetic reproduction in which one egg can be sufficient to establish a new population (Brochet et al. 2010). An example of invasive species of ostracods from South America to Europe is *Strandesia bicuspis*, one of the most abundant species in the passive community in the present study, which was introduced in the other continent by aquatic plants (Matzke-Karasz et al. 2014).

Drought events are common in the Upper Paraná River floodplain (see manuscript two). Such adverse conditions are responsible for the formation of the egg banks (Williams 1997; Brock et al. 2003). Our results showed that richness and abundance of the passive community of ostracod was higher, compared to the active community, in the temporary lake (Osmar Lake).These findings are in agreement with those of Palazzo et al. (2008b) and Sternet et al. (2017), who found that drought periods and hydrodynamic stress (e.g. reduction of connectivity between the environments) seem to cause higher richness of zooplankton and cladocerans, respectively.

The local oscillations of the water level affect the metabolism of aquatic organisms, and in response to it, the organisms might increase the production of resistant eggs (Brendock & De Meester 2003; Radzikowski 2013).

The passive ostracod community of Osmar Lake may contain eggs of species which accumulated of several generations and seasons, due to recurrent wet/drought oscillations (Bertoncin et al. 2019; manuscript two). Six of the eight ostracod species, hatched from the egg bank, were not yet recorded in the active community in this temporary lake, reflecting the "ecological memory" of this environment. The eggs of these species might have washed in to this lake during flooding, or were produced by past active populations of these species in the lake (or by both or other mechanisms). Even though these species seemingly are not now recruited in the active community, this might still happen in the future. Thus, events that occurred in the past can form present or future communities through the set of species that remain dormant in the sediment (Padisák 1992).

2.4.2 Active community

Richness and abundance of ostracods of the active community in the temporary lake of the Upper Paraná River floodplain were low. The hydrological dynamics (frequent and long

periods of hydric stress) (Bertoncin et al. 2019) can, on the one hand, contribute to the increase of egg production for some species, but on the other hand, may also have contributed to the low richness of ostracods, since it can act as environmental filters for the establishment of other species. Drought periods have been reported as an environmental filter (a natural barrier) of the habitat to macroinvertebrate communities, blocking the influx or survival of species that do not tolerate or adapt to these conditions (Giam et al. 2017; Rosset et al. 2017).

Cytheridella ilosvayi (Family Limnocytheridae), the most abundant species of the active community of the temporary lake, is common and abundant in aquatic environments of the Upper Paraná River floodplain (Higuti et al. 2017), mainly in isolated lakes (Conceição et al. 2017). Probably there are biological (e.g. adaptations, strategies) and environmental (e.g. flooding) mechanisms that contribute to the presence or abundance of this species after drought periods. Some possible mechanisms could be listed. *Cytheridella ilosvayi* could have resistant life stages, as suggested for *Gomphocythere* species from South Africa, which species also have brood care, just as *C. ilosvayi* does (Smith et al. 2015). Some species of *Gomphocythere* occur in fully desiccating temporary rock pools (Smith et al. 2015). However, we tested this hypothesis for *C. ilosvayi* and this species did not produce resting eggs or torpid stages (either as adults or as juvenile) this experiment.

Eggs of *C. ilosvayi* are retained within the brood chambers until hatching and often through the first two or three instars. This strategy could ensure their survival for a moderate period of time, keeping the eggs and juveniles inside its carapace (Smith et al. 2015), while the sediment of the lake remains moist. However, it is highly unlikely that animals and juveniles can survive fully desiccated conditions in such a state.

We must therefore hypothesize that *C. ilosvayi* is re-introduced by flooding after each drought period. Biotic and abiotic dispersal in floodplains is indeed a factor that should be considered (Brochet et al. 2010). Osmar Lake is an environment adjacent to the Paraná River and its hydrological dynamics, and the resulting water level fluctuations, are strongly influenced by the main channel of this river (Mormul et al. 2012). The dispersal can occur mainly by pleuston (free floating aquatic macrophytes and their associated biota) through drifting, when the water level of the Paraná River exceeds 350 cm and overflows into adjacent areas (Souza Filho 2009). Ostracods indeed have a strong association with the roots of such plants, which serve to them as a substrate (Higuti et al. 2010; Matsuda et al. 2015).

It is also worth noting that there is a narrow channel that connects the lake with the Paraná River, which is inhabited by floating aquatic plants. The connection between river,

channel and lake occurs when the Paraná River is one meter above of a marginal dike (Mormul, personal comm.).This small channel should be investigated in future studies, to see if it can act as refuge during drought periods.

The dispersal of Candonidae species can also occur through drifting plants during flooding. Besides that, some Candonidae species may inhabit the hyporheic zone (Dole-Olivier et al., 2000), and we cannot rule out the possibility that these species could use this environment as refuge during drought. However, the existence of these two types of putative refuges is as yet purely speculative.

Osmar Lake has been the focus of long-term research encompassing several aquatic communities. Mormul et al. (2012) showed that there are different ecological states possible in this lake (turbid to clear-water conditions) and associated the clear water phase with migration of benthic fish out of the lake, together with the establishment of submerged plants during the period of rising water levels. This condition might have contributed to the establishment of the active community of ostracods in March 2004, 2017 and November 2004 (during the rainy season, November to March), owing to decreased predation by fish and increased availability of the macrophyte substrate. In contrast, no ostracods were recorded in the active community in September 2017 (during the dry season, April to October), when the lake was in a turbid state and there was a high biomass of benthic fish during this period of low water level (Mormul et al. 2012).

2.4.3 Passive and active communities

Our findings showed highest richness and abundance of ostracods in the passive community, although these differences were not significant. Similarly, Martens et al. (1992) and Havel et al. (2000) observed the highest number of ostracod species in the passive compared to the active community in temporary lakes of Israel and USA, respectively. The same studies have shown differences in species composition between passive and active communities, and the existence of species occurring exclusively in the passive community (Martens et al. 1992; Havel et al. 2000). This is also true for the ostracod community of this temporary lake in the Paraná River floodplain.

