



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

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

3

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18

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

34

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

36

37

## 39 **Introduction**

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

45 Differential accumulation of resting eggs in the sediment can lead to differences in the  
46 composition and abundance of the passive communities in distinct compartments of the  
47 water bodies (Brendonck and De Meester 2003; Vandekerkhove et al. 2005; Gerhard et  
48 al. 2017). The spatial patterns of the active microfaunal communities are well-studied.  
49 However, the passive communities generally remain ill - known (Gerhard et al. 2017;  
50 Portinho et al., 2017). Nevertheless, some studies on the active communities suggested  
51 that the spatial variation in the environment is regulated by the production of resting eggs  
52 and the dispersal of these propagules (Hairston et al. 1996).

53 The spatial variation within lakes and pools in the abundance and composition of resting  
54 eggs can occur as a result of two processes. Firstly, the resting eggs can become part of  
55 floating debris in drying pools and can thus accumulate along the edges (Martens et al.  
56 1992). Secondly, during the drying of these environments, the eggs accumulate in the  
57 deepest regions, mostly the centre of the water bodies, where the water remains the  
58 longest, prior to full desiccation (Martens et al. 1992; Bright and Bergey 2015). However,  
59 in floodplains, during flood periods of high-water level, most of the environments are  
60 inundated and become connected to a main river channel. It is then expected that the water  
61 flow promotes the dispersal of the resting eggs and consequently homogenizes the egg  
62 banks in the sediments of the lakes (Thomaz et al., 2007; Bozelli et al. 2015), so that  
63 composition of edge and centre of the water body would become similar in any season of  
64 the year.

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

73 Some groups of freshwater ostracods can produce resting eggs. Most species of the family  
74 Cyprididae, which comprises about half the total number of extant non-marine ostracod  
75 species (Meisch et al., 2019), are known to produce resting eggs (Horne and Martens  
76 1998). Several experiments have been carried out on the viability and/or the hatching  
77 phenology of ostracod eggs of *Eucypris virens* (Jurine, 1820) and *Heterocypris*  
78 *incongruens* (Ramdohr, 1808) in temperate regions in the Palaearctic (e.g. Rossi et al.  
79 2012; Vandekerkhove et al. 2013). There are no studies focusing specifically on resting  
80 eggs of ostracods in the Neotropical region. However, some studies on the resting eggs  
81 of zooplankton and other invertebrates have occasionally also recorded ostracods  
82 hatching from egg banks (Stenert et al. 2010; Ávila et al., 2015; Santangelo et al. 2015;  
83 Vargas et al. 2019).

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

95

## 96 **Material and methods**

### 97 *Study area*

98 The Paraná River is formed by the junction of the Grande and Parnaíba rivers, in South-  
99 central Brazil, and is the second largest river in South America (4,695 km long)  
100 (Agostinho et al. 2008). The Upper Paraná River has a large catchment (approx. 802,150  
101 km<sup>2</sup>) in Brazil, which encompasses large parts of the states of Paraná, São Paulo, Mato  
102 Grosso do Sul, Minas Gerais and Goiás (Souza-Filho and Steuvax 2004). The upper part  
103 of this river comprises a floodplain, which includes a series of small islands and a variety  
104 of environments, such as channels, rivers, connected and isolated lakes, as well as  
105 temporary lakes. The Upper Paraná River floodplain is located in the “Área de Proteção  
106 Ambiental das Ilhas de Várzea do Rio Paraná” (Environmental Protection Area)

107 (Agostinho et al. 2004b). The climate of the region is tropical-subtropical with distinct  
108 rainy (November to March) and dry (April to October) seasons (Cfa) (Eletrosul, 1986;  
109 Agostinho et al. 2004b).

110 Here, we study the passive ostracod communities of five temporary and isolated lakes  
111 located on the Porto Rico island (Pontal, 22°45 '05.7" S/ 053°15'23.6" W; Clara,  
112 22°45'20.7" S/ 053°15'27.7" W; Figueira, 22°45'22.7" S/ 053°15'34.0" W) and Mutum  
113 island (Pousada, 22°44'43.4" S/ 053°14'06.9 W; Osmar, 22°46'28.6" S/ 053°19'58.8" W)  
114 in the Upper Paraná River floodplain (Fig. 1). These lakes are shallow (not exceeding 2.2  
115 meters depth) and small (areas of 0.15 hectares or less). The limnological variables (e.g.  
116 dissolved oxygen, electrical conductivity, pH) and vegetation cover formed by  
117 herbaceous and arboreal species, with higher presence of emergent macrophytes of the  
118 family Poaceae (Kita and De Souza 2003) are similar amongst the five lakes. In the dry  
119 season of 2017, four lakes were dry (Pontal, Clara, Figueira and Pousada) and one lake  
120 (Osmar) had a low water level (less than 30 cm) during the sampling.

121

122 >>> Figure 1

123

#### 124 *Sampling and hatching procedures*

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

132 Twelve samples were collected at the edge of each of the lakes, in order to cover the entire  
133 edge region of the lake, while five samples were collected in the centre. These samples  
134 were taken every three meters (approximately 4.250 kg of sediment per lake). A larger  
135 number of samples were collected at the edge of each lake compared to the central region,  
136 to better cover the spatial representation of this variable environment. From each lake,  
137 the samples of each region (edge and centre) were separately pooled to form a composite  
138 sample, totalling 10 samples (2 x 5 lakes). The sediment was stored in plastic bottles and  
139 was kept refrigerated for two months, following the methods described by Maia-Barbosa

140 et al. (2003). Despite the fact that the lakes were dry (no or very little water), the sediment  
141 was mostly still moist.

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

151 The incubation period was monitored weekly. Every 7 days, the water from the  
152 microcosm was filtered using a plankton net (68 µm) (Fig. S1C). As lifecycles of non-  
153 marine ostracods take at least 3 weeks (Meisch, 2000), the time of 7 days was not  
154 sufficient for the sexual maturation of individuals for reproduction. After that, the water  
155 of the microcosm was replaced with fresh distilled water. The filtered material, retained  
156 in the net, was sorted with a stereoscope microscope. Hatched juveniles were grown  
157 separately in glass bottles with distilled water, fed with fresh spinach and reared to the  
158 adult stage (when the juveniles did not die) in separate chambers, for identification and  
159 counting. The ostracod species were identified following Higuti et al. (2010, 2013) and  
160 using the references in Martens and Behen (1994).

161

#### 162 *Abiotic variables*

163 Sediment from each region (edge and centre) was also used to determine the particle size  
164 and organic matter content. Sediment composition was determined according to the  
165 method of Suguio (1973), using the Wentworth scale (Wentworth 1922). The samples  
166 were sorted in a nested series of sieves (size range between 2 mm and < 0.63 mm) and  
167 weighed. The size of sediment particles was classified as gravel (> 2 mm), very coarse  
168 sand (2-1 mm), coarse sand (1.0-0.5 mm), medium sand (0.50-0.25 mm), fine sand  
169 (0.250-0.125 mm), very fine sand (0.125-0.063 mm) and mud (<0.063 mm). Organic  
170 matter in the sediment was obtained from 10 g of dry sediment by incineration at 560 °C  
171 for four hours (Moretto et al. 2013). The difference between the initial and final weights  
172 of the sediment indicates the amount of organic matter that was present in the sediment.

173 Dissolved oxygen ( $\text{mg.L}^{-1}$ ) (YSI oximeter 550A), electrical conductivity ( $\mu\text{S.cm}^{-1}$ ) and  
174 pH (using Conductivimeter-Digimed, Digimed, São Paulo, Brazil and pHmeter-Digimed,  
175 Digimed, São Paulo, Brazil) were measured weekly in the microcosms.

176

#### 177 *Data analysis*

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

183 The frequency of ostracod species that hatched from resting eggs was calculated using  
184 the constancy index (Dajoz 1973) through the expression:  $C = n/N \times 100$ , where:  $C =$   
185 constancy;  $n =$  number of samples in which the species was recorded and  $N =$  total number  
186 of samples. The following categories were assigned: constant ( $C \geq 50\%$ ); accessory ( $50\%$   
187  $> C \geq 25\%$ ); accidental or rare ( $C < 25\%$ ) according to Dajoz (1973).

188 A principal coordinate analysis (PCoA) was performed to visualize (dis)similarity of  
189 ostracod species composition between the lakes' regions (edge and centre), using data  
190 from presence and absence of the ostracods hatched weekly (Legendre and Legendre  
191 1998). A Multivariate Permutational Variance Analysis (PERMANOVA) was performed  
192 to evaluate differences in ostracod species composition between edge and centre  
193 (Anderson 2005). The test was based on a dissimilarity matrix using the Jaccard distance.  
194 A total of 999 permutations were performed to assess significance.

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

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



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

213 Analyses of variance, PCoA and Models GAM analyses were carried out in R 3.4  
214 software (R Development Core Team 2013) using the vegan (Oksanen et al. 2018),  
215 permute (Simpson 2018) and mgcv (Wood 2018) packages.

216

## 217 **Results**

### 218 *Composition and abundance of ostracod resting egg banks*

219 Twelve species of ostracods hatched from the egg banks of the five temporary lakes.  
220 Cyprididae was the richest and most abundant family, represented by 11 species. The  
221 family Candonidae was represented only by the species *Physocypria schubarti* Farkas,  
222 1958. *Cypridopsis vidua* (O. F. Müller, 1776), *Cypricercus* sp. nov. and *Bradleytriebella*  
223 *trispinosa* (Pinto & Purper, 1965) were only reared from sediment from the centre of the  
224 lakes (Table 1).

225

226 >>> Table 1

227

228 A total of 553 ostracod specimens hatched from the sediments of the five temporary lakes;  
229 144 and 409 ostracods were recorded from the edges and centres, respectively (Figure 2).  
230 There was no significant difference in the number of individuals between the two regions  
231 (Wilcoxon Test,  $p = 0.07$ ). *Chlamydotheca colombiensis* (Sars, 1901) was the most  
232 abundant species at the edge and *Strandesia mutica* (Sars, 1901) in the centre of the lakes.  
233 According to the constancy index, *S. mutica* and *C. colombiensis* were the most common  
234 species in both regions, while *Strandesia velhoi* Higuti & Martens, 2013 was common  
235 only in the centre of the lakes (Table 1). The results of the PERMANOVA did not show  
236 significant differences in the species composition of the egg banks between the centre and  
237 edge regions ( $F = 0.62$ ,  $p = 0.87$ ) (Fig. 3).

