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Spatial variation of ostracod (Crustacea, Ostracoda) egg banks in temporary lakes of a tropical floodplain

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Abstract. Ostracods are microcrustaceans that produce resting eggs under adverse conditions. We evaluated the spatial variation of ostracod resting eggs in different regions of temporary lakes in a Brazilian floodplain. Based on the homogenization effect of flood pulses on aquatic communities in floodplains, we hypothesized that the composition and abundance of ostracod eggs in the centre of the temporary lakes would be similar to those in the edge regions. Samples were collected in the centre and edge regions of five temporary lakes. Sediment was oven-dried, re-hydrated and hatching was monitored in germinating chambers. Twelve ostracod species hatched from the egg banks during our experiments. Our results show that the abundance and species composition were similar between the two regions of the lakes. Flood events may be responsible for the homogenization of the egg banks, owing to the connection of the lakes with the principal river channels. During flooding, water masses powerfully enter the lakes and can redistribute the sediments. Our results show that egg banks have the potential to contribute to the maintenance of the local biodiversity and the resilience of the biodiversity of temporary lake ecosystems.

Additional keywords: Microcrustaceans. Hatching. Dormancy. Passive community.

Introduction

Some microcrustaceans produce resting eggs to survive periods of desiccation of the habitats (Brendonck and De Meester 2003). These eggs accumulate in the sediment, forming egg banks (Brock et al. 2003). Such resting egg banks constitute ecological and evolutionary reservoirs which 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). The spatial patterns of the active microfaunal communities are well-studied. However, the passive communities generally remain ill - known (Gehard et al. 2017; Portinho et al., 2017). Nevertheless, some studies on the active communities suggested that the spatial variation in the environment is regulated by the production of resting eggs and the dispersal of these propagules (Hairston et al. 1996).

The spatial variation within lakes and pools in the abundance and composition of resting eggs can occur as a result of two processes. Firstly, the resting eggs can become part of floating debris in drying pools and can thus accumulate along the edges (Martens et al. 1992). Secondly, during the drying of these environments, the eggs accumulate in the deepest regions, mostly the centre of the water bodies, where the water remains the longest, prior to full desiccation (Martens et al. 1992; Bright and Bergey 2015). However, in floodplains, during flood periods of high-water level, most of the environments are inundated and become connected to a main river channel. It is then expected that the water flow promotes the dispersal of the resting eggs and consequently homogenizes the egg banks in the sediments of the lakes (Thomaz et al., 2007; Bozelli et al. 2015), so that composition of edge and centre of the water body would become similar in any season of the year.

Resting eggs that settle at the water-sediment interface of the water body, may adhere to particles of sediment (e.g. organic matter) and may also be covered by them (Brendonck & De Meester, 2003). The abiotic variables of the sediment may thus influence the viability, dispersal and hatchability of resting eggs. For example, the amount of organic matter has an effect on the oxygen concentrations in the sediment, influencing the viability and hatchability of the resting eggs (Rossi et al. 2004). In addition, the smaller the particles of sediment, the greater their capacity of suspension, which increases the probability of dispersal of resting eggs (Constable 1999).

Some groups of freshwater ostracods can produce resting eggs. Most species of the family Cyprididae, which comprises about half the total number of extant non-marine ostracod species (Meisch et al., 2019), are known to produce resting eggs (Horne and Martens 1998). Several experiments have been carried out on the viability and/or the hatching phenology of ostracod eggs of *Eucypris virens* (Jurine, 1820) and *Heterocypris incongruens* (Ramdohr, 1808) in temperate regions in the Palaearctic (e.g. Rossi et al. 2012; Vandekerkhove et al. 2013). There are no studies focusing specifically on resting eggs of ostracods in the Neotropical region. However, some studies on the resting eggs of zooplankton and other invertebrates have occasionally also recorded ostracods hatching from egg banks (Stenert et al. 2010; Ávila et al., 2015; Santangelo et al. 2015; Vargas et al. 2019).

Studies on ostracod resting eggs can provide vital information on the biology of the group. The ecological information about the recruitment of organisms from egg banks can fuel scientifically underpinned recommendations on the conservation of aquatic environments, such as temporary lakes in floodplains. These ecosystems are threatened by anthropic impacts (e.g. the construction of artificial dams) that affect the hydrological dynamics of the ecosystem (droughts and flood events) (Agostinho et al. 2004a), and thus require protective measures. Here, we evaluate the spatial variation of ostracod resting eggs in different regions of five temporary lakes of the Upper Paraná River floodplain. Based on the homogenization effect of flooding in the floodplain lakes, we hypothesized that the composition and abundance of ostracod eggs in the centre of the temporary lakes would be similar to those in the edge regions.

Material and methods

Study area

The Paraná River is formed by the junction of 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, which encompasses large parts of 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. 2004b). The climate of the region is tropical-subtropical with distinct rainy (November to March) and dry (April to October) seasons (Cfa) (Eletrosul, 1986; Agostinho et al. 2004b).

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). These lakes are shallow (not exceding 2.2 meters depth) and small (areas of 0.15 hectares or less). The limnological variables (e.g. dissolved oxygen, electrical conductivity, pH) and vegetation cover formed by herbaceous and arboreal species, with higher presence of emergent macrophytes of the family Poaceae (Kita and De Souza 2003) are similar amongst the five lakes. In the dry season of 2017, four lakes were dry (Pontal, Clara, Figueira and Pousada) and one lake (Osmar) had a low water level (less than 30 cm) during the sampling.

>>> Figure 1

Sampling and hatching procedures

Sediments were collected during the dry season (September 2017), at the edge and in the centre of the five temporary lakes. The edge region was defined as the area in direct contact with the adjacent terrestrial ecosystem, mostly with shallower sites, while the centre region was defined as the open area of the lake, associated with deeper sites. We sampled six cm of sediment depth (c 250 g) using a core sampler (194.5 cm^3 volume) in each region of the lakes, as the higher viability of resting eggs is usually found in the top three cm of the sediment (Garcia-Roger et al. 2006).

Twelve samples were collected at the edge of each of the lakes, in order to cover the entire edge region of the lake, while five samples were collected in the centre. These samples were taken every three meters (approximately 4.250 kg of sediment per lake). A larger number of samples were collected at the edge of each lake compared to the central region, to better cover the spatial representation of this variable environment. From each lake, the samples of each region (edge and centre) were separately pooled to form a composite sample, totalling 10 samples (2 x 5 lakes). The sediment was stored in plastic bottles and was kept refrigerated for two months, following the methods described by Maia-Barbosa et al. (2003). Despite the fact that the lakes were dry (no or very little water), the sediment was mostly still moist.

For the hatching procedure in the laboratory, the composite samples from each region (edge and centre) of the five lakes were separately homogenized and 300 g of sediment was individually oven-dried at 50°C (this sediment temperature can be easily reached in the floodplain lakes on hot and dry days) and then placed in individual plastic trays, which acted as artificial microcosms, totalling 10 microcosms. Each dry sediment sample was hydrated with 500 ml of distilled water (Fig. S1A) and was maintained in the microcosm at 25° C (Rossi et al., 2004) for 91 days (Fig. S1B) in a germination chamber (Model SOLAB, SL.225). Photoperiods were maintained at 12 hours light/12 hours dark (Rossi et al., 2012).

The incubation period was monitored weekly. Every 7 days, the water from the microcosm was filtered using a plankton net (68 µm) (Fig. S1C). As lifecycles of non-marine ostracods take at least 3 weeks (Meisch, 2000), the time of 7 days was not sufficient for the sexual maturation of individuals for reproduction. After that, the water of the microcosm was replaced with fresh distilled water. The filtered material, retained in the net, was sorted with a stereoscope microscope. Hatched juveniles were grown separately in glass bottles with distilled water, fed with fresh spinach and reared to the adult stage (when the juveniles did not die) 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).

Abiotic variables

Sediment from each region (edge and centre) was also used to determine the particle size and organic matter content. 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. The size of sediment particles was classified as gravel (> 2 mm), very coarse sand (2-1 mm), coarse sand (1.0-0.5 mm), medium sand (0.50-0.25 mm), fine sand (0.250-0.125 mm), very fine sand (0.125-0.063 mm) and mud (<0.063 mm). Organic matter in the sediment was obtained from 10 g of dry sediment by incineration at 560 °C for four hours (Moretto et al. 2013). 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 Conductivimeter-Digimed, Digimed, São Paulo, Brazil and pHmeter-Digimed, Digimed, São Paulo, Brazil) were measured weekly in the microcosms.

Data analysis

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

The frequency of ostracod species that hatched from resting eggs 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%) according to Dajoz (1973).

A principal coordinate analysis (PCoA) was performed to visualize (dis)similarity of ostracod species composition between the lakes' regions (edge and centre), using data from presence and absence of the ostracods hatched weekly (Legendre and Legendre 1998). A Multivariate Permutational Variance Analysis (PERMANOVA) was performed to evaluate differences in ostracod species composition between edge and centre (Anderson 2005). The test was based on a dissimilarity matrix using the Jaccard distance. A total of 999 permutations were performed to assess significance.

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 for 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 significant values of the variance test (ANOVA).

Finally, Kruskal-Wallis test was used to evaluate possible significant differences of each limnological variable between edge and centre. For this, we used the data of dissolved oxygen, pH, and electrical conductivity measured weekly in each microcosm of the edge and centre. In addition, to evaluate possible differences in organic matter content between edge and centre regions, the T test for paired samples was applied. We used a t-test because this test is appropriate for the number and dependency of samples of the present study.

Analyses of variance, 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.

Results

Composition and abundance of ostracod resting egg banks

Twelve species of ostracods hatched from the egg banks of the five 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* sp. nov. and *Bradleytriebella trispinosa* (Pinto & Purper, 1965) were only reared from sediment from the centre of the lakes (Table 1).

>>> Table 1

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 (Figure 2). 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 common species in both regions, while *Strandesia velhoi* Higuti & Martens, 2013 was common only in the centre of the lakes (Table 1). The results of the PERMANOVA did not show significant differences in the species composition of the egg banks between the centre and edge regions ($F= 0.62$, $p= 0.87$) (Fig. 3).

>>>Figure 2

>>> Figure 3

Effects of the abiotic variables on ostracod resting egg banks

Very fine sand was the most dominant type of sediment in the edge regions, whereas mud (clay and silt) was the most dominant type of sediment in the centre regions of the five temporary lakes (Fig. 4). In general, the sediment of the lakes was composed mainly of particles of sediment smaller than 0.25 mm.

>>> Figure 4

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 test, $t= -21.92$, $p= 0.00$) (Fig. 5).

>>> Figure 5

The non-generalized linear model showed positive effects of very fine sand on the number of hatchlings. 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

Environmental variables did not vary significantly between the microcosms of the edge and centre regions (Kruskal-Wallis, 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 $\mu S.cm^{-1}$ (electrical conductivity), and in the "centre" microcosms were 4.45 $mg.L^{-1}$ (dissolved oxygen), 6.68 (pH), 18.27 $\mu S.cm^{-1}$ (electrical conductivity) (Table S1).
Macrophytes germinated from sediments of both regions of the lakes, during the incubation period (Fig. S2). *Nymphaea amazonum* Mart. & Zucc. was the most common macrophyte species recorded in eight of the ten microcosms (Table S1).

Discussion

Composition and abundance of ostracod resting egg banks

We herewith corroborate the hypothesis that the composition and abundance of ostracod resting eggs is similar between the two regions of the temporary lakes in the Upper Paraná River floodplain. A practical implication based on our results is that further studies about egg banks can be performed with sediment sampled at any region (centre and edge) of temporary lakes in floodplains, due to the similar spatial distribution of resting eggs. Theoretically, flood pulses promote the homogenization of active communities (Thomaz et al. 2007) and similarly, this fact might have contributed to the homogenization of the passive community (the egg bank). The similar spatial distribution of the egg bank between edge and centre of the lakes may increase the probability of dispersal of ostracod resting eggs by biotic vectors, when compared to the dispersal of an egg bank that only accumulate eggs in a specific region of the lake. Since several animals (e.g. birds) visit this type of environment, they can promote the dispersal of these structures (Morais Junior et al. 2019).

Resting eggs have shown spatial variation of occurrence in water bodies with both horizontal (between the edge and centre), and vertical distribution, when the sediment floats on the surface of the water after rains, as observed by Martens et al. (1992) in a temporary pool in Israel. The same study also showed that the floating sediment contained more eggs than the submerged sediments, although the composition did not differ between the regions. In our study, only samples of submerged sediment (dry or wet) were sampled owing to the fact that the lakes were dry or had very low water level during the sampling period.

The presence of resting eggs in the centre and at the edge of temporary water bodies may depend on several factors. For example, drought resting eggs can accumulate along the floating debris along 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 in these remaining pools. Ostracods may thus produce a greater number of eggs in the deepest part (centre) of the ponds and lakes (Bright and Bergey 2015).

However, both of these processes are mostly relevant for rain-filled, isolated temporary water bodies. In the temporary lakes of the Upper Paraná River floodplain, the effect of flood pulses may nullify such processes and may lead to homogenization (Thomaz et al. 2007), also with respect to the composition and abundance of ostracods between the edge and centre of these lakes. This is so because during high water periods, the main river waters will invade the lakes, and homogenize and disperse the propagule bank of the dormant communities (Gurnell et al. 2008). The floodwaters will mix the sediments of these temporary lakes and 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 morphology of the temporary floodplain lakes. They are invariably elongated and narrow, and this possibly facilitates the homogenization between the regions of these temporary lakes, as edge and centre are only a few meters apart.

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 regions 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 owing to environmental factors and passive dispersal by wind and inundation.

All 12 ostracod species hatching from the resting eggs in our experiments have previously been recorded from other 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 regions 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* hatching from sediment egg banks (Havel et al. 2000).

Most studies on production and hatching of resting eggs of ostracods are from the temperate regions of the Palaearctic (Martens et al. 1992; Horne and Martens 1998; Valls et al. 2016) and few studies focus on (sub-) tropical regions. Ostracods are known to lay mixed batches of subitanous and resting eggs, and it is possible that the ratio of these two types of eggs can be influenced by environmental factors (Dumont et al. 2002; Schön et al. 2012). This is unlike, for example, Cladocera, where resting eggs (ephippia) are only produced by the final sexual population at the end of the reproductive period (mostly summer).

Effect of the abiotic variables on hatching of ostracod resting eggs

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 they 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 for suspension (Constable 1999). 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 resting 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. In addition, Tilbert et al. (2019) also found a positive association between the active ostracods and fine and very fine sand in a small tropical estuary in Brazil.

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) material, provided by riparian vegetation, mostly leaves of trees, 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 all microcosms might also contribute to the ecological succession, because these aquatic plants provide substrate and food for ostracods (juveniles) after hatching of the resting eggs. 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).