The overwhelming abundance of *C. ilosvayi* in the active community and the absence of six Cyprididae species, present in the passive community, from the active community, is especially puzzling if we compare the reproductive strategies of these species. *Cytheridella ilosvayi* is considered a k-strategist species, with sexual reproduction and parental care where females carry the offspring and eggs into the brood chambers, causing a limited number of

offspring per female (Horne et al. 1998). On the other hand, the Cyprididae species recorded in the egg bank are considered r strategists, since their reproduction is mostly asexual, and they generally produce large amounts of resting eggs (Horne & Martens 1998). One could thus have expected that these Cyprididae would easily outcompete *C. ilosvayi*, but it appears that the opposite is the case. During our experiments, no specimens of Candonidae hatched from the passive community. This indicates that also *Candobrasilopsis brasiliensis* (Sars, 1901), *Candobrasilopsis rochai* Higuti & Martens, 2012, *Pseudocandona agostinhoi* Higuti & Martens, 2014 and *Cytheridella ilosvayi*, present in the active community can be allochthonous species.

Thus, we stress that, when performing biodiversity surveys in areas such as floodplains, where fluctuating water levels can cause the existence of temporary habitats, both active and passive communities of such environments must be investigated. This is especially important for monitoring programs leading to conservation and management actions of such temporary aquatic environments. At the same time we encourage long-term research to understand the inter-annual dynamics of temporary lakes of the floodplain, as, with climate change, extreme drought events could become more frequent and longer which can have effects on (meta-) communities (Campos et al., 2019). Since, the temporary lakes contribute to biodiversity, ecosystem functioning and contribute to the dynamics of the main channel and floodplain.

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3 SPATIAL VARIATION OF OSTRACOD EGG BANKS (OSTRACODA, CRUSTACEA) IN TEMPORARY LAKES OF A TROPICAL FLOODPLAIN

ABSTRACT

Some aquatic invertebrates can survive extreme disturbances, such as extended periods of droughts, for example by the production of drought-resistant stages. We evaluate the spatial variation of the ostracod resting eggs in different regions of temporary lakes of the Upper Paraná River floodplain, Brazil. We hypothesize a similarity in composition and abundance of ostracod eggs between the centre and peripheral areas (edge) of the temporary lakes. Samples were collected in the dry season, in these regions of five temporary lakes. Sediment was oven-dried, then re-hydrated and kept and monitored in germinating chamber to hatching of the eggs. A total of 12 species, belonging to families Cyprididae and Candonidae, were recorded from the egg banks of the lakes. Our results showed that the abundance and species composition was similar between the regions of the lakes. Flood events may be responsible for the homogenization of the species in egg banks, owing to the connection of temporary lakes with the principal channel of the Paraná River. During flooding, water masses powerfully enter the temporary lakes and can redistribute the sediments. In addition, our results also showed that egg banks have the potential to contribute to the maintenance of the local fauna and the resilience of the temporary lake ecosystems.

*Keywords***:** Microcrustaceans. Resting eggs. Hatching. Dormancy. Passive community.

3.1 INTRODUCTION

Floodplains are characterized by seasonal variation in hydrological regimes, including the so-called "flood pulses" (Junk et al. 1989). Flooding tends to homogenize aquatic communities (Thomaz et al. 2007), while extended periods of drought allow communities to become differentiated (Boulton 2003; Acuña et al. 2005).

Some microcrustaceans produce drought-resistant eggs to survive periods of desiccation of the habitats. These eggs accumulate in the sediment, forming the egg banks. Such resting egg banks constitute ecological and evolutionary reservoirs and contribute to the (re-) colonization and resilience of aquatic environments after disturbances (Brendonck and De Meester 2003; Brock et al. 2003).

Differential accumulation of resting eggs in the sediment can lead to differences in the composition and abundance of the passive communities in distinct compartments of the water bodies (Brendonck and De Meester 2003; Vandekerkhove et al. 2005; Gerhard et al. 2017). These passive communities of drought-resistant stages are also influenced by oscillation and duration of high and low water levels, which are crucial factors for the survival of the active community and for hatching and subsequent production of these resting eggs (Stenert et al. 2017). Furthermore, environmental variables of water (pH, oxygen, temperature, salinity, light intensity and duration) and sediment (e.g. grain size, organic matter) can induce the incubation, hatching and the viability of resting eggs, because they have a pronounced capacity to detect changes in aquatic ecosystem over short periods of time (McLay 1978; Brendonck 1996).

Under favourable environmental conditions, resting eggs will hatch (Brendonck 1996). However, some eggs may remain in dormancy for longer periods of time, owing, on the one hand, to the ability to remain viable for several dozens of years (Martens 1994; Brock et al. 2003), but on the other hand also to the need of several wet/dry cycles before certain eggs can hatch. This life history strategy of some eggs hatching quickly, while others stay dormant for longer periods is known as bet-hedging and serves as protection against potential catastrophic events in the life time of a habitat, for example inundation periods that are too short to support complete life cycles and subsequent enrichment of the egg banks (Evans and Dennehy 2005; Pinto et al., 2007). In addition to providing a mechanism for the long-term maintenance of populations in temporary aquatic habitats, resting eggs are also dispersed by several vectors, such as wind, rain (Moreno et al. 2016), flowing water (Havel et al. 2000), water birds (Valls et al. 2017), floating macrophytes (Battauz et al. 2017), mammals (Vanschoenwinkel et al. 2008) and human activities (Valls et al. 2016).

Freshwater ostracods are amongst the micro-crustaceans that can survive extended periods of drought, mainly by the production of drought-resistant eggs. Most species of the family Cyprididae, comprising about half of the total number of extant non-marine ostracods (Meisch et al., 2019), are known to produce desiccation resistant stages (Horne and Martens 1998). Several experiments have been carried out on viability and/or the hatching phenology of eggs of *Eucypris virens* (Jurine, 1820) and *Heterocypris incongruens* (Ramdohr, 1808) in temperate regions in the Palaearctic (e.g. Vandekerkhove et al. 2013; Rossi et al. 2012). However, in general there are few studies focusing on resting eggs of ostracods, especially in the Neotropical region.

Here, we aim to evaluate the spatial variation of the ostracod resting eggs in different regions of temporary lakes of the Upper Paraná River floodplain. Spatial variation within lakes and pools in the abundance and composition of resting egg can occur as a result of two processes. It has been shown that dried resting eggs can become part of floating debris in drying pools and can thus accumulate along the edges (Martens et al. 1992). Drying pools will accumulate biota at the deepest (mostly central) point where resting eggs will be produced by adults and accumulate in the final stage of the life of the temporary lakes adds to spatial heterogeneity in the passive community.