238

239 >>>Figure 2

240

241 &gt;&gt;&gt; Figure 3

242

243 *Effects of the abiotic variables on ostracod resting egg banks*

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

248

249 &gt;&gt;&gt; Figure 4

250

251 Coarse particulate organic matter (roots and leaves) was observed in all five temporary  
252 lakes. The organic matter content of the sediment was higher at the edge, when compared  
253 to the centre (T test,  $t = -21.92$ ,  $p = 0.00$ ) (Fig. 5).

254

255 &gt;&gt;&gt; Figure 5

256

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

260

261 &gt;&gt;&gt; Table 2

262

263 Environmental variables did not vary significantly between the microcosms of the edge  
264 and centre regions (Kruskal-Wallis, oxygen:  $H = 3.58$ ,  $p = 0.058$ ; pH:  $H = 0.07$ ,  $p = 0.79$ ;  
265 electrical conductivity:  $H = 0.01$ ,  $p = 0.91$ ). The mean values for these variables in the  
266 “edge” microcosms were 4.95 mg.L<sup>-1</sup> (dissolved oxygen), 6.74 (pH), 20.77 μS.cm<sup>-1</sup>  
267 (electrical conductivity), and in the “centre” microcosms were 4.45 mg.L<sup>-1</sup> (dissolved  
268 oxygen), 6.68 (pH), 18.27 μS.cm<sup>-1</sup> (electrical conductivity) (Table S1).

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

272

273 **Discussion**

274 *Composition and abundance of ostracod resting egg banks*

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

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

296 The presence of resting eggs in the centre and at the edge of temporary water bodies may  
297 depend on several factors. For example, drought resting eggs can accumulate along the  
298 floating debris along the edges (Martens et al. 1992). But drying pools will also  
299 concentrate fauna at their deepest (mostly central) point towards the end of the  
300 hydrological cycle, and then resting eggs can be produced at a higher rate in these  
301 remaining pools. Ostracods may thus produce a greater number of eggs in the deepest  
302 part (centre) of the ponds and lakes (Bright and Bergey 2015).

303 However, both of these processes are mostly relevant for rain-filled, isolated temporary  
304 water bodies. In the temporary lakes of the Upper Paraná River floodplain, the effect of  
305 flood pulses may nullify such processes and may lead to homogenization (Thomaz et al.  
306 2007), also with respect to the composition and abundance of ostracods between the edge  
307 and centre of these lakes. This is so because during high water periods, the main river

308 waters will invade the lakes, and homogenize and disperse the propagule bank of the  
309 dormant communities (Gurnell et al. 2008). The floodwaters will mix the sediments of  
310 these temporary lakes and distribute the ostracod resting eggs over the entire lake, thus  
311 resulting in a similar composition of the passive ostracod community in central and  
312 peripheral parts of the water body. Another factor that may contribute to the similarity of  
313 the species composition is the morphology of the temporary floodplain lakes. They are  
314 invariably elongated and narrow, and this possibly facilitates the homogenization  
315 between the regions of these temporary lakes, as edge and centre are only a few meters  
316 apart.

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

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

340 Most studies on production and hatching of resting eggs of ostracods are from the  
341 temperate regions of the Palaeartic (Martens et al. 1992; Horne and Martens 1998; Valls

342 et al. 2016) and few studies focus on (sub-) tropical regions. Ostracods are known to lay  
343 mixed batches of subitaneous and resting eggs, and it is possible that the ratio of these two  
344 types of eggs can be influenced by environmental factors (Dumont et al. 2002; Schön et  
345 al. 2012). This is unlike, for example, Cladocera, where resting eggs (ephippia) are only  
346 produced by the final sexual population at the end of the reproductive period (mostly  
347 summer).

348

349

### 350 *Effect of the abiotic variables on hatching of ostracod resting eggs*

351 Abiotic characteristics of the sediment are important for the active community of benthic  
352 invertebrates, mainly by providing habitats and substrate for organisms (Hauer et al.  
353 2018), and thus they might also have an influence on the dormant egg banks. Here, the  
354 positive relationship between the numbers of the hatched ostracod resting eggs and the  
355 size of particles (very fine sand) might be related to the fact that this type of sediment has  
356 a greater capacity for suspension (Constable 1999). This is owing to the movement of  
357 water or bioturbation activities, which provide oxygenation of the substrate and a higher  
358 concentration of water in the sediment (Constable 1999). It can thus provide better  
359 conditions for the hatching and the dispersal of the resting eggs. These results agree with  
360 those of Masero and Villate (2004), who found a positive correlation between the density  
361 of calanoid eggs on the one hand and smaller sediment particles on the other hand, thus  
362 showing that sediment characteristics can affect the egg banks. In addition, Tilbert et al.  
363 (2019) also found a positive association between the active ostracods and fine and very  
364 fine sand in a small tropical estuary in Brazil.

365 The negative effect of organic matter content on the hatching of ostracod resting eggs  
366 may be linked to increased decomposition and hypoxia in the sediment and in the water  
367 column, which can negatively affect the hatching (Rossi et al. 2004; Watkins et al. 2011).  
368 In addition, the organic matter in the sediment of the temporary lakes was mainly  
369 composed of allochthonous (non-aquatic) material, provided by riparian vegetation,  
370 mostly leaves of trees, since they have a dense vegetation cover (Kita and De Souza  
371 2003). The layers of leaves accumulated in the sediment can also cause burial and  
372 smothering of the egg banks, reducing the hatchability and viability of resting eggs  
373 (Gleason et al. 2003).

374 The germination of macrophytes in all microcosms might also contribute to the ecological  
375 succession, because these aquatic plants provide substrate and food for ostracods

376 (juveniles) after hatching of the resting eggs. Several studies have shown the important  
377 effect of macrophytes on the structure of the active ostracod communities (Higuti et al.  
378 2010; Matsuda et al. 2015).

379 A possible limitation of the present study was that the experimental condition imposed  
380 for artificial incubation may not have provided the required environmental cues to the  
381 hatching of all ostracod species present in the egg banks. Although some abiotic variables  
382 are controlled in the laboratory, this still does not exactly reflect the characteristics of the  
383 natural environment. However, artificial incubations have been used as an effective  
384 method to study the egg bank of different communities, such as rotifers (Fernandes et al.,  
385 2012), cladocerans (Stenert et al., 2017) and branchiopods (Pinceel et al., 2019).

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

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

#### 400 **Conflicts of interest**

401 The authors declare that they have no conflicts of interest.

402

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418

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601  
602

603 **Captions of tables and figures**

604

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

609

610 **Table 2.** Generalized Additive Models (GAMs). Model-averaged standardized  
611 coefficients, 95% confidence intervals (CI), Akaike's Information Criterion (AIC) and  
612 ANOVA p-values of predictors from ostracod resting eggs hatched. Significant level ( $p$   
613  $< 0.05$ ) in bold.

614

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

617

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

620

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

623

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

628

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

631




















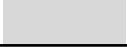
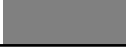
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635 Table 1

636

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

637

638

639 Table 2

640

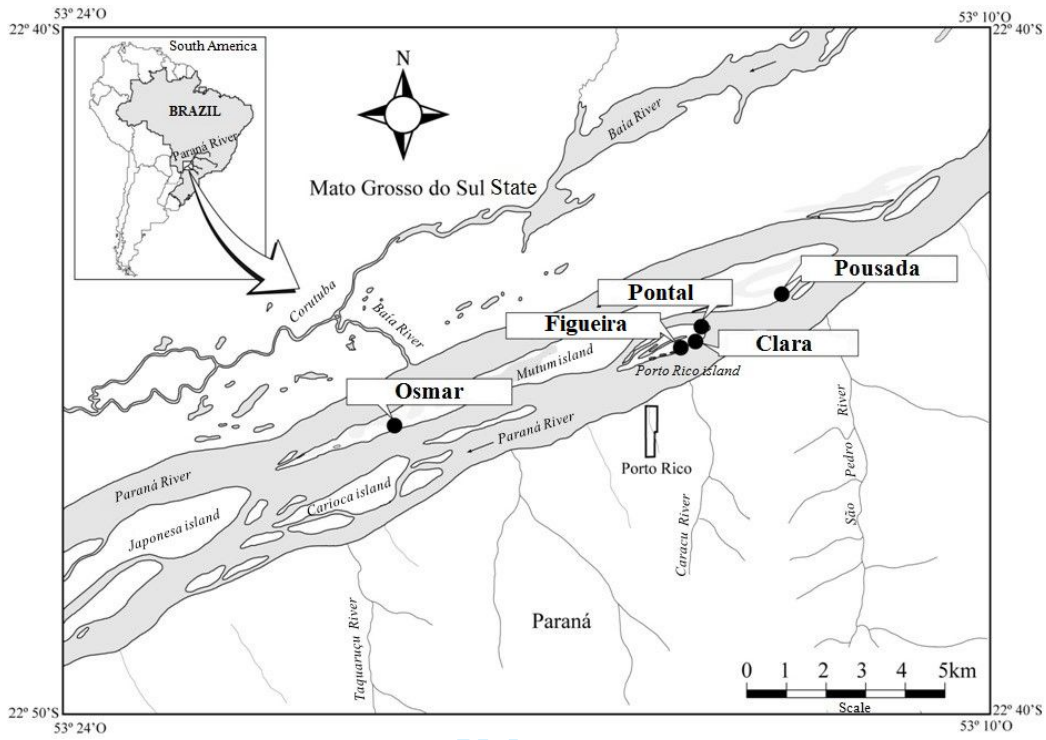
	Standardised Coefficient	95% CI		AIC	ANOVA
		2.5%	97.5%		
Gravel	0.487	-0.867	0.524	101.967	0.285
Very coarse sand	0.611	-0.251	0.141	102.116	0.322
Coarse sand	0.716	-0.264	0.041	100.877	0.123
Medium sand	0.559	-0.389	-0.164	90.734	<b>0.000</b>
Fine sand	1.462	-0.181	0.502	102.441	0.426
Very fine sand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