A possible limitation of the present study was that the experimental condition imposed for artificial incubation may not have provided the required environmental cues to the hatching of all ostracod species present in the egg banks. Although some abiotic variables are controlled in the laboratory, this still does not exactly reflect the characteristics of the natural environment. However, artificial incubations have been used as an effective method to study the egg bank of different communities, such as rotifers (Fernandes et al., 2012), cladocerans (Stenert et al., 2017) and branchiopods (Pinceel et al., 2019).

In conclusion, the composition and abundance of ostracod resting eggs are similar between the edge and central regions in temporary lakes of riverine floodplains, most likely because flood pulses can lead to homogenization of the ostracod egg banks. However, natural floods are becoming less frequent in this region owing to the influence of a cascade of reservoirs upstream of the floodplain. In addition, because of longer periods of drought, reservoirs will retain the water for the production of energy for longer periods of time. Thus, we can infer that reduction of floods, caused by both natural and anthropogenic effects, would influence the structure and spatial variation of ostracod egg banks in the future.

For now, however, the homogenised distribution of ostracod resting eggs between lake regions may increase the dispersal of these structures by biotic vectors (e.g. birds), owing to the larger distribution area in the environment. In addition, this result also has practical implications for the sampling of the ostracod egg banks in floodplain lakes, suggesting that the sediment sampling can be performed at any region of such lakes (edge or centre).

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Captions of tables and figures

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).

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.

Figure 1. Location of the temporary lakes of the Upper Paraná River floodplain. Flow direction is from right to left.

Figure 2. Mean values and standard error of abundance of ostracod hatched from egg banks at the edge and in the centre of the temporary lakes.

Figure 3. Ordination diagram of the principal coordinate analysis of the passive ostracod communities at the edge (dots) and in the centre (squares) of the five temporary lakes.

Figure 4. Mean values and standard error of sediment composition at 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.

Figure 5. Mean values and standard error of organic matter of the sediment at the edge and in the centre of the temporary lakes.

Table 1

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

Table 2

	Standardised Coefficient	95% CI 2.5%	97.5%	AIC	ANOVA
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**

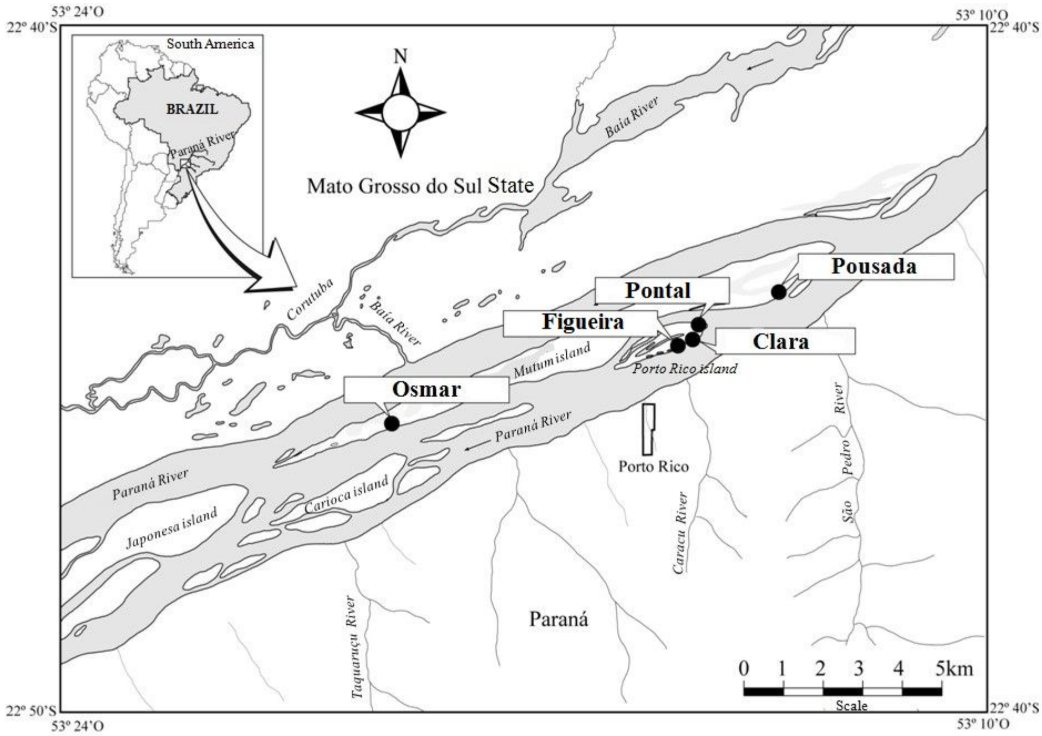

53° 24'O
22° 40'S
53° 10'O
22° 40'S
South America
BRAZIL
Paraná River
N
Mato Grosso do Sul State
Baia River
Corutuba
Baia River
Pousada
Pontal
Figueira
Clara
Mutum island
Porto Rico island
Osmar
Paraná River
Porto Rico
River
Pedro
São
Paraná River
Carioca island
Japonesa island
Caracu River
Taquaruçu River
Paraná
0 1 2 3 4 5km
Scale
22° 50'S
53° 24'O
22° 40'S
53° 10'O

Figure 1

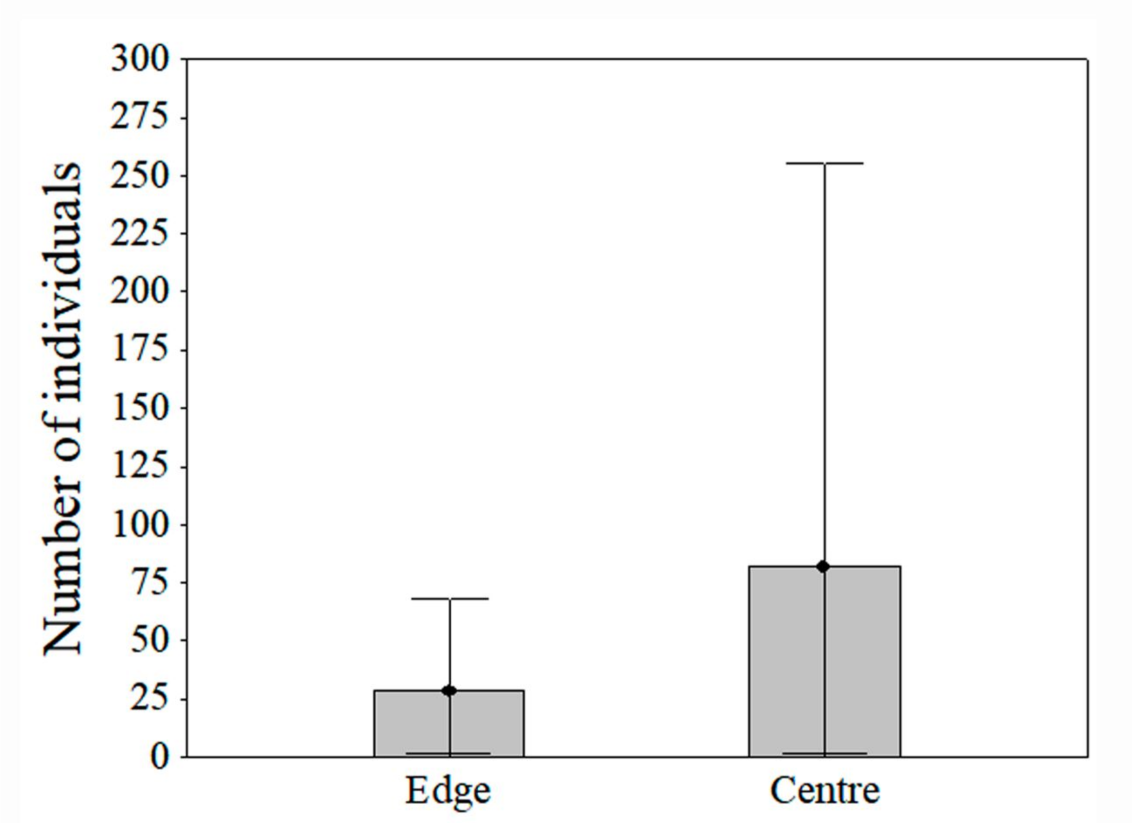

300
275
250
225
200
175
150
125
100
75
50
25
0
Number of individuals
Edge
Centre

Figure 2

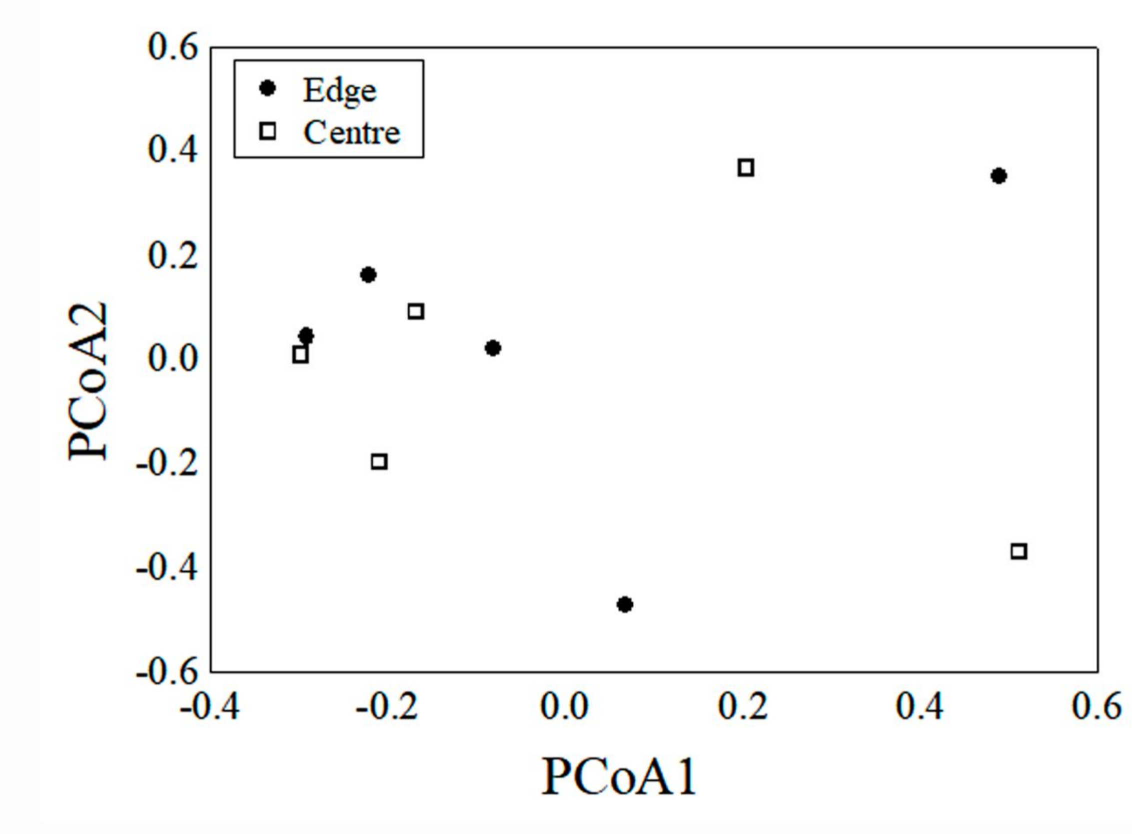


Figure 3

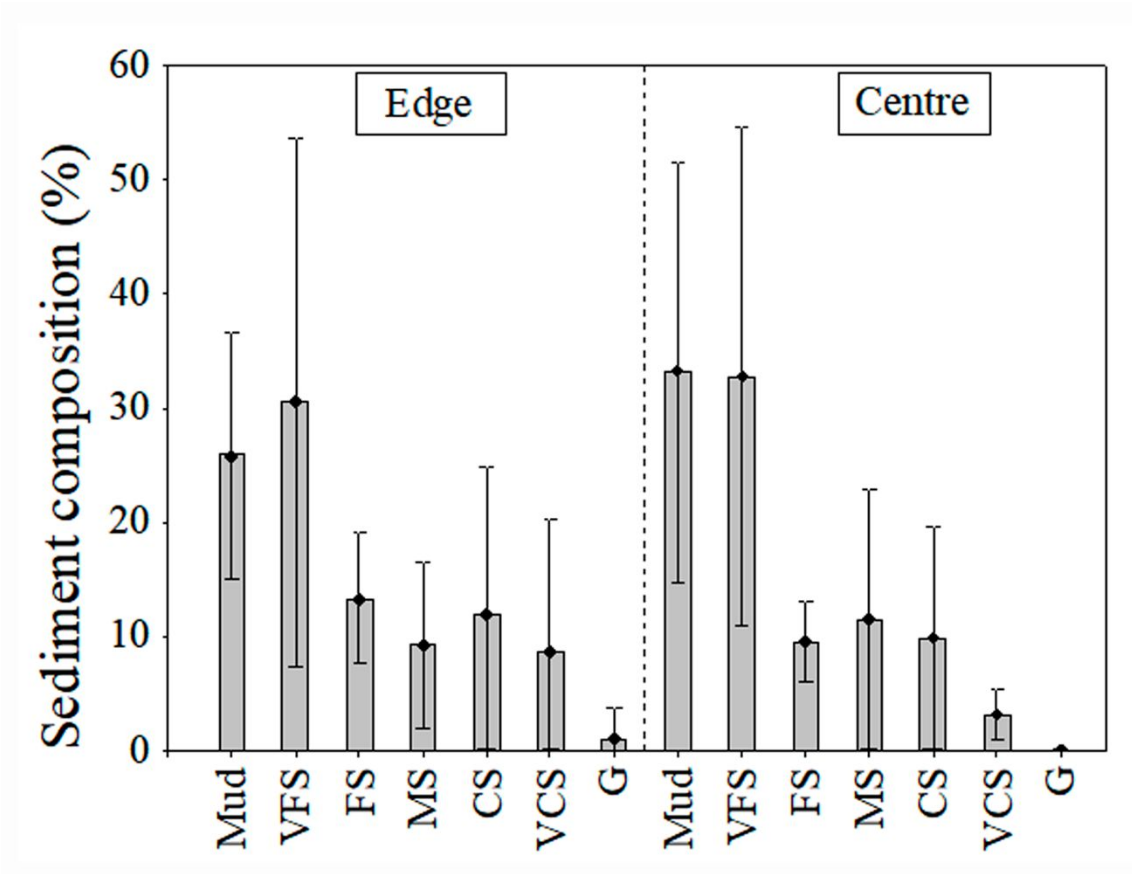
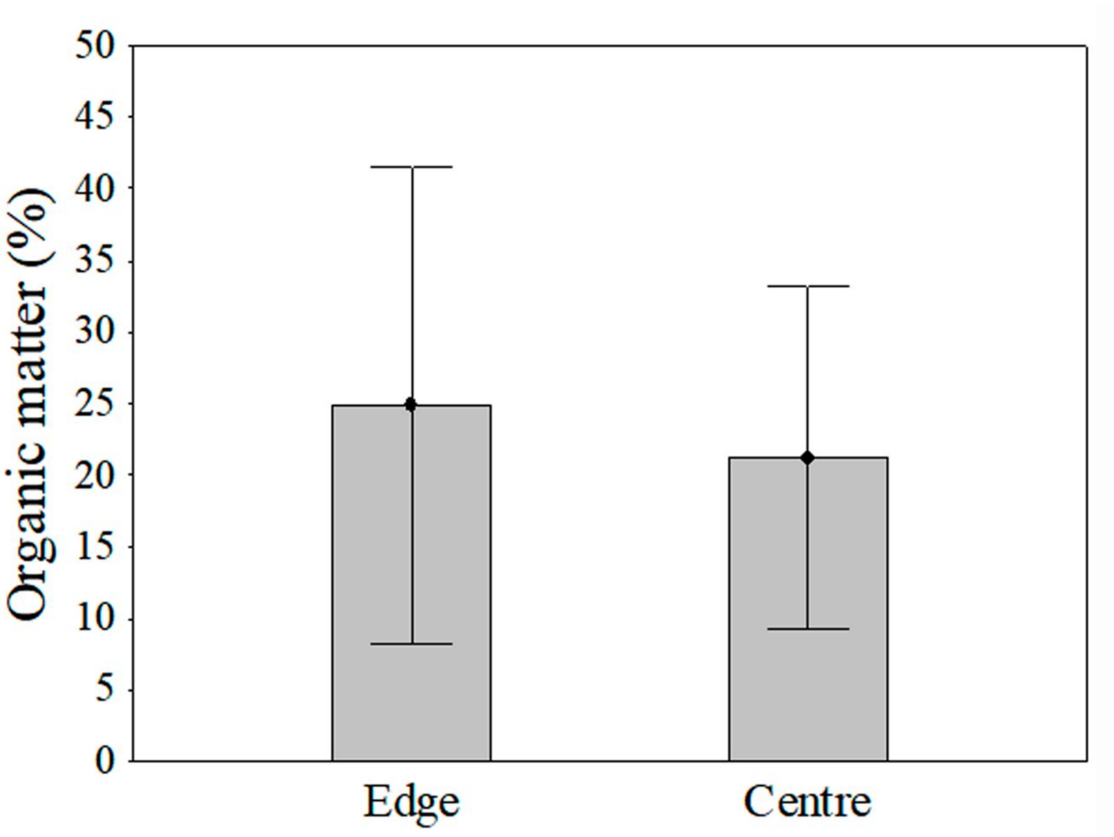