Based on the homogenization effect of floodings in floodplains, that can promote the entrance of water mixing the sediments of these lakes, and thus distribute the resting eggs and the potential to increase production of resting eggs in response to droughts, we hypothesize a similarity in composition and abundance of ostracod eggs between the centre and peripheral areas (edge) of the temporary lakes.

3.2 MATERIAL AND METHODS

3.2.1 Study area

The Paraná River is formed by the Grande and Parnaíba rivers, in South-central Brazil, and is the second largest river in South America (4,695 km long) (Agostinho et al. 2008). The Upper Paraná River has a large catchment (approx. 802,150 km^2) in Brazil, including the states of Paraná, São Paulo, Mato Grosso do Sul, Minas Gerais and Goiás (Souza-Filho and Steuvax 2004). The upper part of this river comprises a floodplain, which includes a series of small islands and a variety of environments, such as channels, rivers, connected and isolated lakes, as well as temporary lakes. The Upper Paraná River floodplain is located in the "Area de Proteção Ambiental das Ilhas de Várzea do Rio Paraná" (Environmental Protection Area)

(Agostinho et al. 2004). 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).

Here, we study the passive ostracod communities of five temporary and isolated lakes located on the Porto Rico island (Pontal, 22°45 '05.7" S/ 053°15'23.6" W; Clara, 22°45'20.7" S/ 053°15'27.7" W; Figueira, 22°45'22.7" S/ 053°15'34.0" W) and Mutum island (Pousada, 22°44'43.4" S/ 053°14'06.9 W; Osmar, 22°46'28.6" S/ 053°19'58.8" W) in the Upper Paraná River floodplain (Fig. 1).
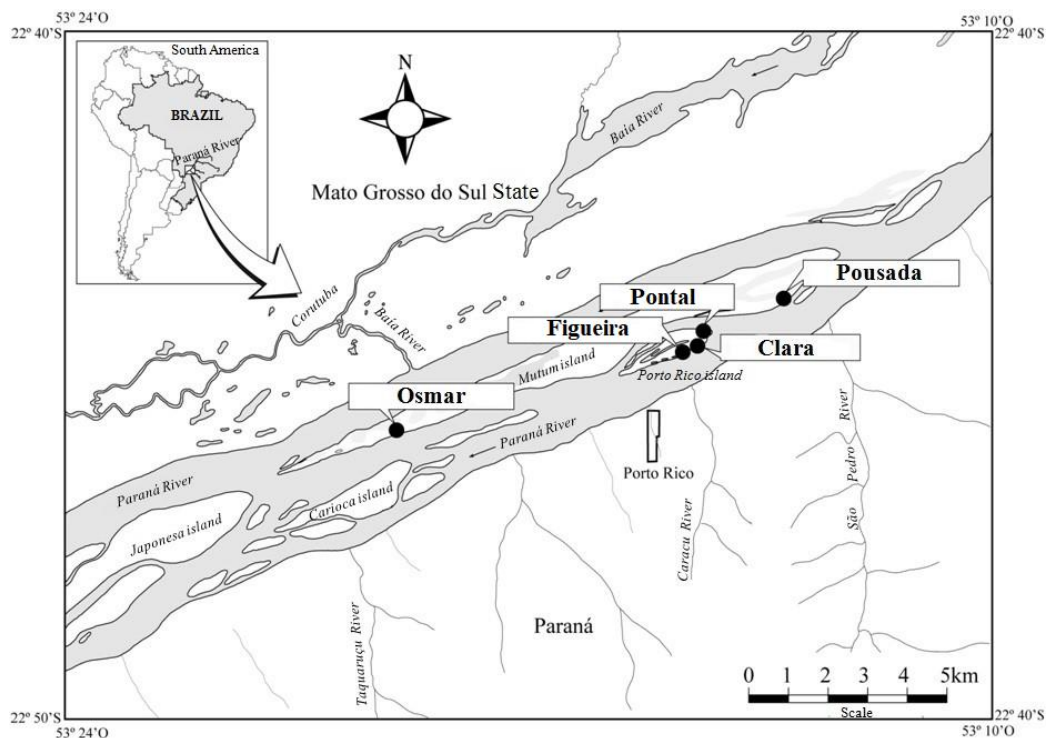
Fig. 1. Location of the temporary lakes of the Upper Paraná River floodplain. Flow direction is from right to left.

3.2.2 Sampling and hatching procedures

Sediments were collected during the dry season (September 2017), at the edge and the centre of the five temporary lakes. Approximately 6 cm depth of moist sediment (c 250 g) were removed from each region of the lakes using a core sampler. Approximatelly five samples were collected in the centre and twelve in the edge of each lake. The lakes were dry or had a low water level. The sediment was stored in plastic bottles and was kept refrigerated following the methods described by Maia-Barbosa et al. (2003).

Samples of each of the regions of the lake were homogenized and approximately 300 g of sediment were then separated from both centre and edge of each lakes, which were

inundated separately in artificial microcosms (plastic trays) with a total of 10 microcosms (5 lakes, each with centre and edge). Sediments were oven-dried at approximately 50°C (Nielsen et al. 2015) prior to hydration. Each dry sediment was hydrated with 500 ml of distilled water (Fig. S1A) and maintained in each microcosms at 25° C (Fig. S1B) for 91 days. Photoperiods were maintained at 12 hours light/12 hours dark.

The incubation period was monitored weekly (these results not shown). Every 7 days, the water from the microcosm was filtered using a plankton net (68 μm) (Fig. S1C). After that, the water of the microcosm was replaced by new distilled water. The filtered material, retained in the net, was sorted with a stereoscope microscope, and hatched juveniles were grown separately in glass bottles with distilled water, fed with fresh spinach and reared until the adult stage in separate chambers, for identification and counting. The ostracod species were identified following Higuti et al. (2010, 2013) and using the references in Martens and Behen (1994).