641

642

For Review Only

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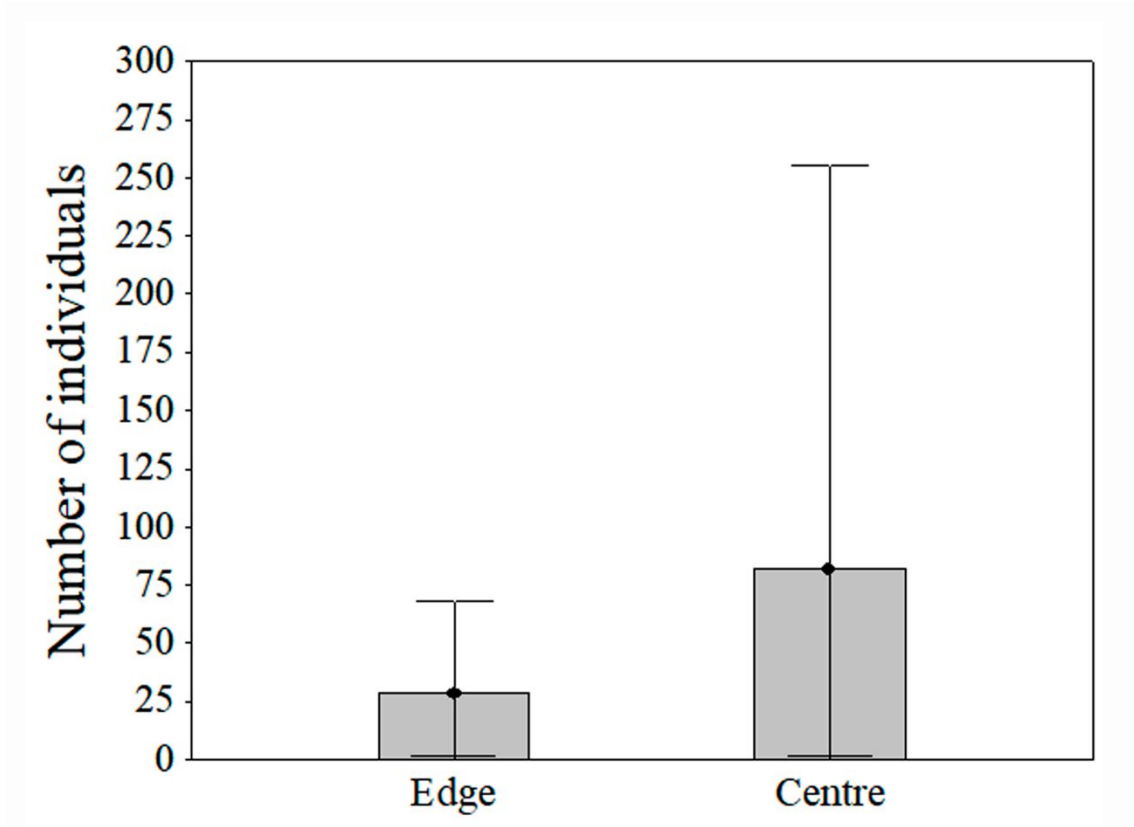
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645 Figure 1

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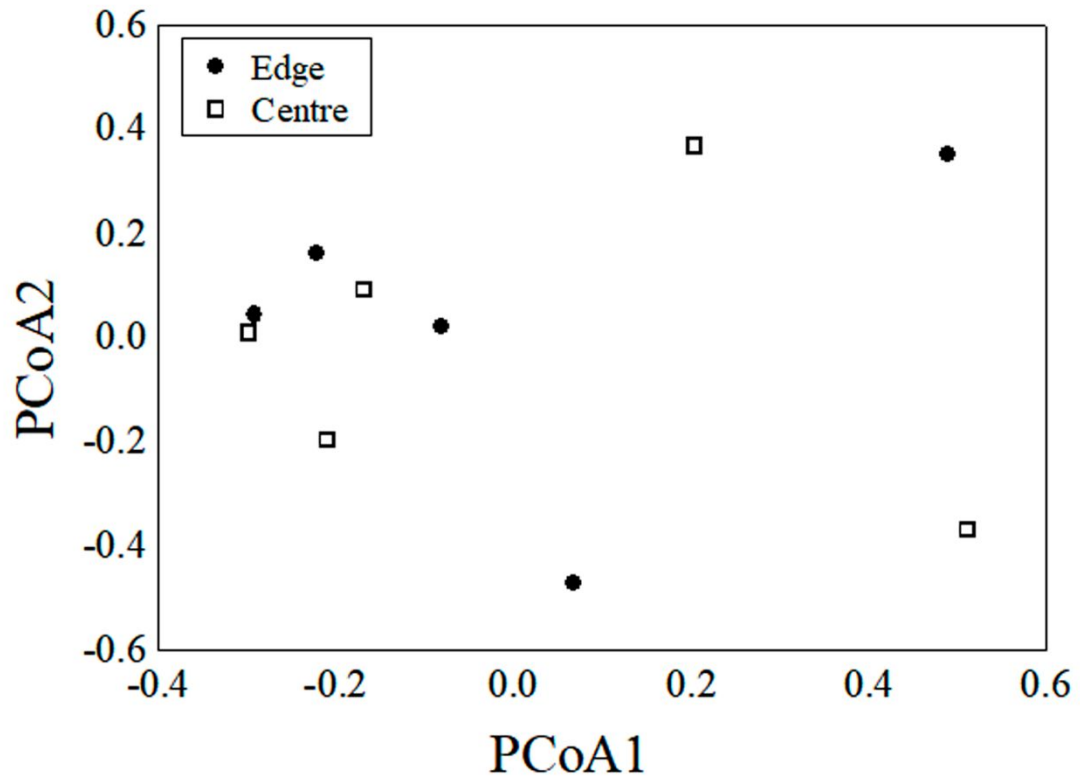
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649 Figure

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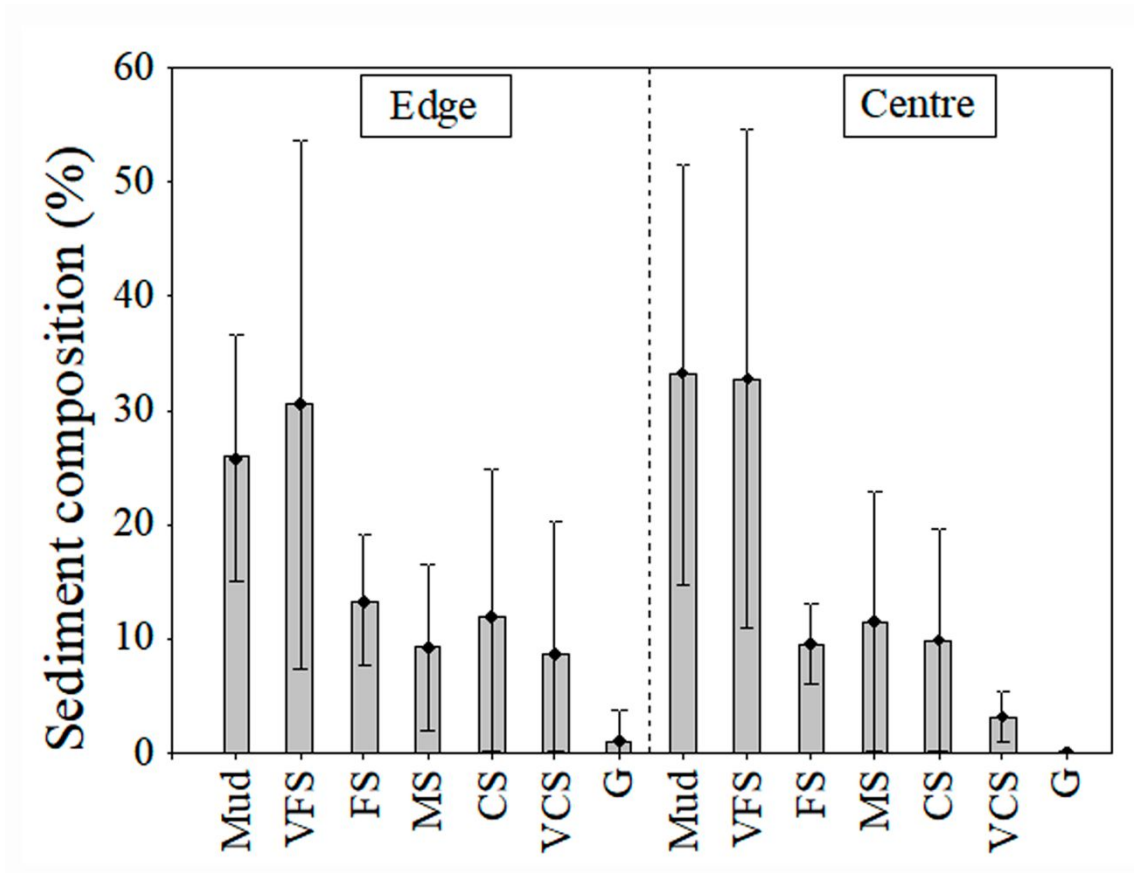


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652 Figure 3

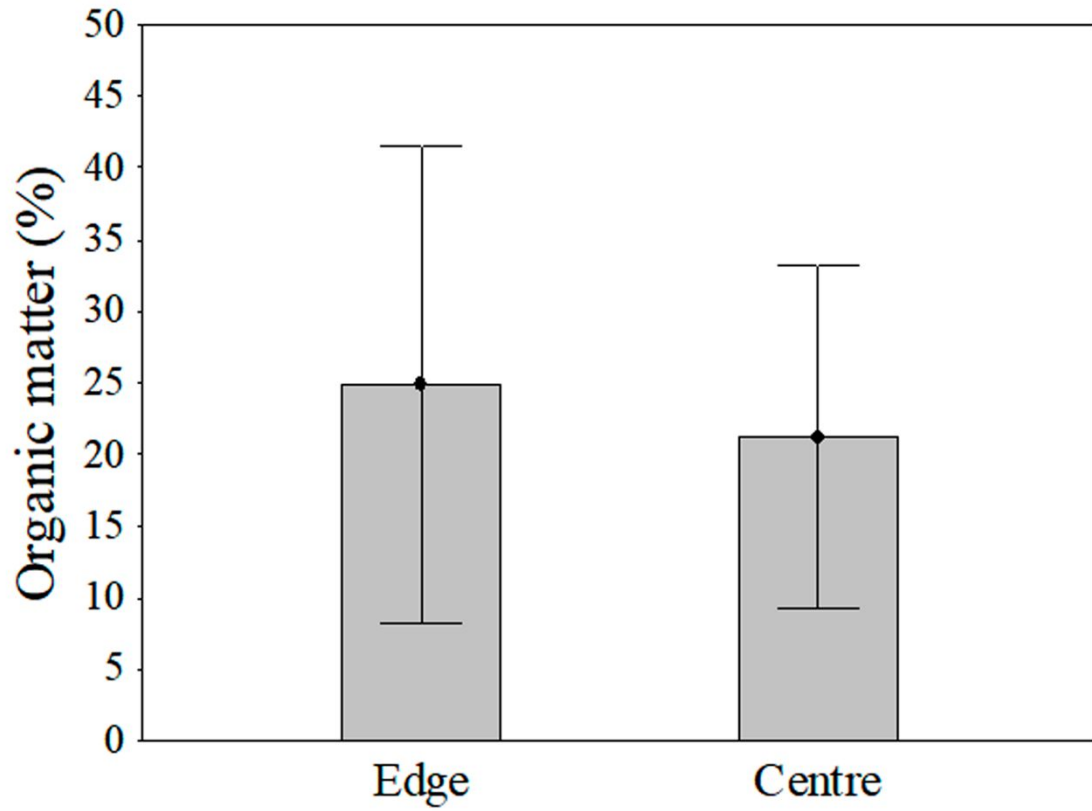
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656 Figure 4



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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.