Sediment composition (%)
60
50
40
30
20
10
0
Edge
Centre
Mud
VFS
FS
MS
CS
VCS
G
Mud
VFS
FS
MS
CS
VCS
G

Figure 4



659 Figure 5

MF19081/ SUMMARY TEXT

Spatial variation of ostracod (Crustacea, Ostracoda) egg banks in temporary lakes of a tropical floodplain

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Abstract. Ostracods are microcrustaceans that produce resting eggs under adverse conditions. We evaluated the spatial variation of ostracod resting eggs in different regions of temporary lakes in a Brazilian floodplain. Based on the homogenization effect of flood pulses on aquatic communities in floodplains, we hypothesized that the composition and abundance of ostracod eggs in the centre of the temporary lakes would be similar to those in the edge regions. Samples were collected in the centre and edge regions of five temporary lakes. Sediment was oven-dried, re-hydrated and hatching was monitored in germinating chambers. Twelve ostracod species hatched from the egg banks during our experiments. Our results show that the abundance and species composition were similar between the two regions of the lakes. Flood events may be responsible for the homogenization of the egg banks, owing to the connection of the lakes with the principal river channels. During flooding, water masses powerfully enter the lakes and can redistribute the sediments. Our results show that egg banks have the potential to contribute to the maintenance of the local biodiversity and the resilience of the biodiversity of temporary lake ecosystems.

Additional keywords: Microcrustaceans. Hatching. Dormancy. Passive community.

Spatial variation of ostracod (Crustacea, Ostracoda) egg banks in temporary lakes of a tropical floodplain

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Abstract. Ostracods are microcrustaceans that produce resting eggs under adverse conditions. ~~In floodplains, the flood pulse is the main driving factor in the variation of organisms, which leads to the homogenization of aquatic communities.~~ ~~as a survival strategy, but the ecology and distribution of these structures remain largely unknown.~~ We evaluated the spatial variation of ostracod resting eggs in different regions of temporary lakes in a ~~of a tropical~~~~of the Upper Paraná River~~ ~~floodplain,~~~~from B~~Brazilian floodplain. Based on the homogenization effect of flood pulses on aquatic communities in floodplains, ~~W~~we hypothesized that the composition and abundance of ostracod eggs in the centre of the temporary lakes would be similar to those in the edge regions. Samples were collected ~~during the dry season,~~ in the centre and edge regions of five temporary lakes. Sediment was oven-dried, ~~and after some time~~ re-hydrated~~.~~ and ~~H~~hatching was monitored in ~~a~~ germinating chambers. Twelve ostracod species~~, belonging to families Cyprididae and Candonidae,~~ hatched from the egg banks during our experiments ~~of the lakes~~. Our results show that the abundance and species composition were similar between the two regions of the lakes. Flood events may be responsible for the homogenization of the egg banks, owing to the connection of the lakes with the principal river channels ~~of the Paraná River~~. During flooding, water masses powerfully enter the lakes and can redistribute the sediments. ~~In addition,~~ ~~o~~Our results show that egg banks have the potential to contribute to the maintenance of the local biodiversity and the resilience of the biodiversity of temporary lake ecosystems.

Additional keywords: Microcrustaceans. Hatching. Dormancy. Passive community.

Introduction

Some microcrustaceans produce resting eggs to survive periods of desiccation of the habitats (Brendonck and De Meester 2003). These eggs accumulate in the sediment, forming egg banks (Brock et al. 2003). Such resting egg banks constitute ecological and evolutionary reservoirs which 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). The spatial patterns of the active microfaunal communities are well-studied. However, the passive communities generally remain ill - known (Gehard et al. 2017; Portinho et al., 2017). Nevertheless, some studies on the active communities suggested that the spatial variation in the environment is regulated by the production of resting eggs and the dispersal of these propagules (Hairston et al. 1996).

The~~is~~ spatial variation within lakes and pools in the abundance and composition of resting eggs can occur as a result of two processes. ~~In the first process~~Firstly, the resting eggs can become part of floating debris in drying pools and can thus accumulate along the edges (Martens et al. 1992). ~~In the second process~~Secondly, during the drying of these environments, the eggs accumulate in the deepest regions, mostly the centre of the water bodies, where the water remains the longest, prior to full ~~dessication~~desiccation (Martens et al. 1992; Bright and Bergey 2015). However, in floodplains, during flood periods of high-water level, most of the environments are inundated and become connected to a main river channel. It is then expected that the water flow promotes the dispersal of the resting eggs and consequently homogenizes the egg banks ~~of the communities~~in the sediments of the lakes (Thomaz et al., 2007; Bozelli et al. 2015), so that composition of edge and centre of the water body would become similar in any season of the year.

Resting eggs that settle at the water-sediment interface of the water body, may adhere to particles of sediment (e.g. organic matter) and may also be covered by them ~~it~~ (Brendonck & De Meester, 2003). The abiotic variables of the sediment may thus influence the viability, dispersal and hatchability of resting eggs. For example, the amount of organic matter has an effect on the oxygen concentrations in the sediment, influencing the viability and hatchability of the resting eggs (Rossi et al. 2004). In addition, ~~Besides,~~ the smaller the particles of sediment, the greater their capacity of suspension, which increases the probability of dispersal of resting eggs (Constable 1999).

~~These passive communities of resting eggs are also influenced by theoscillation and duration of high and low water levels, which are crucial factors for the survival of the active community, and subsequent production of these resting eggs (Stenert et al. 2017). Furthermore, other environmental variables of water (pH, oxygen, temperature, salinity, light intensity and duration) can trigger the development of resting eggs, because such eggs have a pronounced capacity to detect changes in aquatic ecosystems over short periods of time (McLay 1978; Brendonck 1996).~~

~~Under favourable environmental conditions, some resting eggs will hatch (Brendonck 1996). However, other eggs may remain in dormancy (Martens 1994; Brock et al. 2003), requiring several wet/dry cycles before they can hatch. This strategy, in which some eggs hatching quickly, while others stay dormant for long periods, is known as bet-hedging. It serves as protection against future catastrophic events in habitat, for example, short periods of inundation that are too brief to support the complete life cycle of the organisms, i.e. from hatching of eggs to production of new eggs by adults (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), and mammals (Vanschoenwinkel et al. 2008), including human activities (Valls et al. 2016).~~

Some groups of freshwater ostracods can produce resting eggs. Most species of the family Cyprididae, which comprises about half the total number of extant non-marine ostracod species (Meisch et al., 2019), are known to produce resting eggs (Horne and Martens 1998). Several experiments have been carried out on the viability and/or the hatching phenology of ostracod eggs of *Eucypris virens* (Jurine, 1820) and *Heterocypris incongruens* (Ramdohr, 1808) in temperate regions in the Palaearctic (e.g. Rossi et al. 2012; Vandekerkhove et al. 2013). ~~In general, t~~There are no studies focusing specifically on resting eggs of ostracods in the Neotropical region. However, some studies on the resting eggs of zooplankton and other invertebrates have occasionally also recorded ostracods hatching from egg banks (Stenert et al. 2010; Ávila et al., 2015; Santangelo et al. 2015; Vargas et al. 2019).

~~Ostracods and their resting eggs can be used as a model group to understand ecological aspects and process in aquatic ecosystems. The spatial patterns of the active microfaunal communities are well-studied. However, the passive communities generally remain ill-~~

~~known (Gehard et al. 2017; Portinho et al., 2017). Nevertheless, some studies on the active communities suggest that the spatial variation in the environment is regulated by the production of resting eggs and the dispersal of these structures (Hairston et al. 1995). Thus, s~~Studies on ostracod resting eggs can provide vital information on the biology of the group. The ecological information about the recruitment of organisms from egg banks can fuel scientifically underpinned recommendations on the conservation of aquatic environments, such as temporary lakes in floodplains. These ecosystems are threatened by anthropic impacts~~,~~ (~~such as~~e.g. the construction of artificial dams) that affect~~s~~ the hydrological dynamics of the ecosystem (droughts and flood events) (Agostinho et al. 2004a), and thus require protective measures.

Here, we evaluate the spatial variation of ostracod resting eggs in different regions of five temporary ~~five~~ lakes of the Upper Paraná River floodplain. Based on the homogenization effect of flooding in the floodplain lakes, we hypothesized that the composition and abundance of ostracod eggs in the centre of the temporary lakes would be similar to those in the edge regions.

Material and methods

Study area

The Paraná River is formed by the junction of 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~~b~~). The Upper Paraná River has a large catchment (approx. 802,150 km^2) in Brazil, which encompasses large parts of 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. 2004b). The climate of the region is tropical-subtropical with distinct rainy (November to March) and dry (April to October) seasons (Cfa) (Eletrosul, 1986; Agostinho et al. 2004b).

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). These lakes are shallow (not exceeding 2.2 meters depth~~ep~~) and small (areas of 0.15 hectares or less). The limnological variables (e.g. dissolved oxygen, electrical conductivity, pH) and vegetation cover formed by herbaceous and arboreal species, with higher presence of emergent macrophytes of the family Poaceae (Kita and De Souza 2003) are similar amongst the five lakes. In the dry season of 2017, four lakes were dry (Pontal, Clara, Figueira and Pousada) and one lake (Osmar) had a low water level (less than 30 cm) during the sampling.

>>> Figure 1

Sampling and hatching procedures

Sediments were collected during the dry season (September 2017), at the edge and in the centre of the five temporary lakes. The edge region ~~corresponds to the site that is~~ was defined as the area in direct contact with the adjacent terrestrial ecosystem, mostly with shallower sites, while the centre region ~~corresponds to~~ was defined as the open area of the lake, associated with deeper sites. ~~Approximately six cm depth of moist sediment (c 250 g)~~ We sampled six cm of sediment depth (c 250 g) ~~were sampled from each region of the lakes~~ using a core sampler (194.5 cm^3 volume) in each region of the lakes, ~~because~~ as the higher viability of resting eggs is usually found in the top three cm of the sediment (Garcia-Roger et al. 2006).

Twelve samples were collected at the edge of each of the lakes, in order to cover the entire edge region of the lake, while five samples were collected in the centre. These samples were taken every three meters (approximately 4.250 kg of sediment per lake). A larger number of samples were collected at the edge of each lake compared to the central region, to better cover the spatial representation of this variable~~e~~ environment. From each lake, the samples of each region (edge and centre) were separately pooled to form a composite sample, totalling 10 samples (2 x 5 ~~replicates~~lakes). The sediment was stored in plastic bottles and was kept refrigerated for two months, following the methods described by Maia-Barbosa et al. (2003). Despite the fact that the lakes were dry (no or very little water), the sediment was mostly still moist.

For the hatching procedure ~~,~~ in the laboratory, the composite samples from each region (edge and centre) of the five lakes were separately homogenized and 300 g of sediment was individually oven-dried at 50°C (this sediment temperature can be easily reached in the floodplain lakes on hot and dry days)~~(Nielsen et al. 2015)~~ and then placed in individual plastic trays, which acted as artificial microcosms, totallingzing 10 microcosms (replicates). Each dry sediment sample was hydrated with 500 ml of distilled water (Fig. S1A) and was maintained in the microcosm at 25° C (Rossi et al., 2004) for 91 days (Fig. S1B) in a germination chamber (Model SOLAB, SL.225). Photoperiods were maintained at 12 hours light/12 hours dark (Rossi et al., 2012).

The incubation period was monitored weekly. Every 7 days, the water from the microcosm was filtered using a plankton net (68 µm) (Fig. S1C). As lifecycles of non-marine ostracods take at least 3 weeks (Meisch, 2000), the time of 7 days iwas not sufficient for the sexual maturation of individuals for reproduction. After that, the water of the microcosm was replaced with -fresh distilled water. The filtered material, retained in the net, was sorted with a stereoscope microscope. Hatched juveniles were grown separately in glass bottles with distilled water, fed with fresh spinach and reared to the adult stage (when the juveniles did not die) 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).

Abiotic variables

Sediment from each region (edge and centre) was also used to determine the particle size and organic matter content. 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. The size of sediment particles was classified as gravel (≥ 2 mm), very coarse sand (2-1 mm), coarse sand (1.0-0.5 mm), medium sand (0.50-0.25 mm), fine sand (0.250-0.125 mm), very fine sand (0.125-0.063 mm) and mud (<0.063 mm). Organic matter in the sediment was obtained from 10 g of dry sediment by incineration at 560 °C for four hours (Moretto et al. 2013). 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 Conductivimeter-Digimed, Digimed, São Paulo, Brazil and pHmeter-Digimed, Digimed, São Paulo, Brazil) were measured weekly in the microcosms.