3.2.3 Abiotic variables

Sediment from each region (edge and centre) was also used to determine the particle size and organic matter. Sediment composition was determined according to the method of Suguio (1973), using the Wentworth scale (Wentworth 1922). The samples were sorted in a nested series of sieves (size range between 2 mm and < 0.63 mm) and weighed. Organic matter in the sediment was obtained from 10 g of dry sediment by incineration at 560 °C for four hours. The difference between the initial and final weights of the sediment indicates the amount of organic matter that was present in the sediment.

Dissolved oxygen (mg.L^{-1}) (YSI oximeter 550A), electrical conductivity (μS.cm^{-1}) and pH (using conductivity meter Digimed DM-3P and pH meter Digimed DM-3P respectively) were measured weekly in the microcosms.

3.2.4 Data analysis

A non-parametric Wilcoxon Test for paired samples was performed to test differences in ostracods abundance (number of ostracod specimens hatched from resting eggs) between the regions of the lakes, since the assumptions of normality and homoscedasticity for the application parametric test were not fulfilled. For this the total number of ostracods hatched in each region was used.

The frequency of ostracod species that hatched from resting eggs, at the edge and in the centre of the temporary lakes, was calculated using the constancy index (Dajoz 1973) through the expression: C = n/N x 100, where: C = constancy; n = number of samples in

which the species was recorded and N = total number of samples. The following categories were assigned: constant (C ≥ 50%); accessory (50% > C ≥ 25%); accidental or rare (C < 25%).

A principal coordinate analysis (PCoA) was performed to evaluate the (dis)similarity of ostracod species composition between the lakes' regions (edge and centre), using data from presence and absence of the hatched community (every 7 days during 91 days). The axes were retained according to the criteria of the broken-stick method. The samples scores of the axes 1 and 2 were submitted to the variance test (ANOVA) to test for significant differences in species composition between edge and centre.

We examined the relationship between ostracod abundance and sediment quality (sediment composition and organic matter) using Generalized Additive Models (GAMs). We constructed our models with negative binomial distribution to avoid overdispersion using the data of the particle size of the sediment as explanatory variables and ostracod abundance as a response variable. Before that, pairwise correlations among explanatory variables were evaluated using Spearman's rank correlations coefficients to avoid multi-collinearity.

One model was constructed to each explanatory variable, owing to the low number of samples. The best models identified were based on comparisons of Akaike's Information Criterion (AIC), intervals of confidence and values ANOVA.

Finally, to evaluate possible significant differences of the limnological variables between the types of microcosms (edge and centre), the data of dissolved oxygen, pH, and electrical conductivity were tested with a non-parametric Kruskal-Wallis test. In addition, to evaluate possible differences in organic matter content between edge and centre of the lakes, the test T for paired samples was applied.

PCoA and Models GAM analyses were carried out in R 3.4 software (R Development Core Team 2013) using the vegan (Oksanen et al. 2018), permute (Simpson 2018) and mgcv (Wood 2018) packages. Analyses of variance were performed in Statistica 7.1 (Statsoft Inc 2005).

3.3 RESULTS

3.3.1 Composition and abundance of ostracod resting egg banks

A total of 12 species of ostracods hatched from the egg banks of the 5 temporary lakes. Cyprididae was the richest and most abundant family, represented by 11 species. The family Candonidae was represented only by the species *Physocypria schubarti* Farkas, 1958.

Cypridopsis vidua (O. F. Müller, 1776), *Cypricercus centrurus* (Klie, 1940) and *Bradleytriebella trispinosa* (Pinto & Purper, 1965) were only reared from sediment from the centre of the lakes (Table 1, Fig. 2).
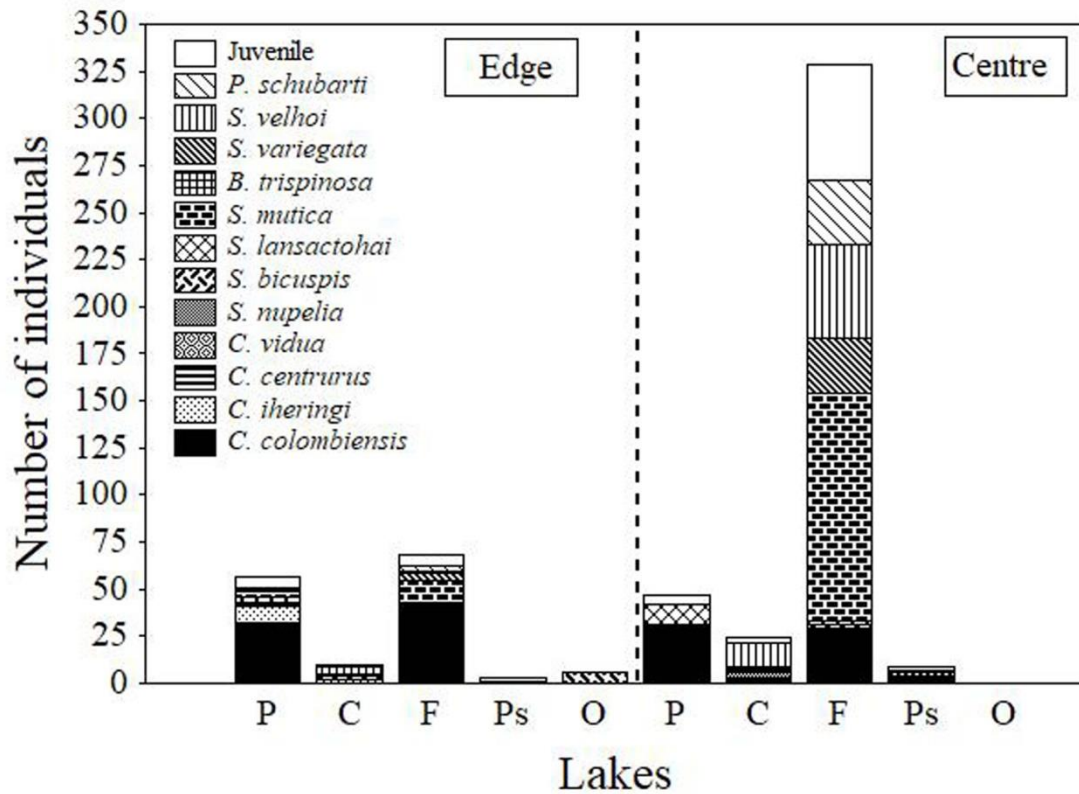
Fig. 2. Composition and abundance of ostracod species hatched from egg banks at the edge and in the centre of the temporary lakes (P = Pontal, C = Clara, F = Figueira, Ps = Pousada, O = Osmar).