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

3

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20

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

41

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

43

44



## 46 **Introduction**

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

52 Differential accumulation of resting eggs in the sediment can lead to differences in the  
53 composition and abundance of the passive communities in distinct compartments of the  
54 water bodies (Brendonck and De Meester 2003; Vandekerckhove et al. 2005; Gerhard et  
55 al. 2017). The spatial patterns of the active microfaunal communities are well-studied.  
56 However, the passive communities generally remain ill - known (Gehard et al. 2017;  
57 Portinho et al., 2017). Nevertheless, some studies on the active communities suggested  
58 that the spatial variation in the environment is regulated by the production of resting eggs  
59 and the dispersal of these propagules (Hairston et al. 1996).

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

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

~~80 These passive communities of resting eggs are also influenced by the oscillation and  
81 duration of high and low water levels, which are crucial factors for the survival of the  
82 active community, and subsequent production of these resting eggs (Stenert et al. 2017).  
83 Furthermore, other environmental variables of water (pH, oxygen, temperature, salinity,  
84 light intensity and duration) can trigger the development of resting eggs, because such  
85 eggs have a pronounced capacity to detect changes in aquatic ecosystems over short  
86 periods of time (McLay 1978; Brendonck 1996).~~

~~87 Under favourable environmental conditions, some resting eggs will hatch (Brendonck  
88 1996). However, other eggs may remain in dormancy (Martens 1994; Broek et al. 2003),  
89 requiring several wet/dry cycles before they can hatch. This strategy, in which some eggs  
90 hatch quickly, while others stay dormant for long periods, is known as bet-hedging. It  
91 serves as protection against future catastrophic events in habitat, for example, short  
92 periods of inundation that are too brief to support the complete life cycle of the organisms,  
93 i.e. from hatching of eggs to production of new eggs by adults (Evans and Dennehy 2005,  
94 Pinto et al., 2007).~~

~~95 In addition to providing a mechanism for the long-term maintenance of populations in  
96 temporary aquatic habitats, resting eggs are also dispersed by several vectors, such as  
97 wind, rain (Moreno et al. 2016), flowing water (Havel et al. 2000), water birds (Valls et  
98 al. 2017), floating macrophytes (Battauz et al. 2017), and mammals (Vanschoenwinkel et  
99 al. 2008), including human activities (Valls et al. 2016).~~

100 Some groups of freshwater ostracods can produce resting eggs. Most species of the family  
101 Cyprididae, which comprises about half the total number of extant non-marine ostracod  
102 species (Meisch et al., 2019), are known to produce resting eggs (Horne and Martens  
103 1998). Several experiments have been carried out on the viability and/or the hatching  
104 phenology of ostracod eggs of *Eucypris virens* (Jurine, 1820) and *Heterocypris*  
105 *incongruens* (Ramdohr, 1808) in temperate regions in the Palaearctic (e.g. Rossi et al.  
106 2012; Vandekerckhove et al. 2013). ~~In general,~~ there are no studies focusing specifically  
107 on resting eggs of ostracods in the Neotropical region. However, some studies on the  
108 resting eggs of zooplankton and other invertebrates have occasionally also recorded  
109 ostracods hatching from egg banks (Stenert et al. 2010; Ávila et al., 2015; Santangelo et  
110 al. 2015; Vargas et al. 2019).

~~111 Ostracods and their resting eggs can be used as a model group to understand ecological  
112 aspects and process in aquatic ecosystems. The spatial patterns of the active microfaunal  
113 communities are well-studied. However, the passive communities generally remain ill-~~

114 ~~known (Gehard et al. 2017; Portinho et al., 2017). Nevertheless, some studies on the~~  
115 ~~active communities suggest that the spatial variation in the environment is regulated by~~  
116 ~~the production of resting eggs and the dispersal of these structures (Hairston et al. 1995).~~  
117 Thus, ~~s~~Studies on ostracod resting eggs can provide vital information on the biology of  
118 the group. The ecological information about the recruitment of organisms from egg banks  
119 can fuel scientifically underpinned recommendations on the conservation of aquatic  
120 environments, such as temporary lakes in floodplains. These ecosystems are threatened  
121 by anthropic impacts, ~~(such as e.g. the construction of artificial dams)~~ that affects the  
122 hydrological dynamics of the ecosystem (droughts and flood events) (Agostinho et al.  
123 2004a), and thus require protective measures.

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

129

## 130 **Material and methods**

### 131 *Study area*

132 The Paraná River is formed by the junction of the Grande and Parnaíba rivers, in South-  
133 central Brazil, and is the second largest river in South America (4,695 km long)  
134 (Agostinho et al. 2008b). The Upper Paraná River has a large catchment (approx. 802,150  
135 km<sup>2</sup>) in Brazil, which encompasses large parts of the states of Paraná, São Paulo, Mato  
136 Grosso do Sul, Minas Gerais and Goiás (Souza-Filho and Steuvax 2004). The upper part  
137 of this river comprises a floodplain, which includes a series of small islands and a variety  
138 of environments, such as channels, rivers, connected and isolated lakes, as well as  
139 temporary lakes. The Upper Paraná River floodplain is located in the “Area de Proteção  
140 Ambiental das Ilhas de Várzea do Rio Paraná” (Environmental Protection Area)  
141 (Agostinho et al. 2004b). The climate of the region is tropical-subtropical with distinct  
142 rainy (November to March) and dry (April to October-) seasons (Cfa) (Eletrosul, 1986;  
143 Agostinho et al. 2004b).

144 Here, we study the passive ostracod communities of five temporary and isolated lakes  
145 located on the Porto Rico island (Pontal, 22°45 '05.7" S/ 053°15'23.6" W; Clara,  
146 22°45'20.7" S/ 053°15'27.7" W; Figueira, 22°45'22.7" S/ 053°15'34.0" W) and Mutum  
147 island (Pousada, 22°44'43.4" S/ 053°14'06.9 W; Osmar, 22°46'28.6" S/ 053°19'58.8" W)

148 in the Upper Paraná River floodplain (Fig. 1). These lakes are shallow (not exceeding 2.2  
149 meters deep) and small (areas of 0.15 hectares or less). The limnological variables  
150 (e.g. dissolved oxygen, electrical conductivity, pH) and vegetation cover formed by  
151 herbaceous and arboreal species, with higher presence of emergent macrophytes of the  
152 family Poaceae (Kita and De Souza 2003) are similar amongst the five lakes. In the dry  
153 season of 2017, four lakes were dry (Pontal, Clara, Figueira and Pousada) and one lake  
154 (Osmar) had a low water level (less than 30 cm) during the sampling.

155

156 >>> Figure 1

157

158 *Sampling and hatching procedures*

159 Sediments were collected during the dry season (September 2017), at the edge and in the  
160 centre of the five temporary lakes. The edge region ~~corresponds to the site that is~~ was  
161 defined as the area in direct contact with the adjacent terrestrial ecosystem, mostly with  
162 shallower sites, while the centre region ~~corresponds to~~ was defined as the open area of  
163 the lake, associated with deeper sites. ~~Approximately six cm depth of moist sediment (c~~  
164 ~~250 g)~~ We sampled six cm of sediment depth (c 250 g) ~~were sampled from each region~~  
165 ~~of the lakes~~ using a core sampler (194.5 cm<sup>3</sup> volume) in each region of the lakes, because  
166 as the higher viability of resting eggs is usually found in the top three cm of the sediment  
167 (Garcia-Roger et al. 2006).

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

178 For the hatching procedure in the laboratory, the composite samples from each region  
179 (edge and centre) of the five lakes were separately homogenized and 300 g of sediment  
180 was individually oven-dried at 50°C (this sediment temperature can be easily reached in  
181 the floodplain lakes on hot and dry days) ~~(Nielsen et al. 2015)~~ and then placed in

182 individual plastic trays, which acted as artificial microcosms, totalling 10  
183 microcosms (replicates). Each dry sediment sample was hydrated with 500 ml of distilled  
184 water (Fig. S1A) and was maintained in the microcosm at 25° C (Rossi et al., 2004) for  
185 91 days (Fig. S1B) in a germination chamber (Model SOLAB, SL.225). Photoperiods  
186 were maintained at 12 hours light/12 hours dark (Rossi et al., 2012).  
187 The incubation period was monitored weekly. Every 7 days, the water from the  
188 microcosm was filtered using a plankton net (68 µm) (Fig. S1C). As lifecycles of non-  
189 marine ostracods take at least 3 weeks (Meisch, 2000), the time of 7 days was not  
190 sufficient for the sexual maturation of individuals for reproduction. After that, the water  
191 of the microcosm was replaced with fresh distilled water. The filtered material, retained  
192 in the net, was sorted with a stereoscope microscope. Hatched juveniles were grown  
193 separately in glass bottles with distilled water, fed with fresh spinach and reared to the  
194 adult stage (when the juveniles did not die) in separate chambers, for identification and  
195 counting. The ostracod species were identified following Higuti et al. (2010, 2013) and  
196 using the references in Martens and Behen (1994).

197

#### 198 *Abiotic variables*

199 Sediment from each region (edge and centre) was also used to determine the particle size  
200 and organic matter content. Sediment composition was determined according to the  
201 method of Suguio (1973), using the Wentworth scale (Wentworth 1922). The samples  
202 were sorted in a nested series of sieves (size range between 2 mm and < 0.63 mm) and  
203 weighed. The size of sediment particles was classified as gravel ( $\geq 2$  mm), very coarse  
204 sand (2-1 mm), coarse sand (1.0-0.5 mm), medium sand (0.50-0.25 mm), fine sand  
205 (0.250-0.125 mm), very fine sand (0.125-0.063 mm) and mud (<0.063 mm). Organic  
206 matter in the sediment was obtained from 10 g of dry sediment by incineration at 560 °C  
207 for four hours (Moretto et al. 2013). The difference between the initial and final weights  
208 of the sediment indicates the amount of organic matter that was present in the sediment.  
209 Dissolved oxygen (mg.L<sup>-1</sup>) (YSI oximeter 550A), electrical conductivity (µS.cm<sup>-1</sup>) and  
210 pH (using Conductivimeter-Digimed, Digimed, São Paulo, Brazil and pHmeter-Digimed,  
211 Digimed, São Paulo, Brazil) were measured weekly in the microcosms.