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, required for parametric tests, were not fulfilled. For this, the total number of ostracods hatched in all weeks for each region and in each lake was used.

The frequency of ostracod species that hatched from resting eggs was calculated using the constancy index (Dajoz 1973) through the expression: $C = n/N \times 100$, 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 ($C \geq 50\%$); accessory ($50\% > C \geq 25\%$); accidental or rare ($C < 25\%$) according to Dajoz (1973).

A principal coordinate analysis (PCoA) was performed to visualize (dis)similarity of ostracod species composition between the lakes' regions (edge and centre), using data from presence and absence of the ostracods hatched weekly (Legendre and Legendre 1998). A Multivariate Permutational Variance Analysis (PERMANOVA) was performed to evaluate differences in ostracod species composition between edge and centre (Anderson 2005). The test was based on a dissimilarity matrix using the Jaccard distance. A total of 999 permutations were performed to assess significance.

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 for 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 significant values of the variance test (ANOVA).

Finally, Kruskal-Wallis test was used to evaluate possible significant differences of each limnological variable between edge and centre. For this, we used the data of dissolved oxygen, pH, and electrical conductivity measured weekly in each microcosm of the edge and centre. In addition, to evaluate possible differences in organic matter content between edge and centre regions, the T test for paired samples was applied. We used a t-test because this test is appropriate for the number and dependency of samples of the present study.

Analyses of variance, 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 software program (version 7.1, Statsoft Inc., 2005, Tulsa, Oklahoma, USA).~~

Results

Composition and abundance of ostracod resting egg banks

Twelve species of ostracods hatched from the egg banks of the five 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* sp. nov. ~~*centrurus* (Klie, 1940)~~ and *Bradleytriebella trispinosa* (Pinto & Purper, 1965) were only reared from sediment from the centre of the lakes (Table 1~~, Fig. 2~~).

>>> Table 1

~~>>> Figure 2~~

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 (Figure 2). 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 common species in both regions, while *Strandesia velhoi* Higuti & Martens, 2013 was common only in the centre of the lakes (Table 1). The results of the PERMANOVA did not show significant differences in the species composition of the egg banks between the centre and edge regions (F= ~~1~~0.62~~53)= 0~~, p= 0.~~9~~87~~)~~) (Fig. 3).

>>>Figure 2

>>> Figure 3

Effects of the abiotic variables on ostracod resting egg banks

Very fine sand was the most dominant type of sediment in the edge regions, whereas mud (clay and silt) was the most dominant type of sediment in the centre regions of the five temporary lakes (Fig. 4). In general, the sediment of the lakes was composed mainly of particles of sediment smaller than 0.25 mm.

>>> Figure 4

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 test, $t= -21.92$, $p= 0.00$) (Fig. 5).

>>> Figure 5

The non-generalized linear model showed positive effects of very fine sand on the number of hatchlings. 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

Environmental variables did not vary significantly between the microcosms of the edge and centre regions (Kruskal-Wallis, 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 $\mu S.cm^{-1}$ (electrical conductivity), and in the “centre” microcosms were 4.45 $mg.L^{-1}$ (dissolved oxygen), 6.68 (pH), 18.27 $\mu S.cm^{-1}$ (electrical conductivity) (Table S1).

Macrophytes germinated from sediments of both regions of the lakes, during the incubation period (Fig. S2). *Nymphaea amazonum* Mart. & Zucc. was the most common macrophyte species recorded in eight of the ten microcosms (Table S1).

Discussion

Composition and abundance of ostracod resting egg banks

We herewith corroborate the hypothesis that the composition and abundance of ostracod resting eggs is similar between the two regions of the –temporary lakes in the Upper Paraná River floodplain. A practical implication based on our results is that further studies about egg banks can be performed with sediment sampled at any region (centre and edge) of temporary lakes in floodplains, due to the similar spatial distribution of resting eggs. Theoretically, flood pulses promote the homogenization of active communities (Thomaz et al. 2007) and similarly, this fact might have contributed to the homogenizsation of the passive community (the egg bank). The similar spatial distribution of the egg bank between edge and centre of the lakes may increase the probability of dispersal of ostracod resting eggs by biotic vectors, when compared to the dispersal of an egg bank that only accumulate eggs in a specific region of the lake. Since several animals (e.g. birds) visit this type of environment, they can promote the dispersal of these structures (Morais Junior et al. 2019).

Resting eggs have shown spatial variation of occurrence in ~~the~~ water bodies with both horizontal (between the edge and centre), and vertical distribution, when the sediment floats on the surface of the water after rains, as observed by Martens et al. (1992) in a temporary pool in Israel. The same study also showed that the floating sediment contained more eggs than the submerged sediments, although the composition did not differ between the regions. In our study, only samples of submerged sediment (dry or wet) were sampled owing to the fact that the lakes were dry or had very low water level during the sampling period.

The presence of resting eggs in the centre and at the edge of temporary water bodies may depend on several factors. For example, drought~~ied~~ resting eggs can accumulate along ~~with~~ the floating debris along 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 in these remaining pools. O~~there and o~~stracods may thus ~~lay~~produce a greater ~~amount~~number of eggs in the deepest part (centre) of the ponds and lakes (Bright and Bergey 2015).

However, both of these processes are mostly relevant for rain-filled, isolated -temporary water bodies. In the temporary ~~floodplain~~ lakes of the Upper Paraná River floodplain, the effect of flood pulses may nullify such processes and may lead to homogenizsation (Thomaz et al. 2007), also with respect to the composition and abundance of ostracods between the edge and centre of these lakes. This is so because during high water periods, the main river waters will invade the lakes, and homogenize and disperse the propagule bank of the dormant communities (Gurnell et al. 2008). The floodwaters will mix the sediments of these temporary lakes and 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 morphology of the temporary floodplain lakes. They are invariably elongated and narrow, and this possibly facilitates the homogenization between the regions of these temporary lakes, as ~~i.e.~~ edge and centre are only a few meters apart.

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 regions 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 owing to environmental factors and passive dispersal by wind and inundation.

All 12 ostracod species hatching from the resting eggs in our experiments have previously been recorded from other 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 regions 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* hatching from sediment egg banks (Havel et al. 2000).

Most studies on production and hatching of resting eggs of ostracods are from the temperate regions of the Palaearctic (Martens et al. 1992; Horne and Martens 1998; Valls et al. 2016) and few studies focus on (sub-) tropical regions. Ostracods are known to lay mixed batches of subitaneous and resting eggs, and it is possible that the ratio of these two types of eggs can be influenced by environmental factors (Dumont et al. 2002; Schön et al. 2012). This is unlike, for example, Cladocera, where resting eggs (ephippia) are only produced by the final sexual population at the end of the reproductive period (mostly summer).

~~Resting eggs have shown variation of occurrence in the water bodies with horizontal (between the edge and centre), and vertical distribution, when the sediment floats on the surface of the water after rains, as observed by Martens et al. (1992) in a temporary pool in Israel. The same study also showed that the floating sediment contained more eggs than the submerged one, although the composition did not differ between the regions. In our study, only samples of submerged sediment (dry or wet) were sampled owing to the fact that the lakes were dried or had very low water level during the sampling period.~~

~~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 along 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 may lay a greater amount of eggs in the deepest part (centre) of the ponds and lakes (Bright and Bergey 2015).~~

~~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 homogenisation (Thomaz et al. 2007), also with respect to the composition and abundance of ostracods between the edge and centre of these lakes. This is so because during high water periods, the main river waters will invade the lakes, and homogenize and disperse the propagule bank of the dormant communities (Gurnell et al. 2008). The floodwaters will mix the sediments of these temporary lakes and 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 morphology of the temporary floodplain lakes. They are invariably elongated and narrow, and this possibly facilitates the homogenization between the regions of these temporary lakes, i.e. edge and centre are only a few meters apart.~~

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Effect of the abiotic variables on hatching of ostracod resting eggs

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 they 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 for suspension (Constable 1999). 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 resting 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. In addition, Tilbert et al. (2019) also found a positive association between the active ostracods and fine and very fine sand in a small tropical estuary in Brazil.

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) material, provided by riparian vegetation, mostly leaves of trees, 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 all microcosms might also contribute to the ecological succession in the microcosms, because these aquatic plants provide substrate and food for ostracods (juveniles) after hatching of the resting eggs. 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).

A possible limitation of the present study was that the experimental condition imposed for artificial incubation may not have provided the required environmental cues to the hatching of all ostracod species present in the egg banks. Although some abiotic variables are controlled in the laboratory, this still does not exactly reflect the characteristics of the natural environment. However, artificial incubations have been used as an effective method to study the egg bank of different communities, such as rotifers (Fernandes et al., 2012), cladocerans (Stenert et al., 2017) and branchiopods (Pinceel et al., 2019).

In conclusion, the composition and abundance of ostracod resting eggs are similar between the edge and central regions in temporary lakes of riverine floodplains, most likely because flood pulses can lead to homogenization of the ostracod egg banks. However, natural floods are becoming less frequent in this region owing to the influence of a cascade of reservoirs upstream of the floodplain. In addition, because of longer periods of drought, reservoirs will retain the water for the production of energy for longer periods of time. Thus, we can infer that reduction of floods, caused by both natural and anthropogenic effects, would influence the structure and spatial variation of ostracod egg banks in the future.

For now, however, the homogenised distribution of ostracod resting eggs between lake regions may increase the dispersal of these structures by biotic vectors (e.g. birds), owing to the larger distribution area in the environment. In addition, this result also has practical implications for the sampling of the ostracod egg banks in floodplain lakes, suggesting that the sediment sampling can be performed at any region of such lakes (edge or centre).In conclusion, the composition and abundance of ostracod resting eggs is similar between the regions in temporary lakes on floodplains and based on the correlations, we can infer that the sediment variables had an effect on the hatching of ostracod resting eggs

In conclusion, the composition and abundance of ostracod resting eggs are similar between the edge and central regions in temporary lakes of riverine floodplains. This results has practical implications for the sampling of ostracod egg banks in floodplain lakes, suggesting that the sediment sampling can be performed at any region of such lakes (edge or centre).

~~Theoretically and based on the results we also infer that flood pulses can lead to homogenization of the ostracod egg banks. The study area has a cascade of reservoirs upstream, which reduce the frequency and duration of flooding periods in this floodplain. this~~ Thus, we can infer that reduction of floods, would influence the ~~structure and spatial variation of ostracod egg bank in the future.~~

~~The homogenised distribution of ostracod resting eggs between lake regions may increase the dispersal of these structures by biotic vectors (e.g. birds), owing to the larger distribution area in the environment.~~

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Captions of tables and figures

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).

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.

Figure 1. Location of the temporary lakes of the Upper Paraná River floodplain. Flow direction is from right to left.

Figure 2. Mean values and standard error of ~~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)~~ .

Figure 3. Ordination diagram of the principal coordinate analysis of the ~~ostracod~~ passive ostracod communities ~~at~~~~in~~at the edge (dots) and in the centre (squares) of the five temporary lakes. ~~Some dots are overlaid.~~

Figure 4. Mean values and standard error of ~~S~~sediment composition ~~of~~ at 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.

Figure 5. Mean values and standard error of ~~O~~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.~~

Table 1

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

Table 2

	Standardised Coefficient	95% CI 2.5%	97.5%	AIC	ANOVA
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**

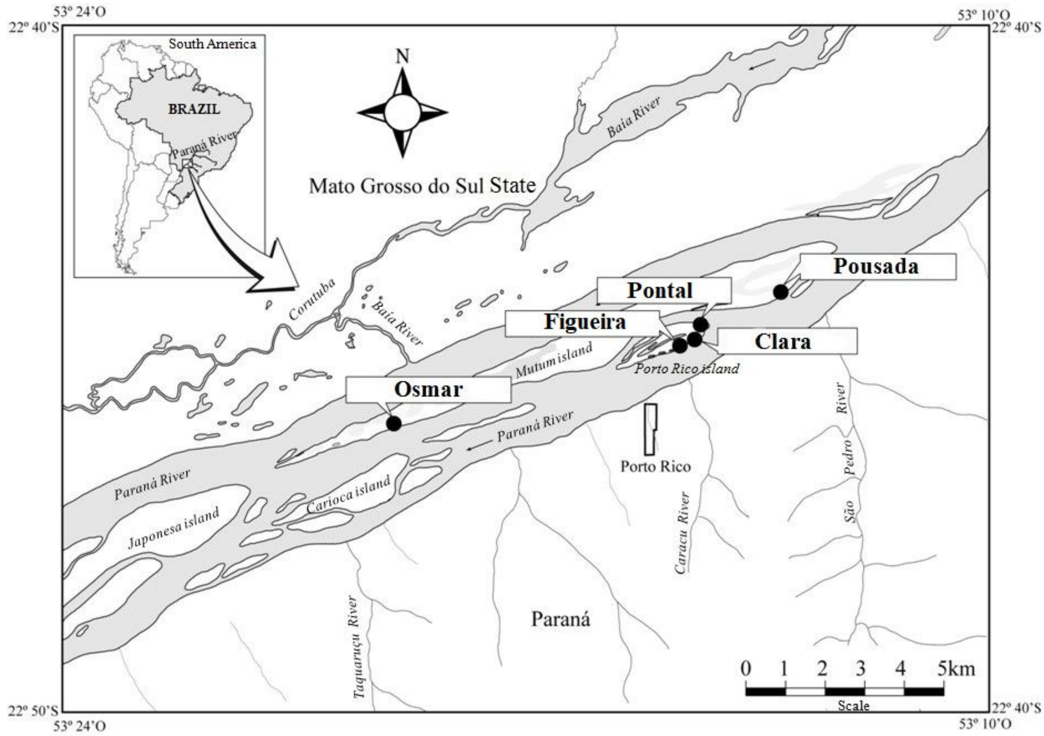
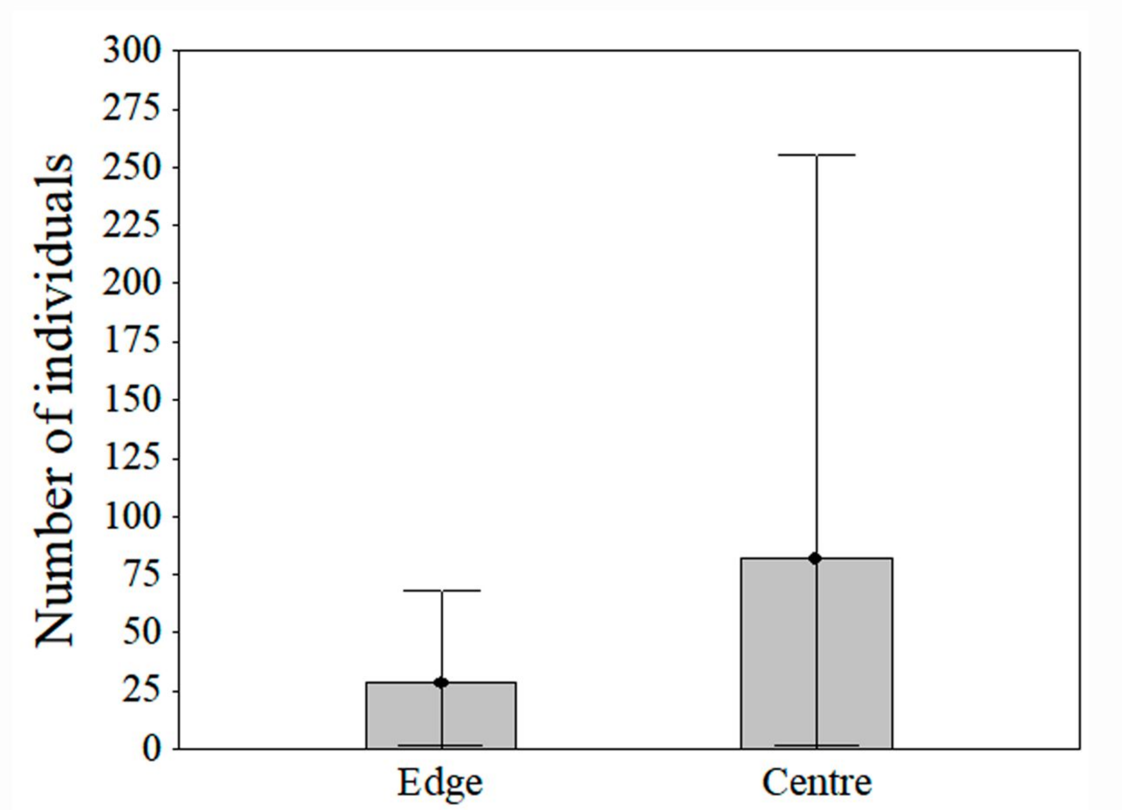