A total of 553 ostracod specimens hatched from the sediments of the five temporary lakes; 144 and 409 ostracods were recorded from the edges and centres, respectively. There was no significant difference in the number of individuals between the two regions (Wilcoxon Test, p = 0.07). *Chlamydotheca colombiensis* (Sars, 1901) was the most abundant species at the edge and *Strandesia mutica* (Sars, 1901) in the centre of the lakes. According to the constancy index, *S. mutica* and *C. colombiensis* were the most frequent species in both regions, while *Strandesia velhoi* Higuti & Martens, 2013 was frequent only in the centre of the lakes (Table 1).

Table 1. Constancy index of ostracod resting eggs hatched in the temporary lakes of the Upper Paraná River floodplain. Constant species were present in more than 50% of the samples, accessory from 25% to 50% of the samples, and accidental less than 25% of the samples (white = absent, light grey = accidental, dark grey = accessory, black = constant).

	Edge	Centre
Family Cyprididae (Baird, 1845)		
Bradleytriebella trispinosa (Pinto & Purper, 1965)		light grey
Chlamydotheca iheringi (Sars, 1901)	dark grey	light grey
Chlamydotheca colombiensis Roessler, 1985	black	black
Cypricercus centrurus (Klie, 1940)		light grey
Cypridopsis vidua (O. F. Müller, 1776)		light grey
Strandesia bicuspis (Claus, 1982)	dark grey	light grey
Strandesia lansactohai Higuti & Martens, 2013	dark grey	dark grey
Strandesia mutica (Sars, 1901)	black	black
Strandesia nupelia Higuti & Martens, 2013	light grey	light grey
Strandesia variegata (Sars, 1901)	light grey	dark grey
Strandesia velhoi Higuti & Martens, 2013	dark grey	black
Family Candonidae (Kaufmann, 1900)		
Physocypria schubarti Farkas, 1958	light grey	dark grey

The results of PCoA did not show significant differences in the dissimilarity of species composition of the egg banks from the centres and edges (F= 0.02, PCoA 1: p= 0.87; PCoA 2: F= 0.17, p= 0.68) (Fig. 3). Thus, the species composition of the passive community of ostracods was similar within these temporary lakes.
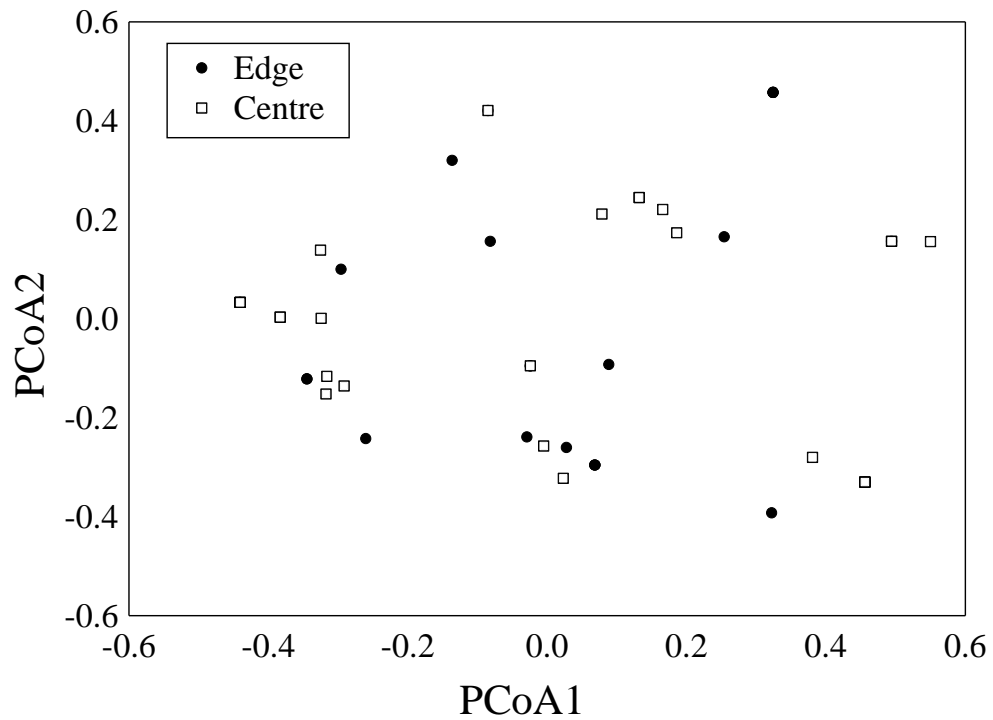
Fig. 3. Ordination diagram of the principal coordinate analysis of the ostracod passive communities at the edge and in the centre of the temporary lakes.