212

#### 213 *Data analysis*

214 A non-parametric Wilcoxon Test for paired samples was performed to test differences in  
215 ostracods abundance (number of ostracod specimens hatched from resting eggs) between

216 the regions of the lakes, since the assumptions of normality and homoscedasticity,  
217 required for parametric tests, were not fulfilled. For this, the total number of ostracods  
218 hatched in all weeks for each region and in each lake was used.

219 The frequency of ostracod species that hatched from resting eggs was calculated using  
220 the constancy index (Dajoz 1973) through the expression:  $C = n/N \times 100$ , where:  $C =$   
221 constancy;  $n =$  number of samples in which the species was recorded and  $N =$  total number  
222 of samples. The following categories were assigned: constant ( $C \geq 50\%$ ); accessory ( $50\%$   
223  $> C \geq 25\%$ ); accidental or rare ( $C < 25\%$ ) according to Dajoz (1973).

224 A principal coordinate analysis (PCoA) was performed to visualize (dis)similarity of  
225 ostracod species composition between the lakes' regions (edge and centre), using data  
226 from presence and absence of the ostracods hatched weekly (Legendre and Legendre  
227 1998). A Multivariate Permutational Variance Analysis (PERMANOVA) was performed  
228 to evaluate differences in ostracod species composition between edge and centre  
229 (Anderson 2005). The test was based on a dissimilarity matrix using the Jaccard distance.  
230 A total of 999 permutations were performed to assess significance.

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

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

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

249 Analyses of variance, PCoA and Models GAM analyses were carried out in R 3.4  
250 software (R Development Core Team 2013) using the vegan (Oksanen et al. 2018),  
251 permute (Simpson 2018) and mgcv (Wood 2018) packages. ~~Analyses of variance were~~  
252 ~~performed in Statistica software program (version 7.1, Statsoft Inc., 2005, Tulsa,~~  
253 ~~Oklahoma, USA).~~

254

255

## 256 **Results**

### 257 *Composition and abundance of ostracod resting egg banks*

258 Twelve species of ostracods hatched from the egg banks of the five temporary lakes.  
259 Cyprididae was the richest and most abundant family, represented by 11 species. The  
260 family Candonidae was represented only by the species *Physocypria schubarti* Farkas,  
261 1958. *Cypridopsis vidua* (O. F. Müller, 1776), *Cypricercus sp. nov. eentrurus* (Klie,  
262 1940) and *Bradleytriebella trispinosa* (Pinto & Purper, 1965) were only reared from  
263 sediment from the centre of the lakes (Table 1, ~~Fig. 2~~).

264

265 >>> Table 1

266

267 >>> ~~Figure 2~~

268

269 A total of 553 ostracod specimens hatched from the sediments of the five temporary lakes;  
270 144 and 409 ostracods were recorded from the edges and centres, respectively (~~Figure 2~~).  
271 There was no significant difference in the number of individuals between the two regions  
272 (Wilcoxon Test,  $p = 0.07$ ). *Chlamydotheca colombiensis* (Sars, 1901) was the most  
273 abundant species at the edge and *Strandesia mutica* (Sars, 1901) in the centre of the lakes.  
274 According to the constancy index, *S. mutica* and *C. colombiensis* were the most common  
275 species in both regions, while *Strandesia velhoi* Higuti & Martens, 2013 was common  
276 only in the centre of the lakes (Table 1). The results of the PERMANOVA did not show  
277 significant differences in the species composition of the egg banks between the centre and  
278 edge regions ( $F = \del{10.6253} = 0$ ,  $p = \del{0.987}$ ) (Fig. 3).

279

280 >>> ~~Figure 2~~

281

282 >>> Figure 3

283

284 *Effects of the abiotic variables on ostracod resting egg banks*

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

289

290 &gt;&gt;&gt; Figure 4

291

292 Coarse particulate organic matter (roots and leaves) was observed in all five temporary  
293 lakes. The organic matter content of the sediment was higher at the edge, when compared  
294 to the centre (T test,  $t = -21.92$ ,  $p = 0.00$ ) (Fig. 5).

295

296 &gt;&gt;&gt; Figure 5

297

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

301

302 &gt;&gt;&gt; Table 2

303

304 Environmental variables did not vary significantly between the microcosms of the edge  
305 and centre regions (Kruskal-Wallis, oxygen:  $H = 3.58$ ,  $p = 0.058$ ; pH:  $H = 0.07$ ,  $p = 0.79$ ;  
306 electrical conductivity:  $H = 0.01$ ,  $p = 0.91$ ). The mean values for these variables in the  
307 “edge” microcosms were 4.95 mg.L<sup>-1</sup> (dissolved oxygen), 6.74 (pH), 20.77 μS.cm<sup>-1</sup>  
308 (electrical conductivity), and in the “centre” microcosms were 4.45 mg.L<sup>-1</sup> (dissolved  
309 oxygen), 6.68 (pH), 18.27 μS.cm<sup>-1</sup> (electrical conductivity) (Table S1).

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

313

314 **Discussion**315 *Composition and abundance of ostracod resting egg banks*



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

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

337

338 The presence of resting eggs in the centre and at the edge of temporary water bodies may  
339 depend on several factors. For example, droughtied resting eggs can accumulate along  
340 with the floating debris along the edges (Martens et al. 1992). But drying pools will also  
341 concentrate fauna at their deepest (mostly central) point towards the end of the  
342 hydrological cycle, and then resting eggs can be produced at a higher rate in these  
343 remaining pools. Othere and ostracods may thus layproduce a greater amountnumber of  
344 eggs in the deepest part (centre) of the ponds and lakes (Bright and Bergey 2015).  
345 However, both of these processes are mostly relevant for rain-filled, isolated -temporary  
346 water bodies. In the temporary floodplain-lakes of the Upper Paraná River floodplain, the  
347 effect of flood pulses may nullify such processes and may lead to homogenization  
348 (Thomaz et al. 2007), also with respect to the composition and abundance of ostracods  
349 between the edge and centre of these lakes. This is so because during high water periods,

350 the main river waters will invade the lakes, and homogenize and disperse the propagule  
351 bank of the dormant communities (Gurnell et al. 2008). The floodwaters will mix the  
352 sediments of these temporary lakes and distribute the ostracod resting eggs over the entire  
353 lake, thus resulting in a similar composition of the passive ostracod community in central  
354 and peripheral parts of the water body. Another factor that may contribute to the similarity  
355 of the species composition is the morphology of the temporary floodplain lakes. They are  
356 invariably elongated and narrow, and this possibly facilitates the homogenization  
357 between the regions of these temporary lakes, as i.e. edge and centre are only a few meters  
358 apart.

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

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

382 Most studies on production and hatching of resting eggs of ostracods are from the  
383 temperate regions of the Palearctic (Martens et al. 1992; Horne and Martens 1998; Valls

384 et al. 2016) and few studies focus on (sub-) tropical regions. Ostracods are known to lay  
385 mixed batches of subitaneous and resting eggs, and it is possible that the ratio of these  
386 two types of eggs can be influenced by environmental factors (Dumont et al. 2002; Schön  
387 et al. 2012). This is unlike, for example, Cladocera, where resting eggs ([ephippia](#)) are  
388 only produced by the final sexual population at the end of the reproductive period (mostly  
389 summer).

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

397 ~~The presence of resting eggs in the centre and at the edge of temporary water bodies may~~  
398 ~~depend on several factors. For example, dried resting eggs can accumulate along with the~~  
399 ~~floating debris along the edges (Martens et al. 1992). But drying pools will also~~  
400 ~~concentrate fauna at their deepest (mostly central) point towards the end of the~~  
401 ~~hydrological cycle, and then resting eggs can be produced at a higher rate there and~~  
402 ~~ostracods may lay a greater amount of eggs in the deepest part (centre) of the ponds and~~  
403 ~~lakes (Bright and Bergey 2015).~~

404 ~~However, these processes are mostly relevant for rain-filled temporary water bodies. In~~  
405 ~~the temporary floodplain lakes of the Upper Paraná River, the effect of flood pulses may~~  
406 ~~nullify such processes and may lead to homogenisation (Thomaz et al. 2007), also with~~  
407 ~~respect to the composition and abundance of ostracods between the edge and centre of~~  
408 ~~these lakes. This is so because during high water periods, the main river waters will invade~~  
409 ~~the lakes, and homogenize and disperse the propagule bank of the dormant communities~~  
410 ~~(Gurnell et al. 2008). The floodwaters will mix the sediments of these temporary lakes~~  
411 ~~and distribute the ostracod resting eggs over the entire lake, thus resulting in a similar~~  
412 ~~composition of the passive ostracod community in central and peripheral parts of the~~  
413 ~~water body. Another factor that may contribute to the similarity of the species~~  
414 ~~composition is the morphology of the temporary floodplain lakes. They are invariably~~  
415 ~~elongated and narrow, and this possibly facilitates the homogenization between the~~  
416 ~~regions of these temporary lakes, i.e. edge and centre are only a few meters apart.~~

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

425

#### 426 *Effect of the abiotic variables on hatching of ostracod resting eggs*

427 Abiotic characteristics of the sediment are important for the active community of benthic  
428 invertebrates, mainly by providing habitats and substrate for organisms (Hauer et al.  
429 2018), and thus they might also have an influence on the dormant egg banks. Here, the  
430 positive relationship between the numbers of the hatched ostracod resting eggs and the  
431 size of particles (very fine sand) might be related to the fact that this type of sediment has  
432 a greater capacity for suspension (Constable 1999). This is owing to the movement of  
433 water or bioturbation activities, which provide oxygenation of the substrate and a higher  
434 concentration of water in the sediment (Constable 1999). It can thus provide better  
435 conditions for the hatching and the dispersal of the resting eggs. These results agree with  
436 those of Masero and Villate (2004), who found a positive correlation between the density  
437 of calanoid eggs on the one hand and smaller sediment particles on the other hand, thus  
438 showing that sediment characteristics can affect the egg banks. In addition, Tilbert et al.  
439 (2019) also found a positive association between the active ostracods and fine and very  
440 fine sand in a small tropical estuary in Brazil.

441 The negative effect of organic matter content on the hatching of ostracod resting eggs  
442 may be linked to increased decomposition and hypoxia in the sediment and in the water  
443 column, which can negatively affect the hatching (Rossi et al. 2004; Watkins et al. 2011).  
444 In addition, the organic matter in the sediment of the temporary lakes was mainly  
445 composed of allochthonous (non-aquatic) material, provided by riparian vegetation,  
446 mostly leaves of trees, since they have a dense vegetation cover (Kita and De Souza  
447 2003). The layers of leaves accumulated in the sediment can also cause burial and  
448 smothering of the egg banks, reducing the hatchability and viability of resting eggs  
449 (Gleason et al. 2003).