53º 24'O
22º 40'S
53º 10'O
22º 40'S
South America
BRAZIL
Paraná River
N
Mato Grosso do Sul State
Baia River
Corutuba
Baia River
Pousada
Pontal
Figueira
Clara
Mutum island
Porto Rico island
Osmar
Paraná River
Porto Rico
Paraná River
Carioca island
Japonesa island
River
Pedro
São
Caracu River
Taquaruçu River
Paraná
0 1 2 3 4 5km
Scale
22º 50'S
53º 24'O
22º 40'S
53º 10'O

Figure 1


Number of individuals
300
275
250
225
200
175
150
125
100
75
50
25
0
Edge
Centre

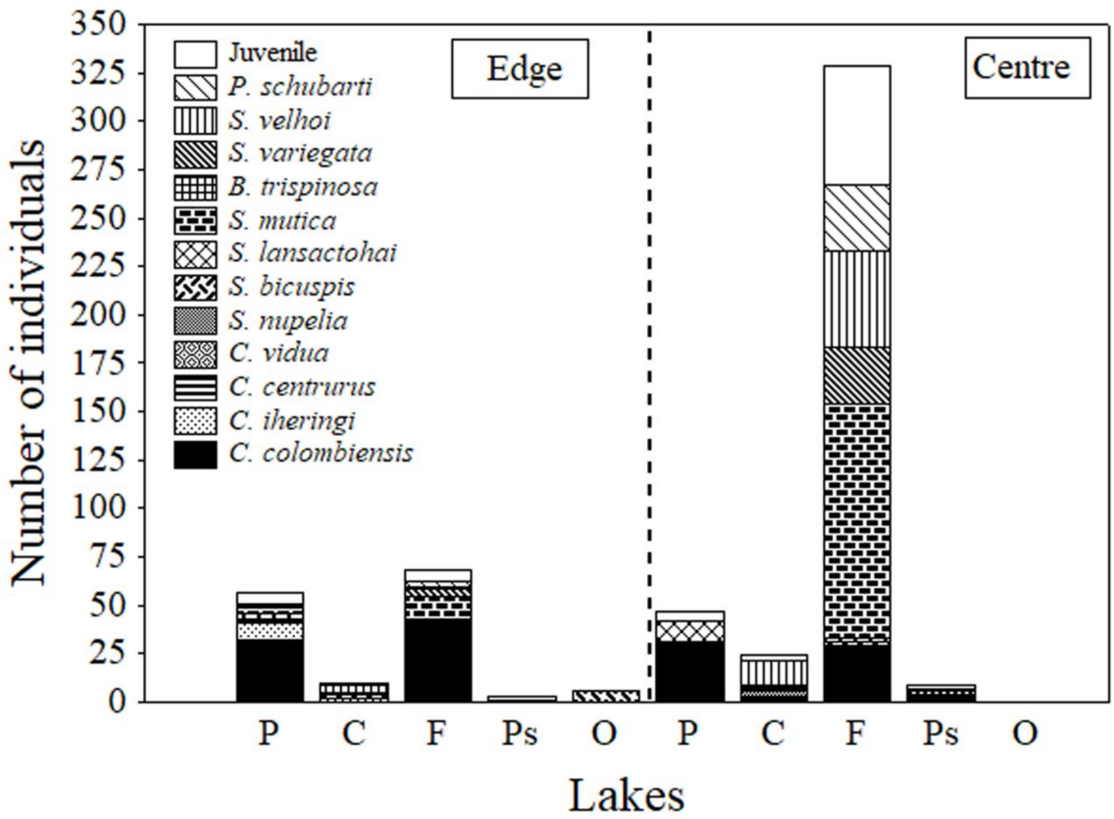
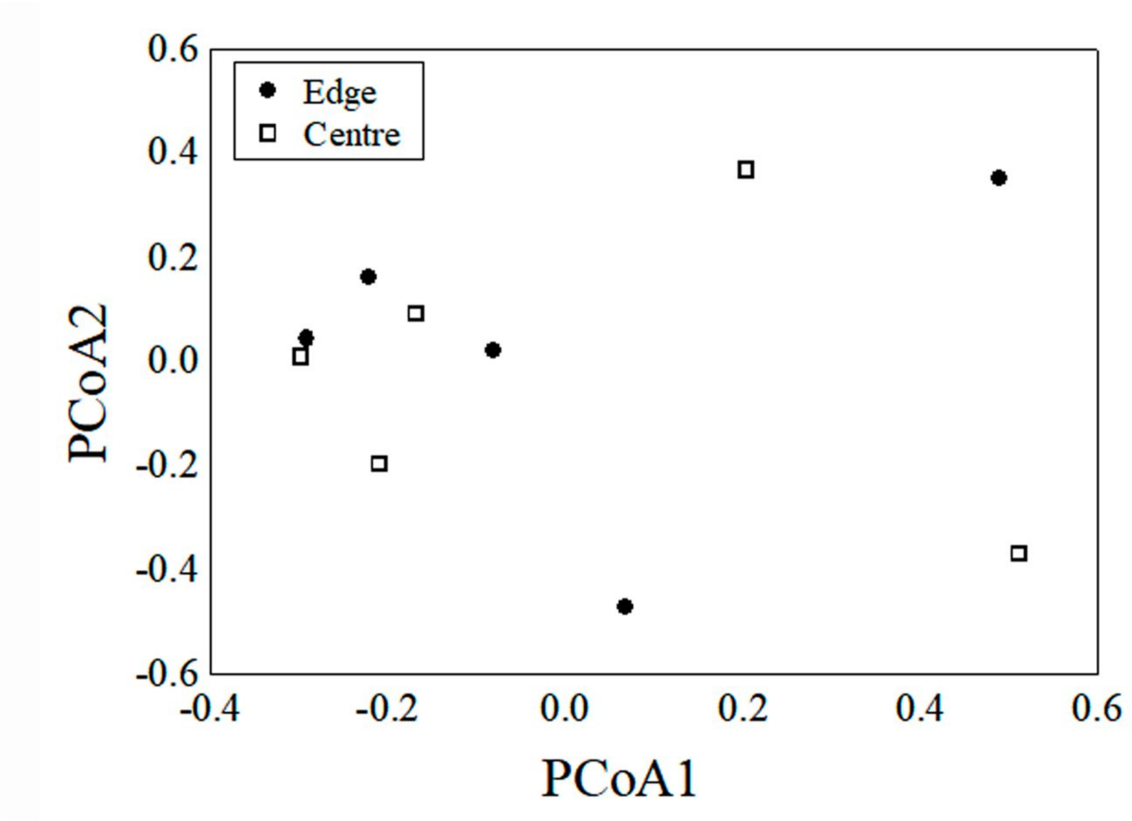

Number of individuals
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
Juvenile
P. schubarti
S. velhoi
S. variegata
B. trispinosa
S. mutica
S. lansactohai
S. bicuspis
S. nupelia
C. vidua
C. centrurus
C. iheringi
C. colombiensis
Edge
Centre
P
C
F
Ps
O
P
C
F
Ps
O
Lakes

Figure 2


Edge
Centre
PCoA2
PCoA1
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6

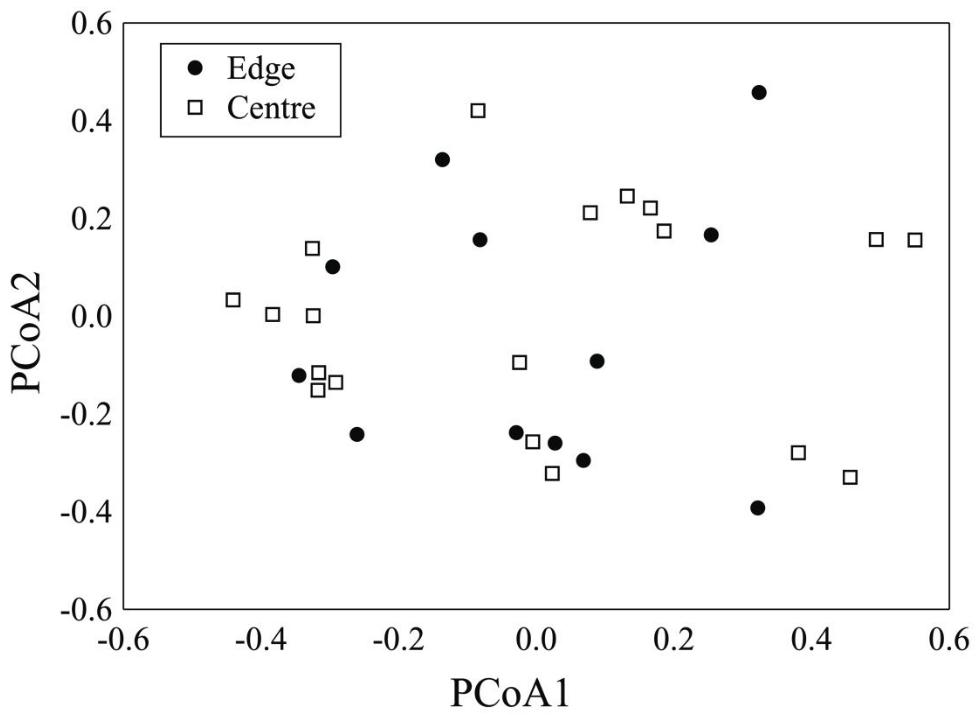

Edge
Centre
PCoA2
PCoA1
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6

Figure 3

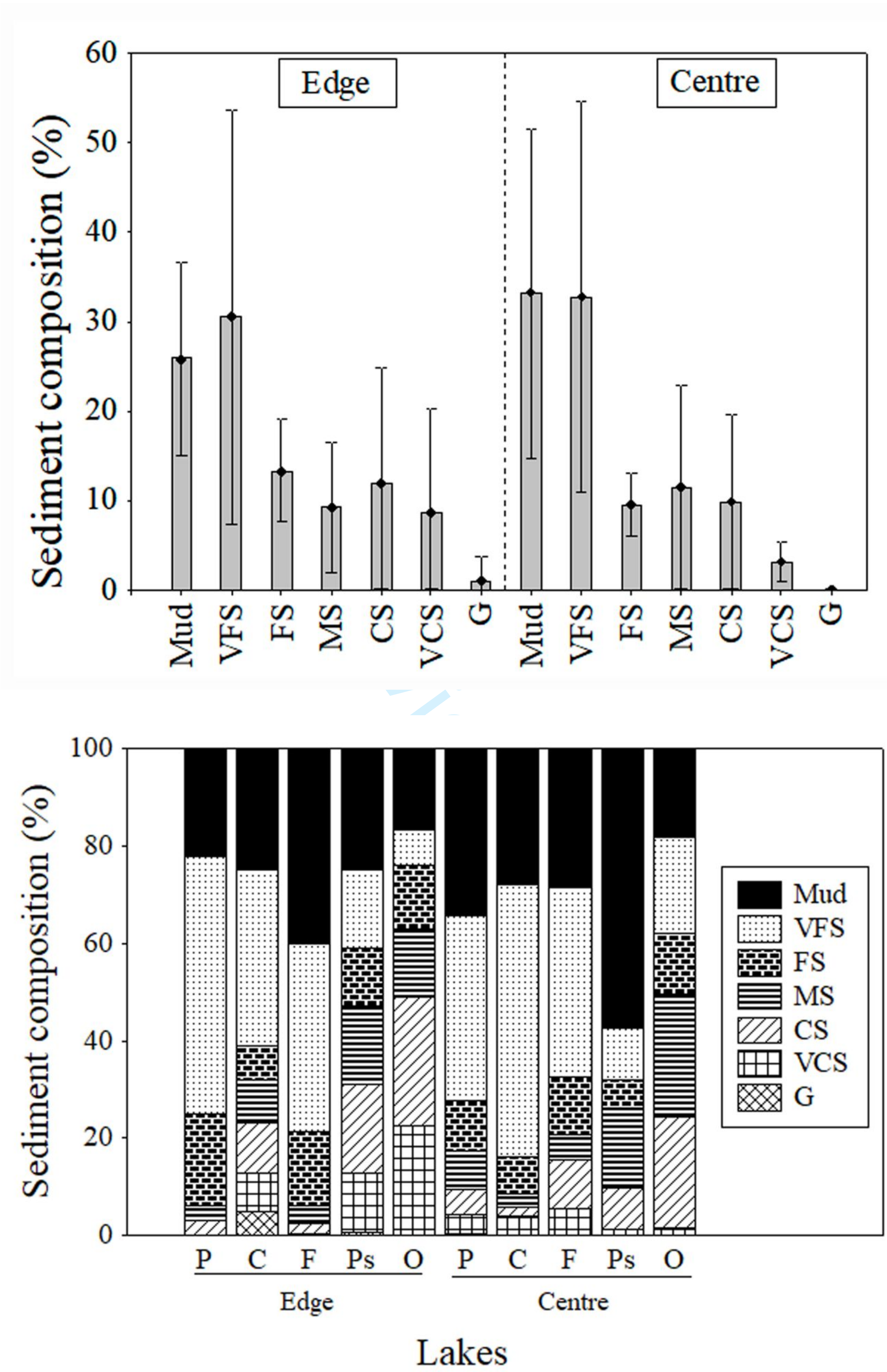

Edge
Centre
Sediment composition (%)
60
50
40
30
20
10
0
Mud
VFS
FS
MS
CS
VCS
G
100
80
Mud
VFS
FS
MS
CS
VCS
G
P
C
F
Ps
O
Edge
Centre
Lakes