3.3.2 Effects of the abiotic variables on hatching of ostracod resting egg

      Very fine sand was the most predominant sediment at the edges and mud (clay and silt) at the centres of the five temporary lakes (Fig. 4). In general, the sediment of the lakes was composed mainly by particles of sediment smaller than 0.25 mm.
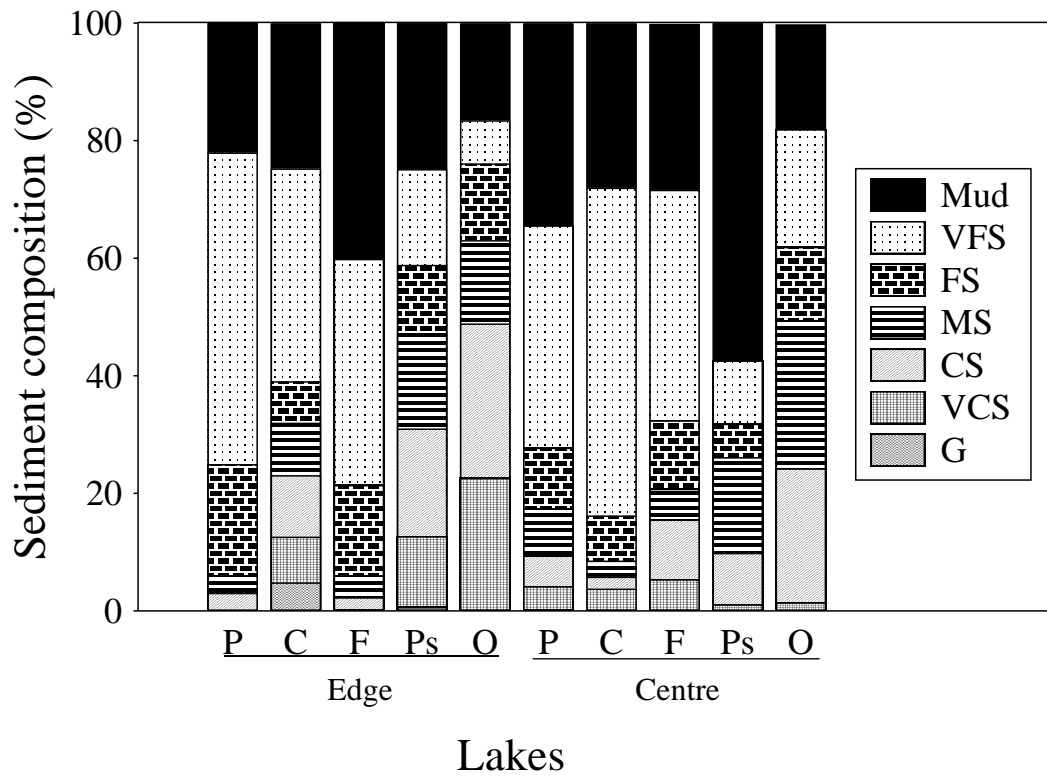
Fig. 4. Sediment composition of the edge and in the centre of the temporary lakes. P = Pontal, C = Clara, F = Figueira, Ps = Pousada, O = Osmar, Mud = Silt and clay, VFS = Very fine Sand, FS = Fine Sand, MS = Medium Sand, CS = Coarse Sand, VCS = Very Coarse Sand, G = Gravel.

Coarse particulate organic matter (roots and leaves) was observed in all five temporary lakes. The organic matter content of the sediment was higher at the edge, when compared to the centre (t= -21.92, p= 0.00) (Fig. 5).
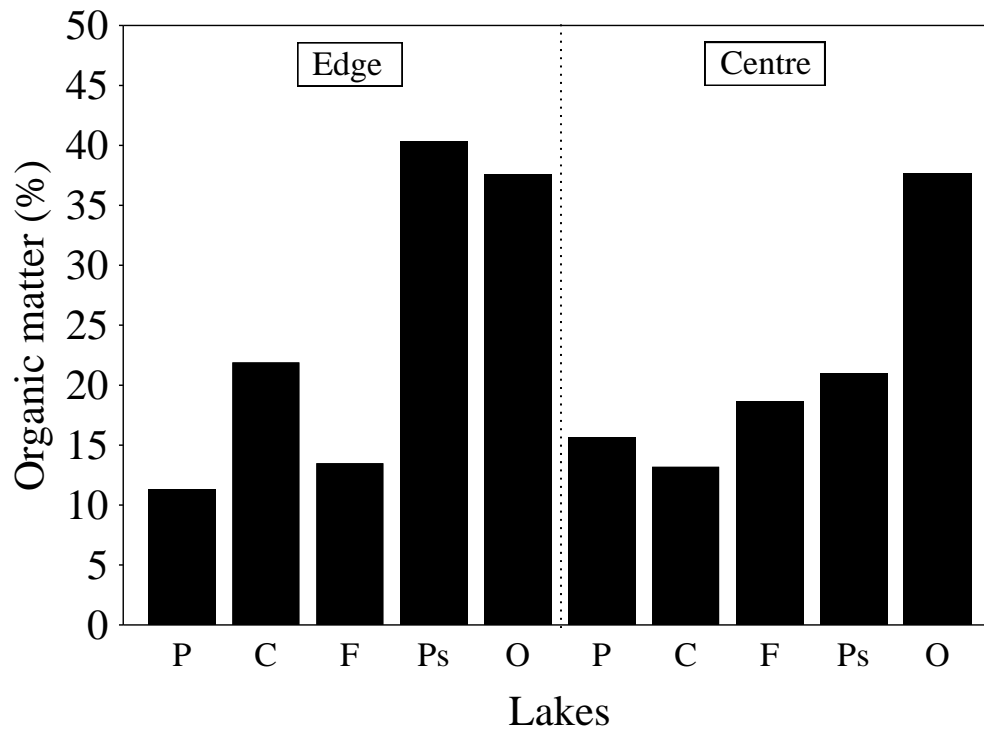
Fig. 5. Organic matter of the sediment at the edge and in the centre of the temporary lakes. P = Pontal, C = Clara, F = Figueira, Ps = Pousada, O = Osmar.

The non-generalized linear model showed positive effects of very fine sand on the hatching of the eggs. On the other hand, the hatching of the eggs was negatively related to the amount of organic matter and the medium grain-size of sand (Table 2).

Table 2. Generalized Additive Models (GAMs). Model-averaged standardized coefficients, 95% confidence intervals (CI), Akaike's Information Criterion (AIC) and ANOVA p-values of predictors from ostracod resting eggs hatched. Significant level ($p < 0.05$) in bold.

	Standardised Coefficient	95% CI		AIC	ANOVA
		2.5%	97.5%		
Gravel	0.487	-0.867	0.524	101.967	0.285
Very coarse sand	0.611	-0.251	0.141	102.116	0.322
Coarse sand	0.716	-0.264	0.041	100.877	0.123
Medium sand	0.559	-0.389	-0.164	90.734	**0.000**
Fine sand	1.462	-0.181	0.502	102.441	0.426
Very fine sand	0.887	0.016	0.138	98.454	**0.018**
Mud (clay + silt)	1.297	-0.114	0.201	103.048	0.917
Organic matter	0.900	-0.222	-0.05	97.014	**0.005**

Environmental variables were not significantly different between the microcosms of the edge and centre of the temporary lakes (oxygen: H = 3.58, p = 0.058; pH: H = 0.07, p = 0.79; electrical conductivity: H = 0.01, p = 0.91). The mean values for these variables in the "edge" microcosms were 4.95 mg.L^{-1} (dissolved oxygen), 6.74 (pH), 20.77 µS.cm^{-1} (electrical conductivity), and in the "centre" microcosms were 4.45 mg.L^{-1} (dissolved oxygen), 6.68 (pH), 18.27 µS.cm^{-1} (electrical conductivity) (Table S2).