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

455 A possible limitation of the present study was that the experimental condition imposed  
456 for artificial incubation may not have provided the required environmental cues to the  
457 hatching of all ostracod species present in the egg banks. Although some abiotic variables  
458 are controlled in the laboratory, this still does not exactly reflect the characteristics of the  
459 natural environment. However, artificial incubations have been used as an effective  
460 method to study the egg bank of different communities, such as rotifers (Fernandes et al.,  
461 2012), cladocerans (Stenert et al., 2017) and branchiopods (Pinceel et al., 2019).

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

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

484 Theoretically and based on the results we also infer that flood pulses can lead to  
485 homogenization of the ostracod egg banks. The study area has a cascade of reservoirs  
486 upstream, which reduce the frequency and duration of flooding periods in this  
487 floodplain. This Thus, we can infer that reduction of floods, would influence the  
488 structure and spatial variation of ostracod egg bank in the future.  
489 The homogenised distribution of ostracod resting eggs between lake regions may increase  
490 the dispersal of these structures by biotic vectors (e.g. birds), owing to the larger  
491 distribution area in the environment.

492

### 493 **Conflicts of interest**

494 The authors declare that they have no conflicts of interest.

495

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511

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735 [org/package=mgcv](http://CRAN.Rproject.org/package=mgcv) [accessed: 20 june 2018].
- 736
- 737

738 **Captions of tables and figures**

739

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

744

745 **Table 2.** Generalized Additive Models (GAMs). Model-averaged standardized  
746 coefficients, 95% confidence intervals (CI), Akaike's Information Criterion (AIC) and  
747 ANOVA p-values of predictors from ostracod resting eggs hatched. Significant level ( $p$   
748  $< 0.05$ ) in bold.

749

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

752

753 **Figure 2.** ~~Mean values and standard error of Composition and~~ abundance of ostracod  
754 ~~species~~ hatched from egg banks at the edge and in the centre of the temporary lakes. (~~P =~~  
755 ~~Pontal, C = Clara, F = Figueira, Ps = Pousada, O = Osmar).~~

756

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

760

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

765

766 **Figure 5.** ~~Mean values and standard error of O~~ organic matter of the sediment at the  
767 edge and in the centre of the temporary lakes. ~~P = Pontal, C = Clara, F = Figueira, Ps =~~  
768 ~~Pousada, O = Osmar.~~

769






















770

771

772

773 Table 1

774

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

775

776

777 Table 2

778

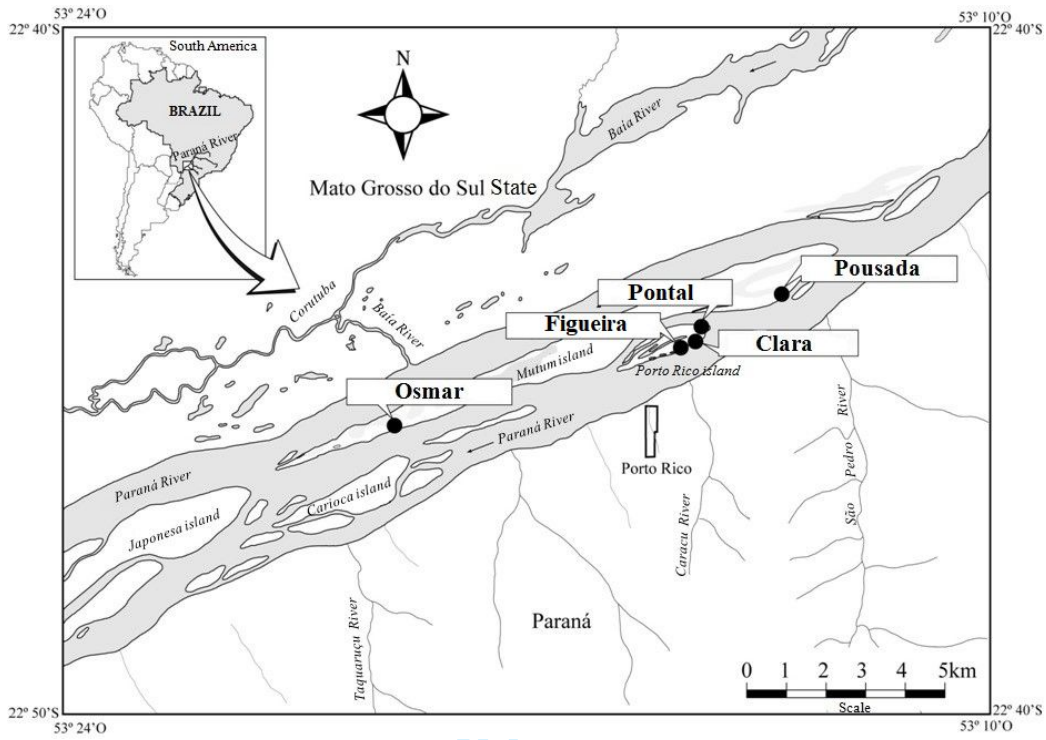
	Standardised Coefficient	95% CI		AIC	ANOVA
		2.5%	97.5%		
Gravel	0.487	-0.867	0.524	101.967	0.285
Very coarse sand	0.611	-0.251	0.141	102.116	0.322
Coarse sand	0.716	-0.264	0.041	100.877	0.123
Medium sand	0.559	-0.389	-0.164	90.734	<b>0.000</b>
Fine sand	1.462	-0.181	0.502	102.441	0.426
Very fine sand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

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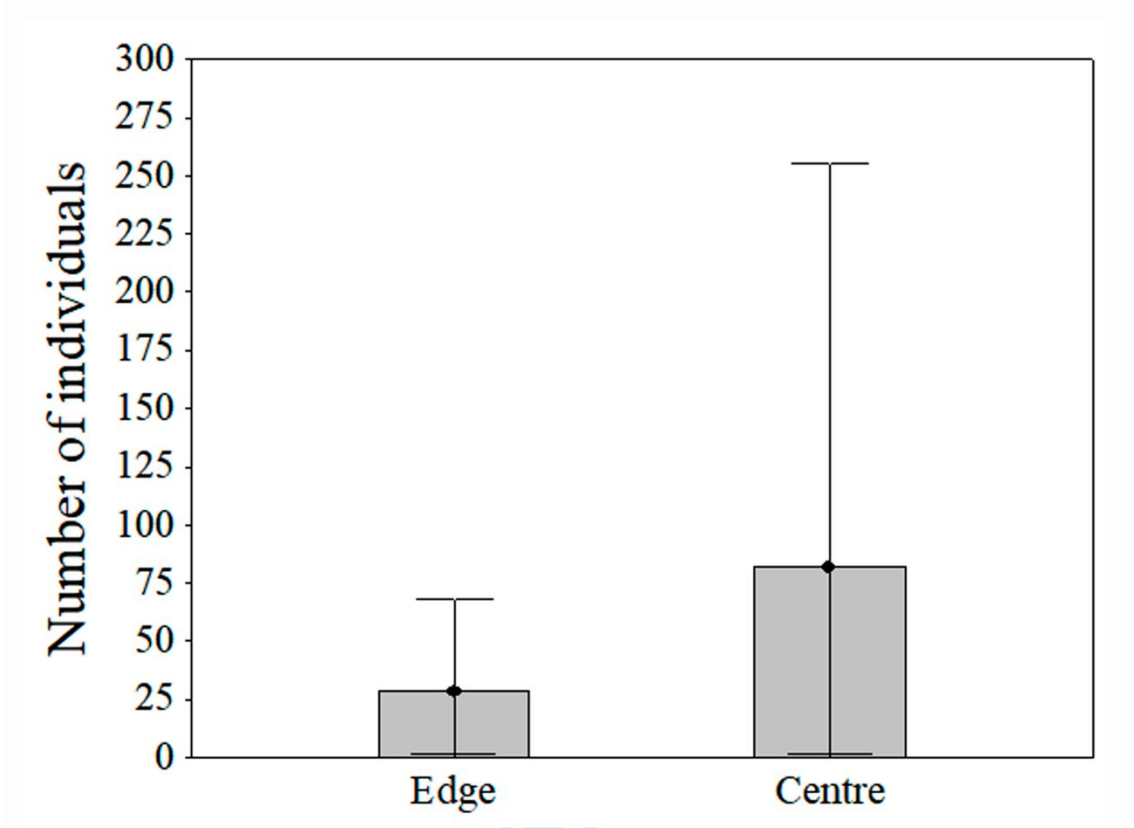


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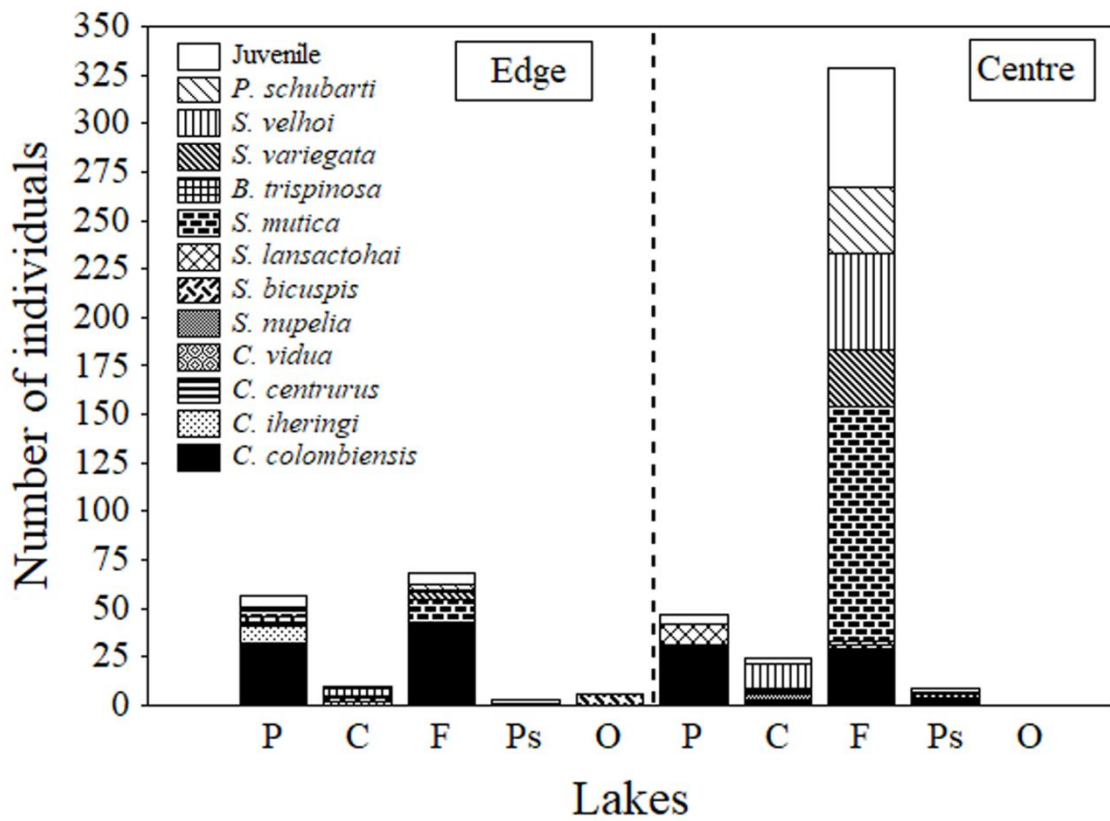
783 Figure 1