Figure 4

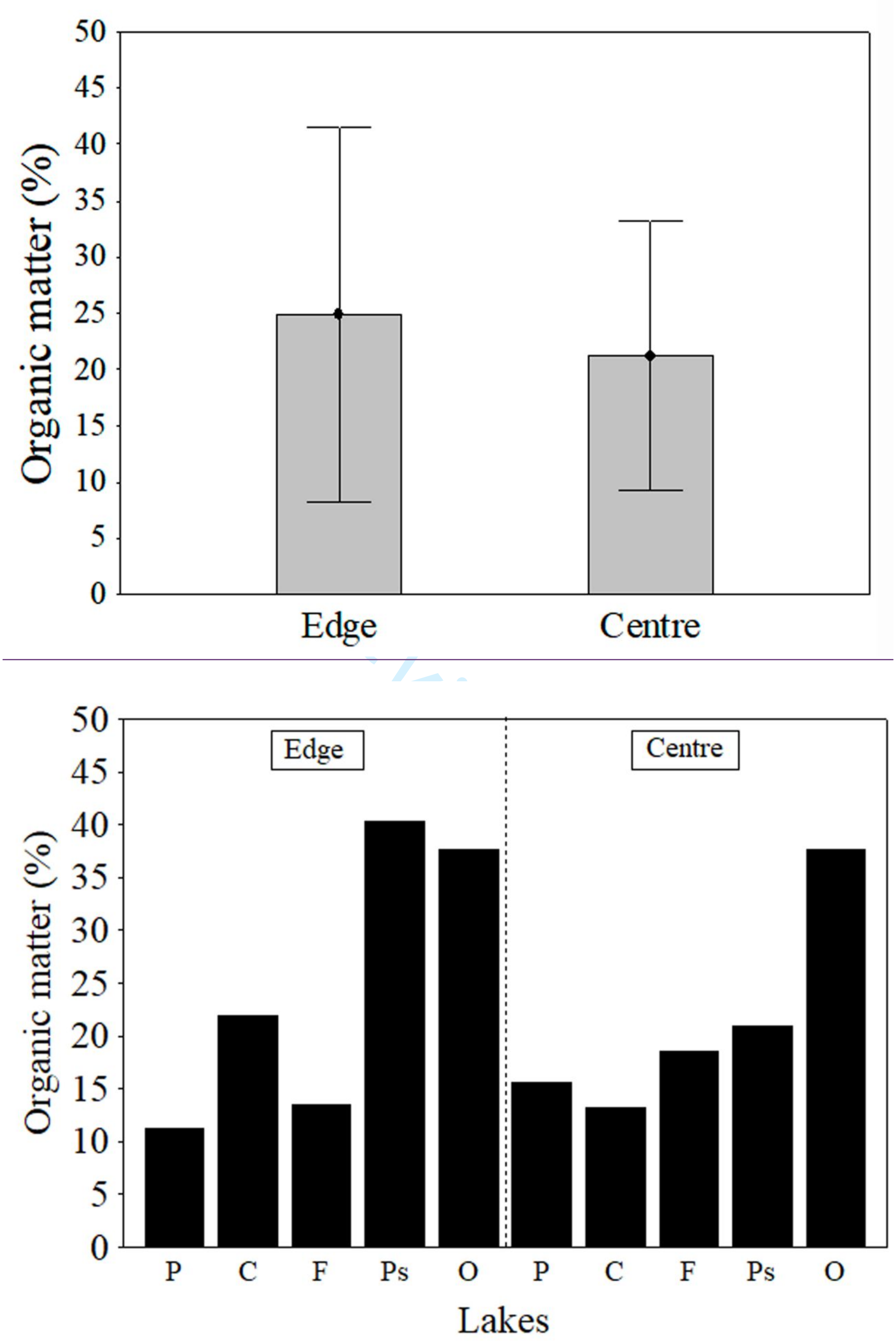

Organic matter (%)
0
5
10
15
20
25
30
35
40
45
50
Edge
Centre
Organic matter (%)
Edge
Centre
P
C
F
Ps
O
P
C
F
Ps
O
Lakes

Figure 5

MF19081

Spatial variation of ostracod (Crustacea, Ostracoda) egg banks in temporary lakes of a tropical floodplain

Jonathan Rosa, Ramiro de Campos, Koen Martens, Janet Higuti

REPLIES TO EDITOR AND REFEREES

COMMENTS FROM DR SAMANTHA CAPON

The reviewer(s) suggest that your manuscript may be suitable for publication with some further revisions. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript. In particular, and as indicated by Reviewer 1, the Introduction needs to be shortened and to highlight the context for your main research questions and hypotheses. Similarly, the discussion needs to more clearly address your findings in light of these as well as their implications.

ANSWER TO ASSOCIATE EDITOR: Thank you very much for your comments. We have revised the manuscript. Besides, we have answered the questions and comments of the reviewers, one by one.

****

Replies to Reviewer(s):

Reviewer 3

General comments

Reviewer 3. Abstract. Need to include a brief explanation as to why it was hypothesised that the composition and abundance of the ostracod egg bank in the centre of the lake would be similar to those in the edge region.

Answer: We rewrote the sentence: "Ostracods are microcrustaceans that produce resting eggs under adverse conditions. We evaluated the spatial variation of ostracod resting eggs in different regions of temporary lakes in a Brazilian floodplain. Based on the homogenization effect of flood pulses on aquatic communities in floodplains, we hypothesized that the composition and abundance of ostracod eggs in the centre of the temporary lakes would be similar to those in the edge regions."

Reviewer 3. Introduction. The intro is very long because it contains too much general ecological information on egg banks. It needs to be shortened to 5 or 6 paragraphs by only including background ecological information that it crucial to showing how the hypothesis was developed.

Answer: Done, we have excluded and rewrote some paragraphs to make the introduction shorter.

Reviewer 3. Methods. It appears that different volumes of sediment were taken from the edge and centre regions (although this has not been explained in the methods so the reader cannot be certain). If this is the case, it would bias any analyses involving species richness and composition, and thus the analyses and interpretations should be treated accordingly.

Answer: In both regions (edge and centre) sampling was performed every three meters. Thus, in order to sample the lake's surroundings, we collected a larger number of samples at the edge (12 samples), compared to the central region (five samples) for a better spatial representation of the environment. We think that the bias of the analyses was reduced by homogenizing the samples separately (edge and centre) and by addition the same proportion of sediment in each microcosm. Other studies about spatial variation of resting eggs used more sediment samples in the edge compared to the centre of the lakes, for a better representation of the environment, because the edge region is always larger (Gehard et al. 2017).

This information was added in the text: "A larger number of samples were collected at the edge of each lake compared to the central region, to better cover the spatial representation of this variable environment. "

Gerhard, M., Iglesias, C., Clemente, J. M., Goyenola, G., Meerhoff, M., Pacheco, J. P., Mello, F. T., and Mazzeo, N. (2017). What can resting egg banks tell about cladoceran diversity in a shallow subtropical lake? *Hydrobiologia* **798,** 75-86.

Reviewer 3. The 50°C sediment drying temperature was just referenced from Nielsen at al. (2015), and no actual justification has been provided for using this temperature.

Nielsen et al. (2015) applied 50°C as a climate change treatment in their study and reported that few microfauna hatched from sediment dried at that temperature.

Answer: We excluded the reference. The citation was used to show that even at high temperatures the hatching still occurred. Besides, Nielsen et al. (2015) have assessed the general microfauna and this might be different for specific groups, for example, ostracods. We used the 50°C sediment drying temperature because when dried eggs are lying on the sandy surface of the dry lake in the blazing mid-day sun, ambient temperatures can reach higher than 50°C.

This information was added in the text: "For the hatching procedure in the laboratory, the composite samples from each region (edge and centre) of the five lakes were separately homogenized and 300 g of sediment was individually oven-dried at 50°C (this sediment temperature can be easily reached in the floodplain lakes on hot and dry days) …"

Reviewer 3. Results. Given that the individual results for the lakes have not been reported in the results, the analyses should just treat the lakes as reps consistently throughout the whole paper. Therefore Figure's 2, 4, and 5 should present the mean and standard error values for each region, rather than the results for each lake (replicate).

Answer: Done. We added new figures with the mean and standard error values of each region. Thank you very much.

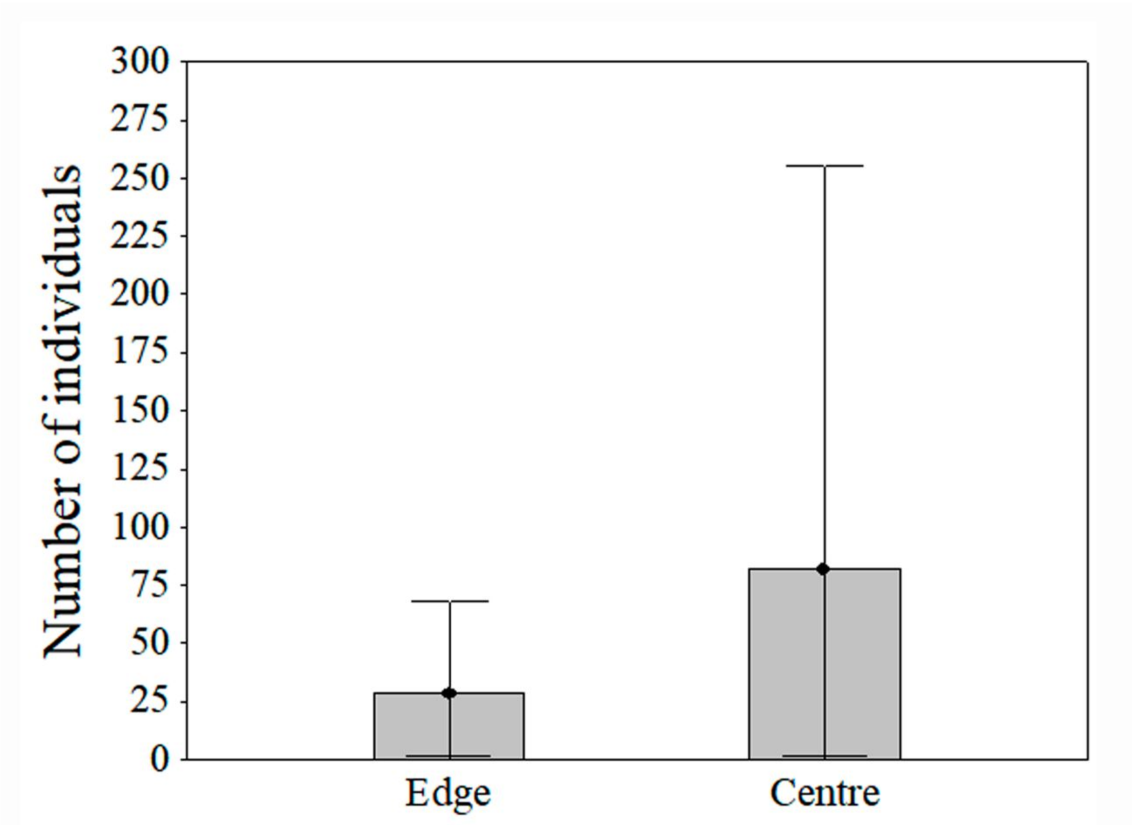

Number of individuals
300
275
250
225
200
175
150
125
100
75
50
25
0
Edge
Centre

Figure 2

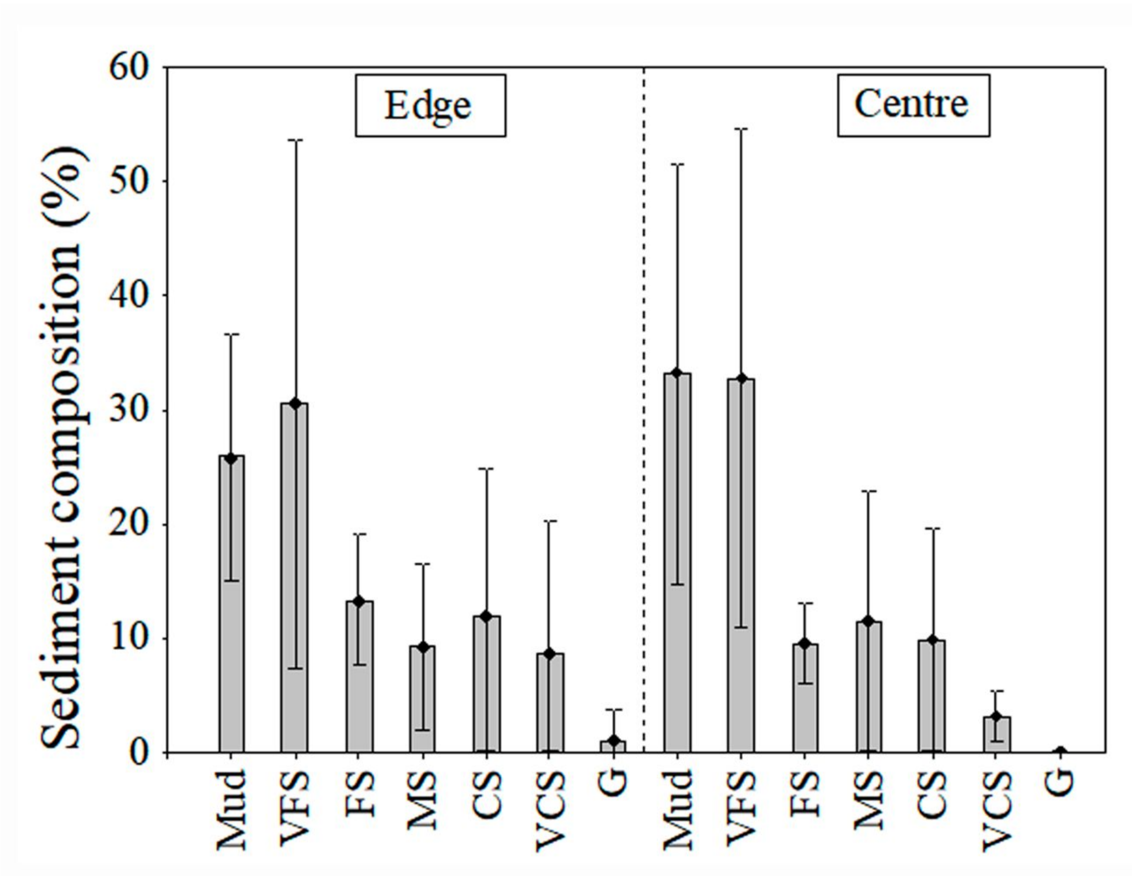

Edge
Centre
Sediment composition (%)
0
10
20
30
40
50
60
Mud
VFS
FS
MS
CS
VCS
G
Mud
VFS
FS
MS
CS
VCS
G