During the incubation period in the microcosms, macrophytes germinated from sediments from both regions of the lakes (Fig. S2). *Nymphaea amazonum* Mart. & Zucc. was the most common macrophyte species.

3.4 DISCUSSION

3.4.1 Composition and abundance of ostracod resting egg banks

All 12 ostracod species which hatched from the resting eggs have previously been recorded from different lotic and lentic environments (e.g. rivers, channels, connected, isolated and temporary lakes) of the river-floodplain system of the Upper Paraná River (Higuti et al. 2010; 2017). Of these 12 species, *Chlamydotheca colombiensis*, *Strandesia mutica*, *S. variegata* (Sars, 1901), and *S. bicuspis* (Claus, 1982) were originally described from specimens that had been hatched from dried sediment (Sars 1901; Roessler 1985). Interestingly, *C. colombiensis* was thus far only found in temporary lakes of the Upper Paraná River (Higuti et al. 2010). In the present study, the species hatched equally successfully from sediments from edge and centre of the temporary lakes, indicating that *C. colombiensis* may be adapted to temporary environments. As expected, our results showed that most ostracod species that hatched from resting eggs belong to the family Cyprididae, while one species belongs to the family Candonidae (*Physocypria schubarti* Farkas, 1958). A previous study on the diversity of crustacean zooplankton in North America also recorded a species of *Physocypria* which hatched from the egg banks of the sediment (Havel et al. 2000).

Most studies on production and hatching of drought-resistant eggs of ostracods are from the temperate regions of the Palaearctic (Martens et al. 1992; Horne and Martens 1998; Valls et al. 2016). Few such studies from (sub-) tropical regions exist (Gerhard et al. 2017).

The presence of resting eggs in the centre and at the edge of temporary water bodies may depend on several factors. For example, dried resting eggs can accumulate along with the floating debris in the edges (Martens et al. 1992). But drying pools will also concentrate fauna at their deepest (mostly central) point towards the end of the hydrological cycle, and then

resting eggs can be produced at a higher rate there and ostracods might lay a greater amount of eggs in the deepest part (centre) of the ponds and lakes.

However, these processes are mostly relevant for rain-filled temporary water bodies. In the temporary floodplain lakes of the Upper Paraná River, the effect of flood pulses may nullify such processes and may lead to the homogenisation in the composition and abundance of ostracods between edge and centre of these lakes. This is so because during high water periods, the main river waters will invade the lakes, and will lead to the homogenization and dispersion of the propagule bank of the communities (Gurnell et al. 2008). The entrance of water will mix the sediments of these temporary lakes and will thus distribute the ostracod resting eggs over the entire lake, thus resulting in a similar composition of the passive ostracod community in central and peripheral parts of the water body. Another factor that may contribute to the similarity of the species composition is the physical appearance of the temporary floodplain lakes. They are invariably of elongated and narrow shape, and this facilitates the homogenization between the regions of these temporary lakes.

Nevertheless, differences in the composition and abundance of species of cladocerans in egg banks have been observed in littoral and pelagic zones of shallow waterbodies (Vandekerkhove et al. 2005; Gerhard et al. 2017). Other studies found no differences in the composition of egg banks of invertebrates, including ostracods, amongst upland, edge and centre of playa wetlands (Bright and Bergey 2015). In addition, the latter authors also showed that the abundance of invertebrate eggs was similar in edge and centre regions attributing to environmental factors and passive dispersal by wind and inundation.

We can herewith corroborate the hypothesis that the composition and abundance of ostracod resting eggs is similar between the two lake regions in the temporary lakes in the Upper Paraná River floodplain.

3.4.2 Effect of the abiotic variables on hatching of ostracod resting egg

Abiotic characteristics of the sediment are important for the active community of benthic invertebrates, mainly by providing habitats and substrate for organisms (Hauer et al. 2018), and thus it might also have an influence on the dormant egg banks. Here, the positive relationship between the numbers of the hatched ostracod resting eggs and the size of particles (very fine sand) might be related to the fact that this type of sediment has a greater capacity of suspension. This is owing to the movement of water or bioturbation activities, which provide oxygenation of the substrate and a higher concentration of water in the sediment (Constable 1999). It can thus provide better conditions for the hatching and the dispersal of the dormant

eggs. These results agree with those of Masero and Villate (2004), who found a positive correlation between the density of calanoid eggs on the one hand and smaller sediment particles on the other hand, thus showing that sediment characteristics can affect the egg banks.

The negative effect of organic matter content on the hatching of ostracod resting eggs may be linked to increased decomposition and hypoxia in the sediment and in the water column, which can negatively affect the hatching (Rossi et al. 2004; Watkins et al. 2011). In addition, the organic matter in the sediment of the temporary lakes was mainly composed of allochthonous (non-aquatic) origin, provided by riparian vegetation, since they have a dense vegetation cover (Kita and De Souza 2003). The layers of leaves accumulated in the sediment can also cause burial and smothering of the egg banks, reducing the hatchability and viability of resting eggs (Gleason et al. 2003).

The germination of macrophytes in the microcosms might also contribute to the hatching of the resting eggs, because these aquatic plants provide substrate and food for ostracods (juveniles and adults). Several studies have shown the important effect of macrophytes on the structure of the active ostracod communities (Higuti et al. 2010; Matsuda et al. 2015).