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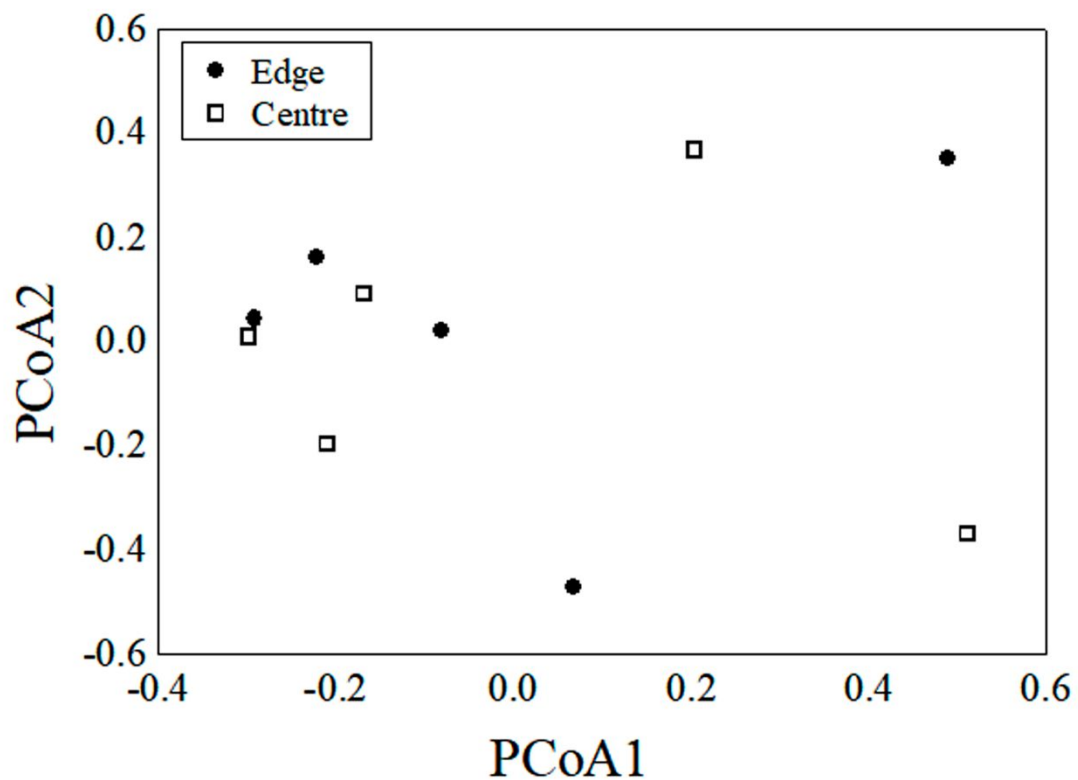


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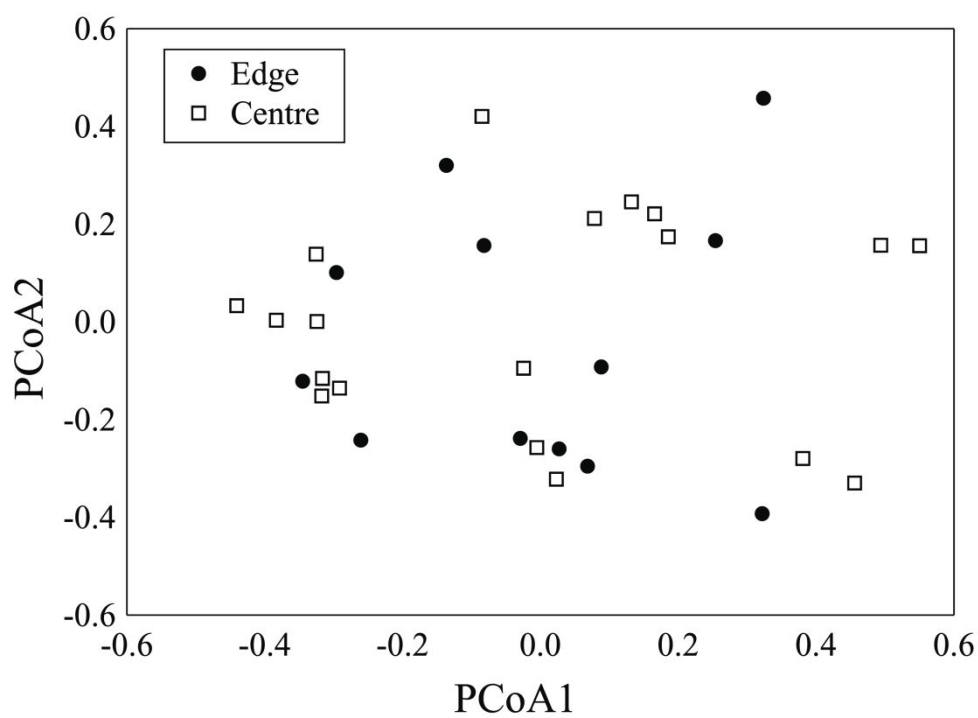
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Figure

2



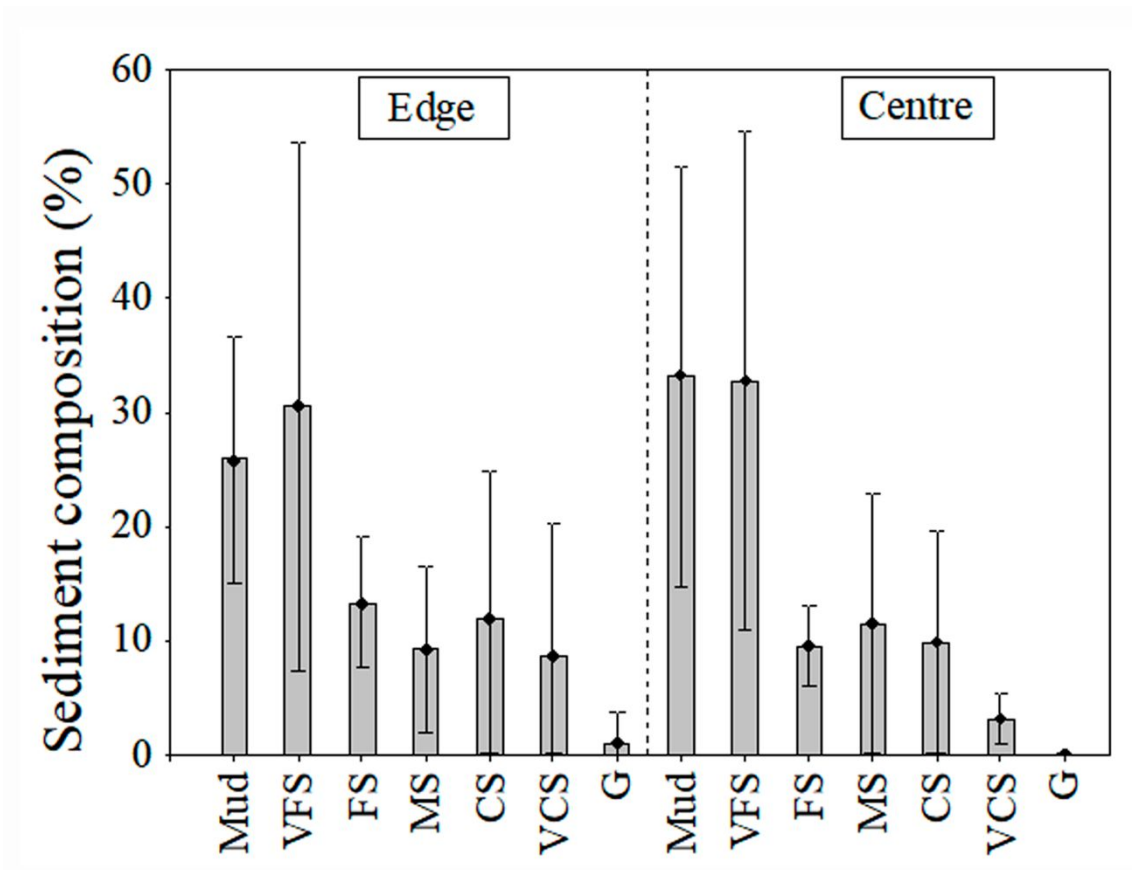
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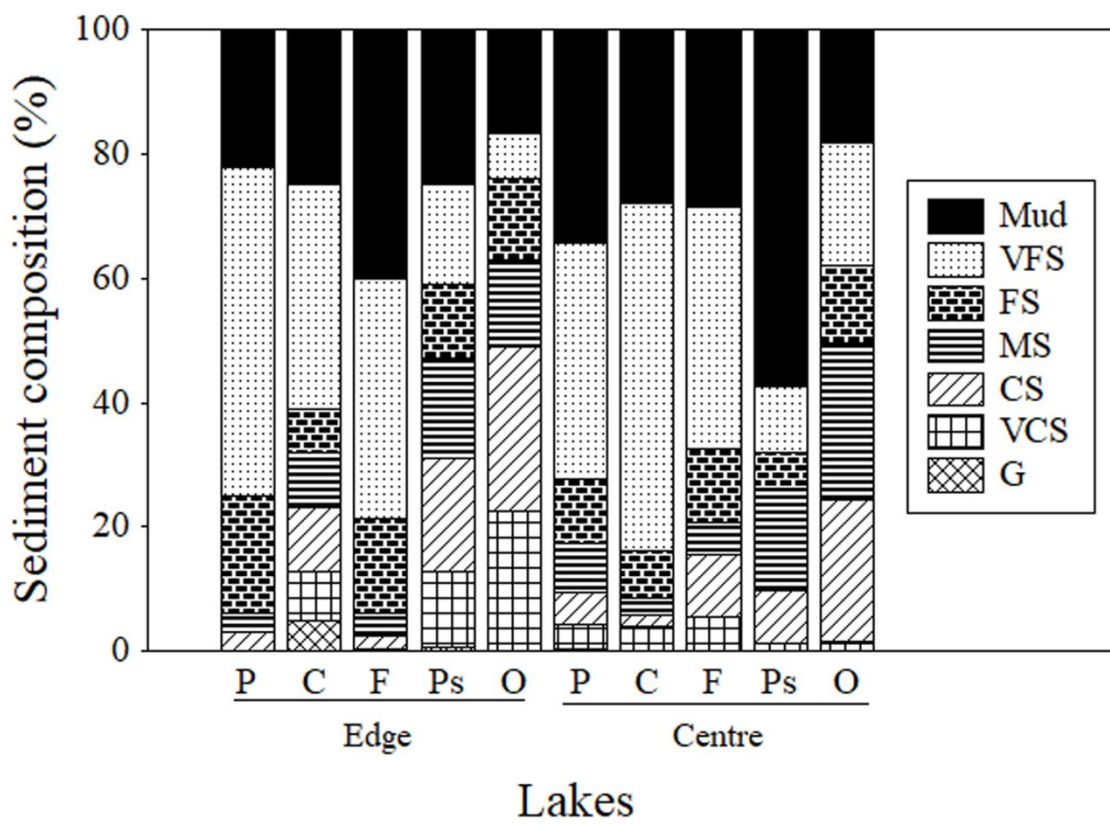
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791 Figure 3