Figure 4

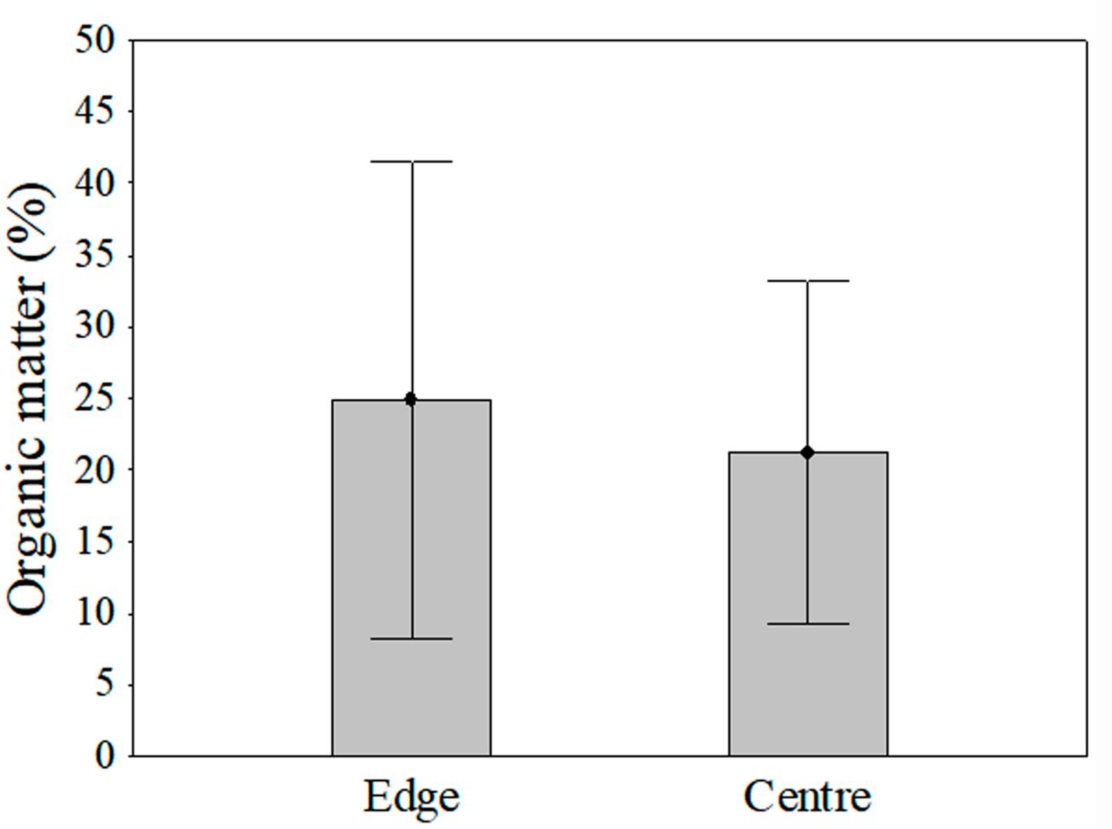


Figure 5

Reviewer 3. The dots currently represent the hatching values for each week. They should instead represent the 5 lakes if the lakes are being treated as the replicates. Also, this should be explained in the figure caption.

Answer: Done. We added new results and new figure in the text showing the species composition of the edge and centre regions of 5 lakes. In addition, we rewrote the legend of Figure 3: "Ordination diagram of the principal coordinate analysis of the passive ostracod communities at the edge (dots) and in the centre (squares) of the five temporary lakes."

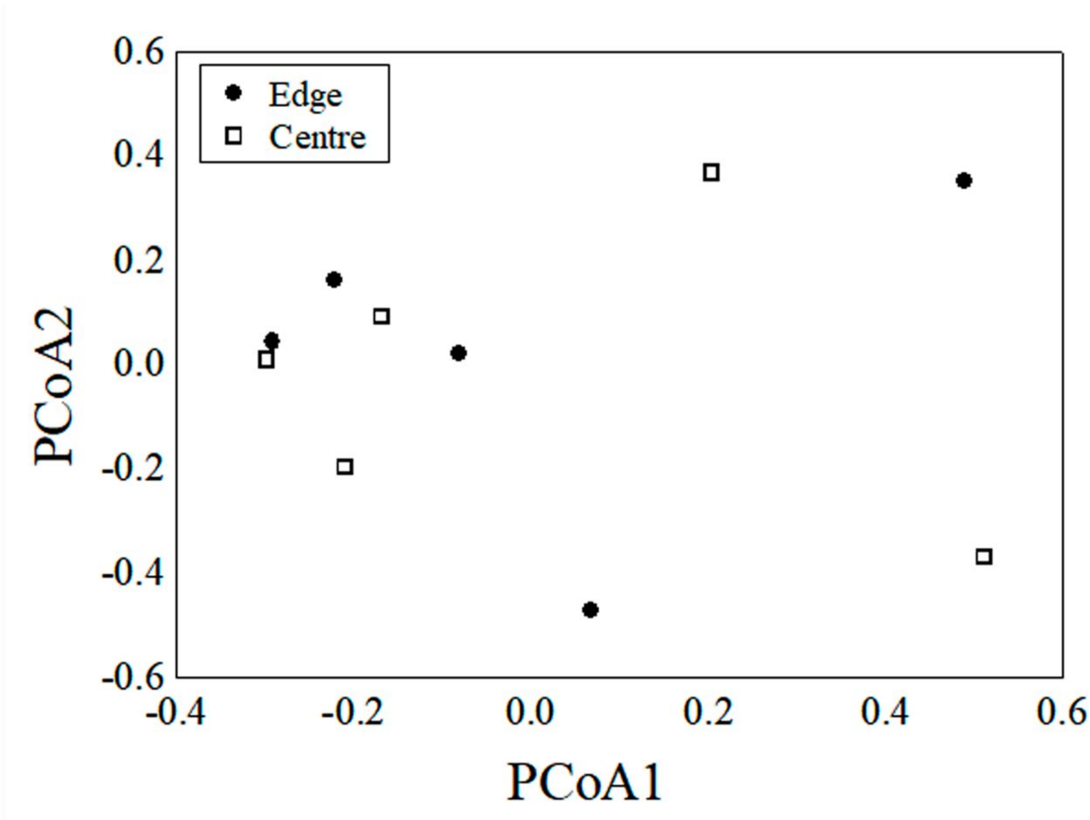


Figure 3

Reviewer 3. Discussion. There is no acknowledgement of the study limitations associated with relying on hatching under artificial conditions. This needs to be included in the discussion.

Answer. We added a new sentence in the text: "A possible limitation of the present study was that the experimental condition imposed for artificial incubation may not have provided the required environmental cues to the hatching of all ostracod species present in the egg banks. Although some abiotic variables are controlled in the laboratory, this still does not exactly reflect the characteristics of the natural environment. However, artificial incubations have been used as an effective method to study the egg bank of different communities, such as rotifers (Fernandes et al., 2012), cladocerans (Stenert et al., 2017) and branchiopods (Pinceel et al., 2019)."

Fernandes, A. P. C., Braghin, L. D. S. M., Nedli, J., Palazzo, F., Lansac-Tôha, F. A., & Bonecker, C. C. (2012). Passive zooplankton community in different environments of a neotropical floodplain. *Acta Scientiarum. Biological Sciences*, *34*(4), 413-418.

Pinceel, T., Vanschoenwinkel, B., Weckx, M., & Brendonck, L. (2019). An empirical test of the impact of drying events and physical disturbance on wind erosion of zooplankton egg banks in temporary ponds. *Aquatic Ecology*, 1-8.

Stenert, C., Wüsth, R., Pires, M. M., Freiry, R. F., Nielsen, N., and Maltchik, L. (2017). Composition of cladoceran dormant stages in intermittent ponds with different hydroperiod lengths. *Ecological Research* **32,** 921-930.

Specific comments

Comment 1. This sentence needs editing because it doesn't make sense.

Answer: Done, we rewrote the sentence: "Ostracods are microcrustaceans that produce resting eggs under adverse conditions. We evaluated the spatial variation of ostracod resting eggs in different regions of temporary lakes in a Brazilian floodplain. Based on the homogenization effect of flood pulses on aquatic communities in floodplains, we hypothesized that the composition and abundance of ostracod eggs in the centre of the temporary lakes would be similar to those in the edge regions."

Comment 2. Be specific with the time.

Answer: In order to adjust the abstract in the journal roles (maximum limit of 200 words), we rewrote the sentence: **"**Samples were collected in the centre and edge regions of five temporary lakes. Sediment was oven-dried, re-hydrated and hatching was monitored in germinating chambers."

Comment 3. Single sentence paragraph.

Answer: We excluded the sentence to shorten the introduction, as suggested by reviewer 3.

Comment 4. How? This statement is too general.

Answer: We excluded the sentence to make the introduction shorter, as suggested by reviewer 3.

Comment 5. NN here 23/10.

Answer: Sorry, we did not understand your comment. We think it could be a personal comment to himself by the reviewer.

Comment 6. Awkward phrasing.

Answer: We rewrote the sentence: "We sampled six cm of sediment depth (c 250 g) using a core sampler (194.5 5 cm^3 volume) in each region of the lakes, as the higher viability of resting eggs is usually found in the top three cm of the sediment (Garcia-Roger et al. 2006)."

Comment 7. Same volume for each region?

Answer: No, a larger number/ volume of samples was collected at the edge (2.500 kg), compared to the central region (1.250 kg) in order to sample the lake's surroundings for a better spatial representation of the environment. This information was added in the methods: "A larger number of samples were collected at the edge of each lake compared to the central region, to better cover the spatial representation of this variable environment."

Comment 8. State where the incubation was carried out.

Answer: The incubation was performed in the laboratory in a germination chamber (Model SOLAB, SL.225).

This information was added in the text: "For the hatching procedure in the laboratory, the composite samples from each region (edge and centre) of the five lakes were separately homogenized and 300 g of sediment was individually oven-dried at 50°C (this sediment temperature that can be easily reached in the floodplain lakes on hot and dry days) and then placed in individual plastic trays, which acted as artificial microcosms, totalling 10 microcosms. Each dry sediment sample was hydrated with 500 ml of distilled water (Fig. S1A) and was maintained in the microcosm at 25° C (Rossi et al., 2004) for 91 days (Fig. S1B) in a germination chamber (Model SOLAB, SL.225). Photoperiods were maintained at 12 hours light/12 hours dark (Rossi et al., 2012)."

Comment 9. Justification?

Answer: The justification about the temperature used for dried sediment was added in the text: "For the hatching procedure in the laboratory, the composite samples from each region (edge and centre) of the five lakes were separately homogenized and 300 g of sediment was individually oven-dried at 50°C (this sediment temperature can be easily reached in the floodplain lakes on hot and dry days) and then placed in individual plastic trays, which acted as artificial microcosms, totalling 10 microcosms."

Comment 10. Also state the theoretical/practical implications of this as the main message. Then repeat this main message in the abstract and conclusion.
Answer: Done. This information was added in the text: "A practical implication based on our results is that further studies about egg banks can be performed with sediment sampled at any region (centre and edge) of temporary lakes in floodplains, due to the similar spatial distribution of resting eggs. Theoretically, flood pulses promote the homogenization of active communities (Thomaz et al. 2007) and similarly, this fact might have contributed to the homogenization of the passive community (the egg bank). The similar spatial distribution of the egg bank between edge and centre of the lakes may increase the probability of dispersal of ostracod resting eggs by biotic vectors, when compared to the dispersal of an egg bank that only accumulate eggs in a specific region of the lake. Since several animals (e.g. birds) visit this type of environment, they can promote the dispersal of these structures (Morais Junior et al. 2019)."

Morais Junior, C. S., Diniz, L. P., Sousa, F. D. R., Gonçalves-Souza, T., Elmoor-Loureiro, L. M. A., and Melo Júnior, M. (2019). Bird feet morphology drives the dispersal of rotifers and microcrustaceans in a Neotropical temporary pond. *Aquatic Sciences*, **81**, 1-9.

Comment 11. The content from L318 to 352 relates directly to the hypothesis and thus should be moved to the start of the discussion following the response to the hypothesis.
Answer: Done. We moved the sentence to the start of the discussion.

Comment 12. Very weak conclusion and only 1 sentence. What are the theoretical or practical implications?
Answer: We rewrote the conclusion:
"In conclusion, the composition and abundance of ostracod resting eggs are similar between the edge and central regions in temporary lakes of riverine floodplains, most likely because flood pulses can lead to homogenization of the ostracod egg banks. However, natural floods are becoming less frequent in this region owing to the influence of a cascade of reservoirs upstream of the floodplain. In addition, because of longer

periods of drought, reservoirs will retain the water for the production of energy for longer periods of time. Thus, we can infer that reduction of floods, caused by both natural and anthropogenic effects, would influence the structure and spatial variation of ostracod egg banks in the future.

For now, however, the homogenised distribution of ostracod resting eggs between lake regions may increase the dispersal of these structures by biotic vectors (e.g. birds), owing to the larger distribution area in the environment. In addition, this result also has practical implications for the sampling of the ostracod egg banks in floodplain lakes, suggesting that the sediment sampling can be performed at any region of such lakes (edge or centre)."

Comment 13. Order?

Answer: We organized the order of the references.

Reviewer: 5

Reviewer 5: Not in bibliography

- Hairston et al. 1995 - Legendre and Legendre 1998 - Anderson 2005

Not in the text

- Battauz, Y. S., Paggi S. B. J., and Paggi, J. C. (2015) Endozoochory by an ilyophagous fish in the Paraná River floodplain: a window for zooplankton dispersal. Hydrobiologia 755, 161–171.

- Hofmann, H., Lorke, A., and Peeters, F. (2008). Ecological Effects of Water-Level Fluctuations in Lakes. In Temporal scales of water-level fluctuations in lakes and their ecological implications. (Eds K. M. Wantzen, K. O. Rothhaupt, M. Mörtl, M. Cantonati, L. G. Tóth, and P. Fischer) pp. 85-96. (Developments in Hydrobiology Springer, Dordrecht). –

Junk, W. J., Bayley, P. B., and Sparks, R. E. (1989). The flood pulse concept in river-floodplain systems. Canadian Journal of Fisheries and Aquatic Science 106, 110-127.

Answer: We organized the references. Thank you very much.

Comment 1. What kind of vegetation is found in the border of the lakes? Are they common among all the lakes studied? This information is important because it influences the richness of species and consequently the resting eggs bank.

Answer: This information was added in the text: "The limnological variables (e.g. dissolved oxygen, electrical conductivity, pH) and vegetation cover formed by herbaceous and arboreal species, with higher presence of emergent macrophytes of the family Poaceae (Kita and De Souza 2003) are similar amongst the five lakes."