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4 CONCLUSIONS

Ostracod egg banks of the temporary lakes of the Upper Paraná River floodplain have potential to harbour eggs of species of several generations and seasons. Propagules of species not present in the active community can still inoculate other environments of the floodplain. Ostracod resting eggs thus play an important role in the maintenance and enrichment of both local and regional faunas and can provide resilience of communities after extreme drought events, since different species can hatch at different times under different circumstances. As such, egg banks provide an alternative source for assessing species diversity in temporary environments.
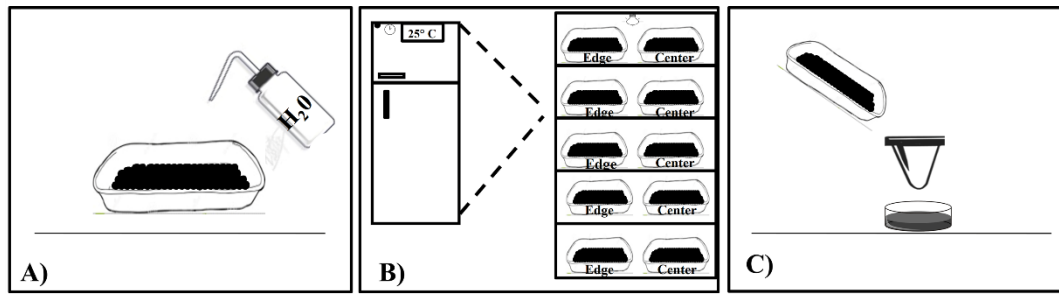
The flood pulse is probably the main factor for the homogenization of passive ostracod communities (abundance and composition) between the edge and centre regions of temporary lakes. The flooding also facilitates the entrance of allochthonous ostracod species (both active and passive communities) in the Osmar Lake, most likely by pleuston (associated communities in root systems of free floating aquatic macrophytes).

We reported 13 ostracod species that produce resting eggs or torpidity stages. These taxa are organisms resilient to extreme events and have the invasive potential, owing to the asexual reproduction (parthenogenesis) of most species and passive dispersal of the drought resistant structures.

It is also worth noting that little is known about resting eggs of ostracods. The drought resistant eggs uncovered when dried are only small pieces of organic matter, which makes them difficult to separate from sediments. In addition, no specific protocol for hatching eggs has been described for ostracods, besides the difficulties to ensure survival of juveniles until the adult stage, thus allowing the specific identification.

APPENDIX A - Table S1. Mean, standard deviation, minimum and maximum (in parentheses) values of the abiotic variables and species of macrophytes which germinated in the microcosms. DO = dissolved oxygen, EC = electrical conductivity, Hr = *Hydrocotyle ranunculoides* L.f.*,* Lv = *Lemna valdiviana* Phil., Ea = *Eichhornia azurea* Kunth*,* Na = *Nymphaea amazonum* Mart. & Zucc., Sm = *Sagittaria montevidensis* Cham. & Schltdl, Ht = *Helanthium tenellum* (Mart.) Britt., Ls = *Lindernia* sp, Nf = *Nitella furcata* (Roxburgh ex Bruzelius) C. Agardh emend. R.D. Wood.

Lakes	Region	DO (mg.L^{-1})	pH	EC (µS.cm^{-1})	Macrophytes
Pontal	Edge	6.31±1.83	8.06±0.97	33.06±21.88	Hr, Lv, Ea,Na,Nf
		(3.34-8.85)	(6.33-9.39)	(8.7-79.9)	
	Centre	5.35±1.35	8.12±0.64	16.49±11.25	Lv, Na
		(3.34-7.08)	(7.22-9)	(8.5-37.7)	
Clara	Edge	5.41±0.88	7.12±0.87	12.89±8.87	Lv, Ea, Nf
		(3.77-6.75)	(5.69-8.56)	(6.5-31.7)	
	Centre	4.89±1.04	6.96±0.91	16.06±9.75	Ea, Na, Ls, Nf
		(3.14-6.5)	(5.16-8.27)	(8.27-33.3)	
Figueira	Edge	3.74±0.89	6.01±0.44	22.69±9.62	Na, Sm, Ht
		(2.1-5.06)	(5.4-6.92)	(5.74-41.2)	
	Centre	3.29±0.63	6.39±0.56	17.67±5.18	Na
		(2-4.32)	(5.92-7.75	(7.75-27)	
Osmar	Edge	5.17±0.55	6.5±0.63	15.11±7.94	Na
		(4.04-6)	(5.22-7.37)	(7.12-33.4)	
	Centre	5.03±0.64	6.15±0.6	17.61±10.46	Na, Ht
		(4.02-5.96)	(5.4-7.08)	(6.49-36)	
Pousada	Edge	4.14±0.46	6.02±0.4	20.11±12.14	Na
		(3.47-4.91)	(5.33-6.56)	(6.28-43.6)	
	Centre	3.7±0.44	5.8±0.38	23.53±11.49	Na, Ht
		(2.94-4.46)	(5.23-6.46)	(5.77-48.7)	
Edge region		4.95±1.39	6.74±1.04	20.77±14.89	
		(2.1-8.85)	(5.22-9.39)	(5.74-79.9)	
Centre region		4.45±1.2	6.68±1.03	18.27±10.26	
		(2-7.08)	(5.16-9)	(5.77-48.7)	

APPENDIX B – Fig. S1. Procedures of hatching of the resting eggs of ostracods. a) hydration of the dry sediment, b) microcosm with hydrated sediment in the germination chamber, c) filtering the water.
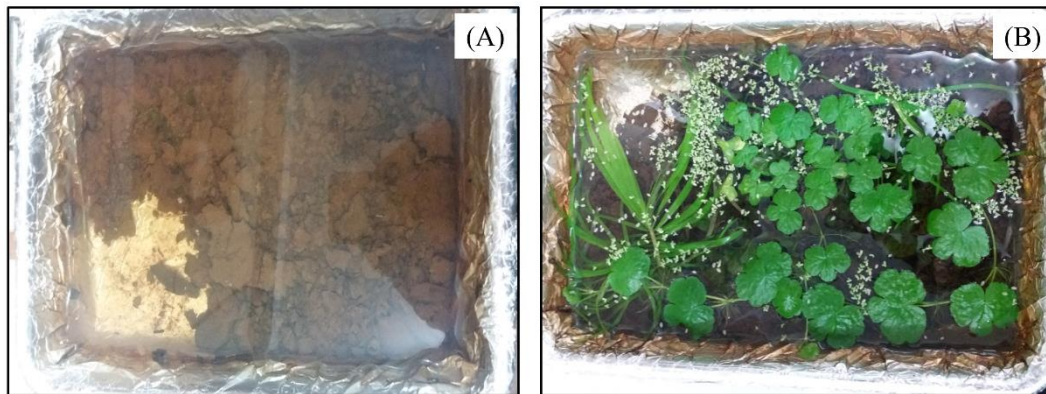
APPENDIX C – Fig. S2. Microcosms in the germination chamber. Beginning of incubation period (A), end of incubation after 91 days (B).