Kita, K. K., and De Souza, C. M. (2003). Floristic survey phytophysiognomy of the Figueira pond in the upper Paraná River floodplain, in Porto Rico, state of Paraná. *Acta Scientiarum Biological Science* **25,** 145-155.

Comment 2. In all microcosms? Add this information.

Answer: No, the germination of the *Nymphaea amazonum* was recorded in eight of the ten microcosms. This information was added in the text: "*Nymphaea amazonum* Mart. & Zucc. was the most common macrophyte species recorded in eight of the ten microcosms (Table S1)."

Comment 3. As the lakes of this flood basin have been studied for a long time, it would be interesting to use the richness data of cladocera species and resistance eggs these lakes and both areas.

Answer: Unfortunately, there are no studies of cladocera (or ostracod) richness from the active community and the egg bank in the edge and centre regions in the lakes of this floodplain.

The Cladocera studies carried out in these lakes do not consider the edge and centre regions, having only general information about the total cladoceran richness per lake. For example, there are data on cladoceran richness from the active (36 species) and passive (22 species) communities in one of the lakes studied (Osmar Lake), however there is no information on richness in the edge and centre regions (Palazzo et al., 2008; Fialek 2018). For this reason these references were not added in the discussion.

Fialek, C. G. 2018. Restabelecimento da comunidade zooplanctônica em uma lagoa temporária Neotropical: regime hidrológico e formas de dormência. Doctoral dissertation, Universidade Estadual de Maringá. Departamento de Biologia. Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais. Maringá, Brazil.

Palazzo, F., Bonecker, C. C., and Fernandes, A. P. C. (2008). Resting cladoceran eggs and their contribution to zooplankton diversity in a lagoon of the Upper Paraná River floodplain. *Lakes & Reservoirs: Research & Management*, *13*(3), 207-214.

Comment 4. It would be very interesting to compare data on species richness and distribution of other invertebrates (eg Cladocera) in this floodplain considering the central and border region of these lakes, before and after the flooding period.
Answer: There are no studies with other invertebrates evaluating the spatial distribution between the edge and centre of the lakes in this floodplain, during the flood and drought periods.
The studies that were carried out in the lakes of this floodplain with other invertebrates (e.g. cladocerans) did not consider the spatial variation of the edge and centre. For this reason, they were not used in the discussion.

Comment 5. Do you have any idea about the bacterial community in this sediment?
Answer: Unfortunately, we don't have any information about the bacterial community of the sediment from these temporary lakes.

Comment 6. Has this happened in all microcosms?
Answer: Yes, the germination of macrophytes was recorded in all microcosms. This information was added in the text: "The germination of macrophytes in all microcosms might also contribute to the ecological succession, because these aquatic plants provide substrate and food for ostracods (juveniles) after hatching of the resting eggs."

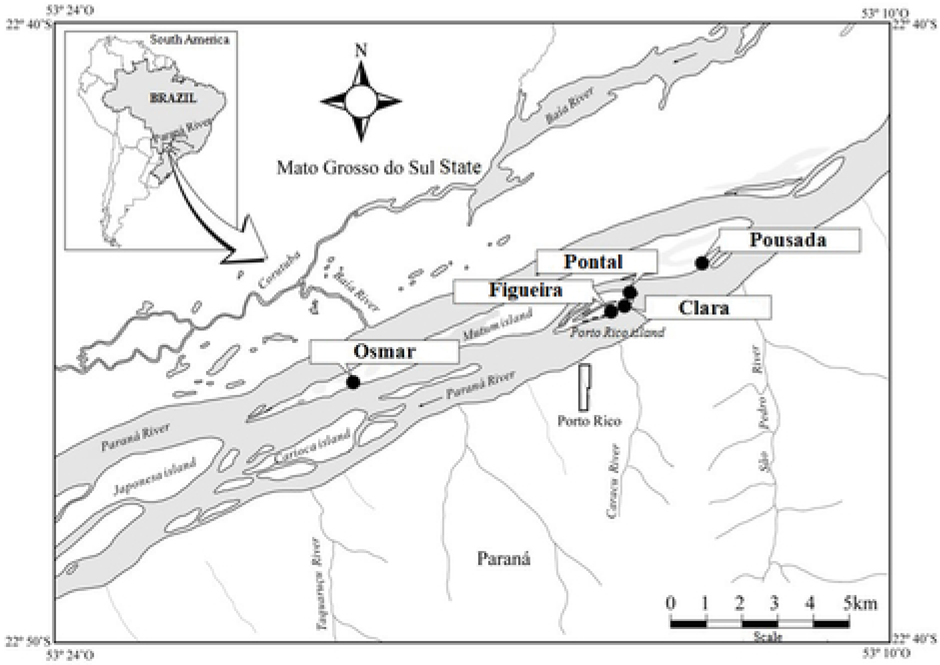


Figure 1. Location of the temporary lakes of the Upper Paraná River floodplain. Flow direction is from right to left.

39x28mm (300 x 300 DPI)

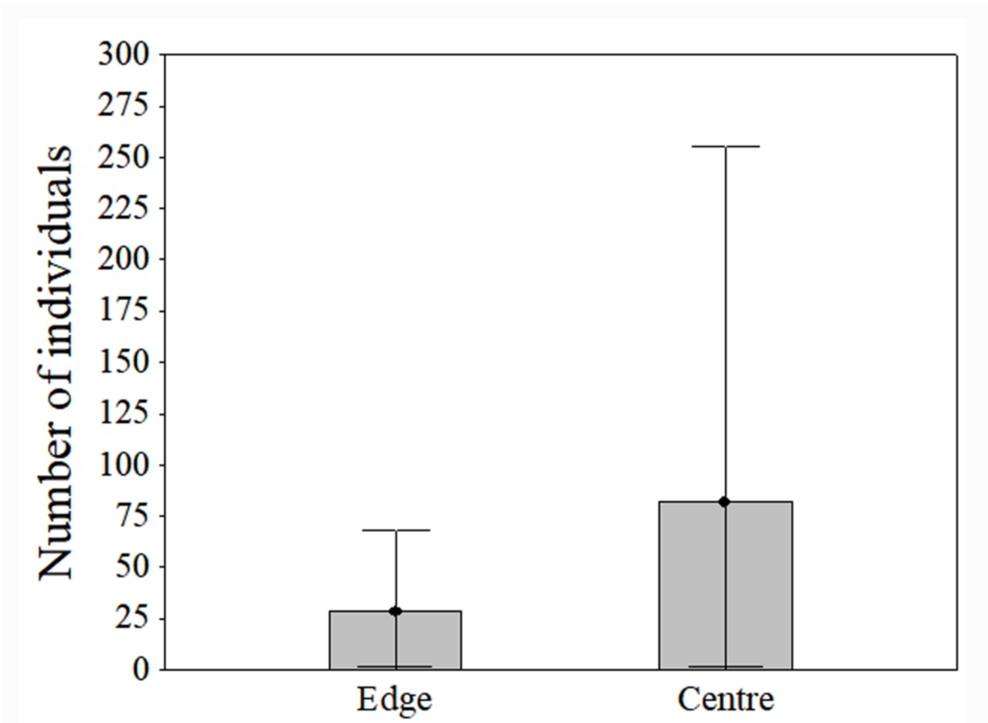


Figure 2. Mean values and standard error of abundance of ostracod hatched from egg banks at the edge and in the centre of the temporary lakes.

165x120mm (300 x 300 DPI)

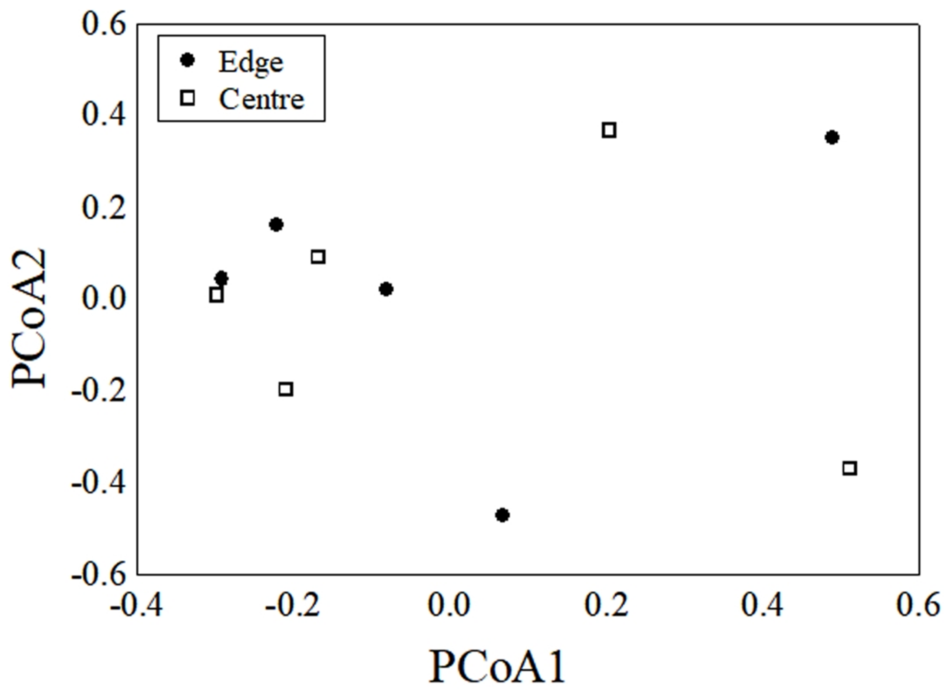


Figure 3. Ordination diagram of the principal coordinate analysis of the passive ostracod communities at the edge (dots) and in the centre (squares) of the five temporary lakes.

168x124mm (300 x 300 DPI)

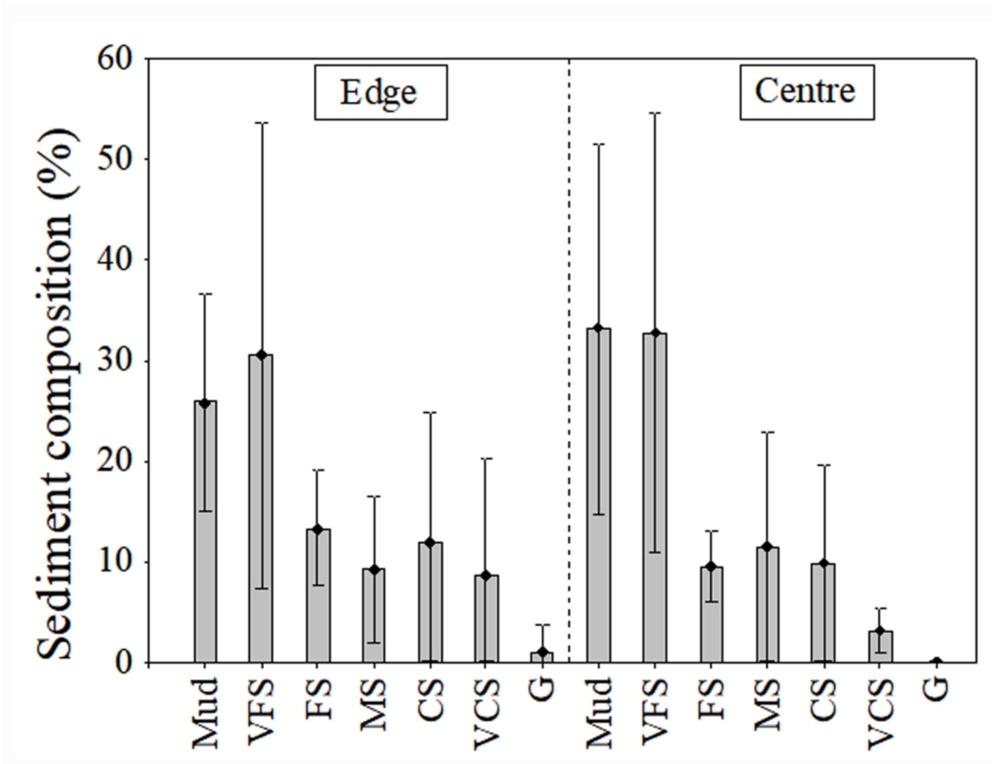


Figure 4. Mean values and standard error of sediment composition at the edge and in the centre of the temporary lakes. P = Pontal, C = Clara, F = Figueira, Ps = Pousada, O = Osmar, Mud = Silt and clay, VFS = Very fine Sand, FS = Fine Sand, MS = Medium Sand, CS = Coarse Sand, VCS = Very Coarse Sand, G = Gravel.

159x122mm (300 x 300 DPI)

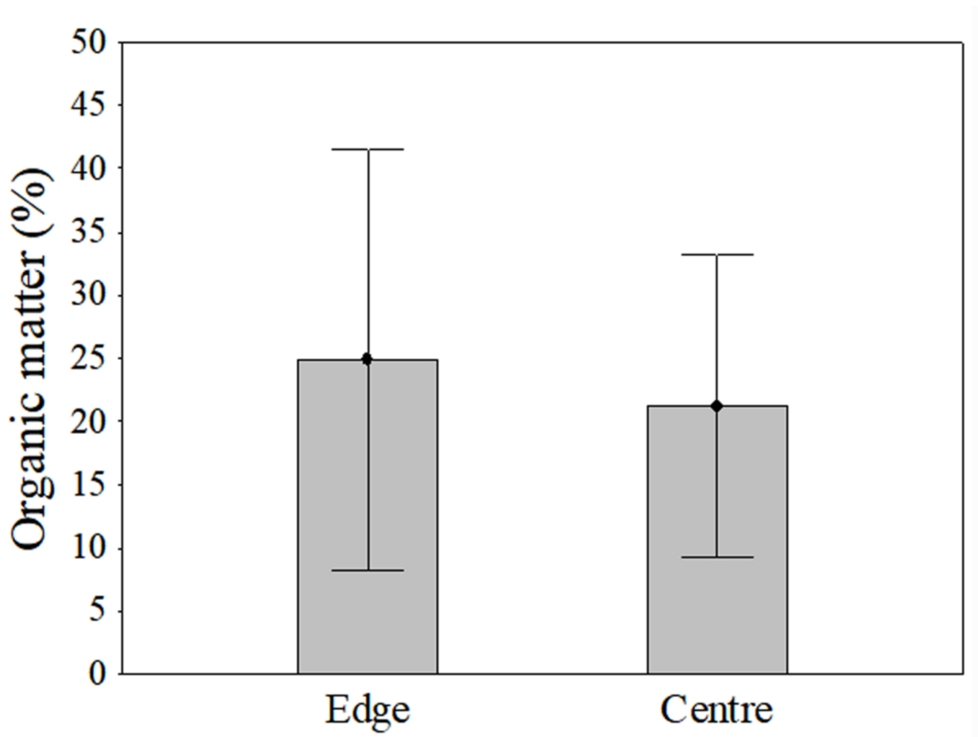


Figure 5. Mean values and standard error of organic matter of the sediment at the edge and in the centre of the temporary lakes.

161x118mm (300 x 300 DPI)