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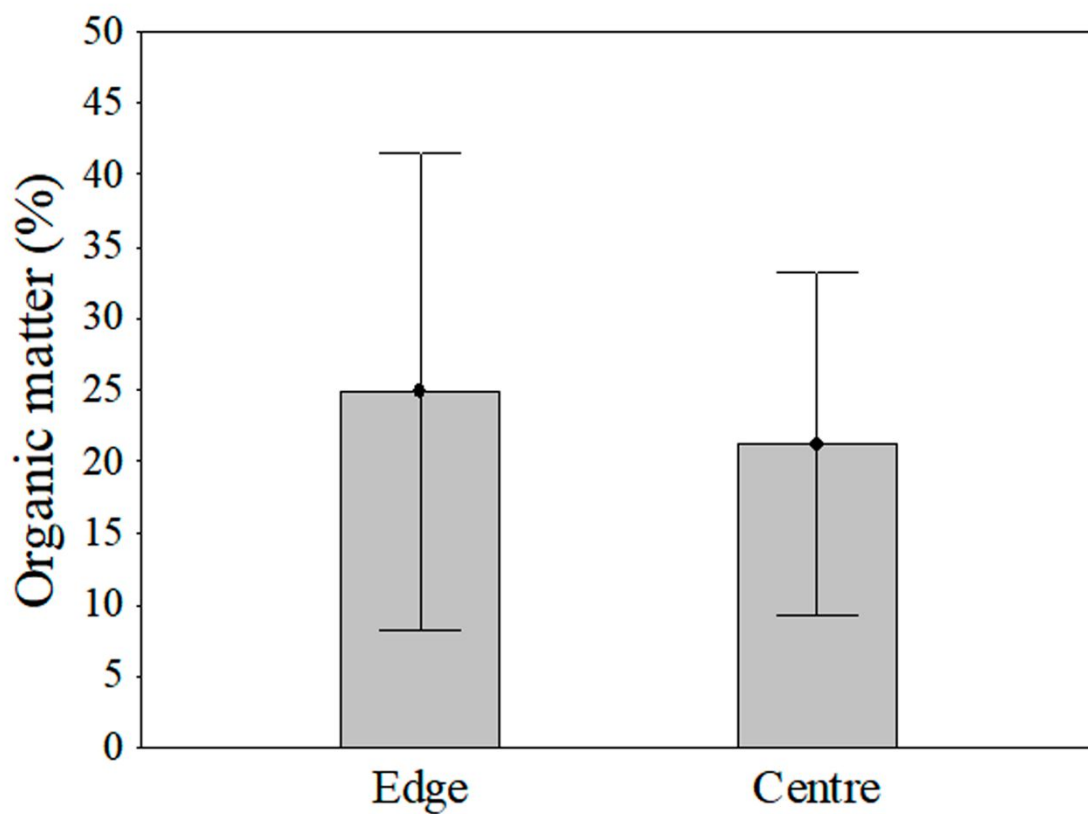


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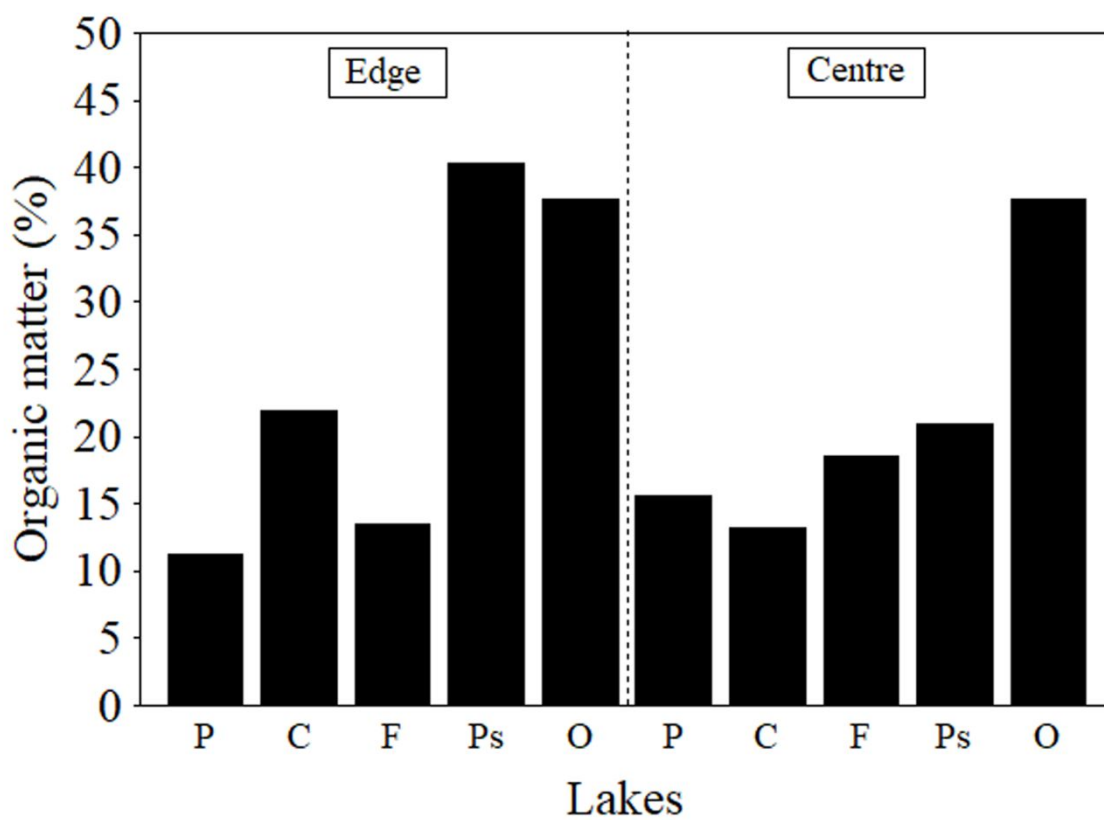


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795 Figure 4



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797

798 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 is 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 (Gerhard 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 et 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.



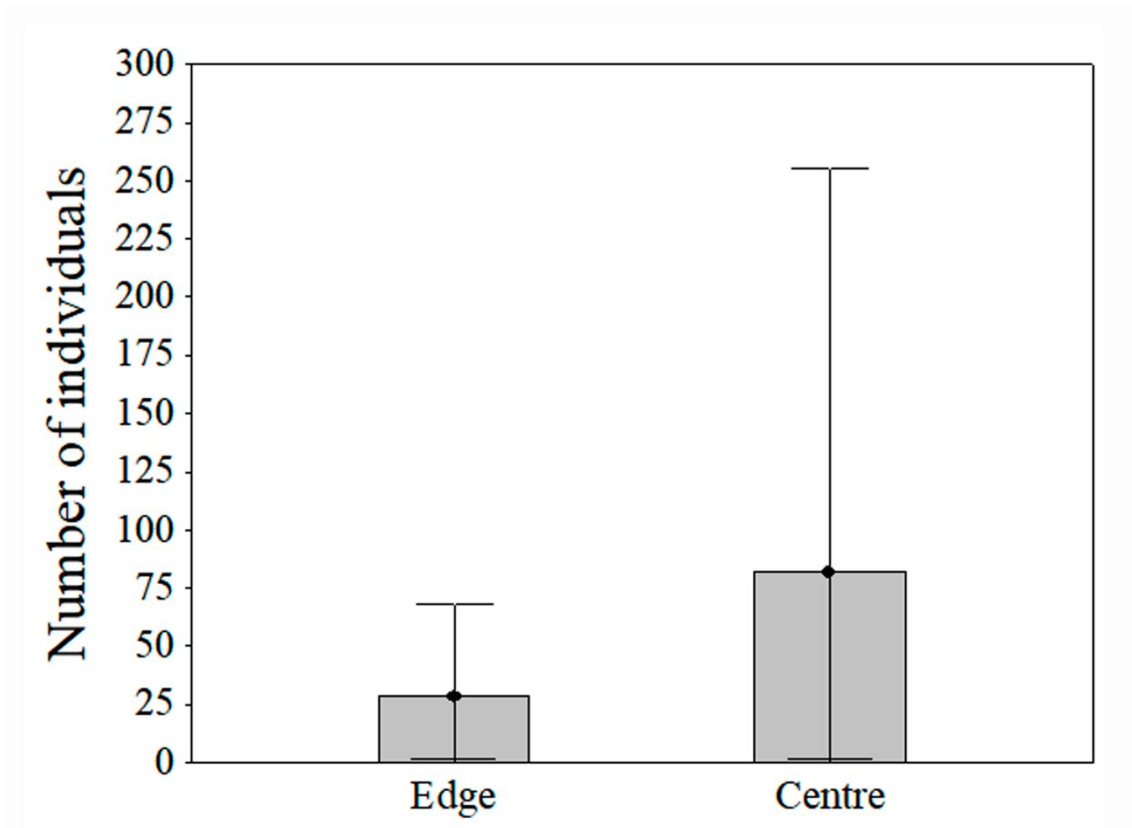


Figure 2

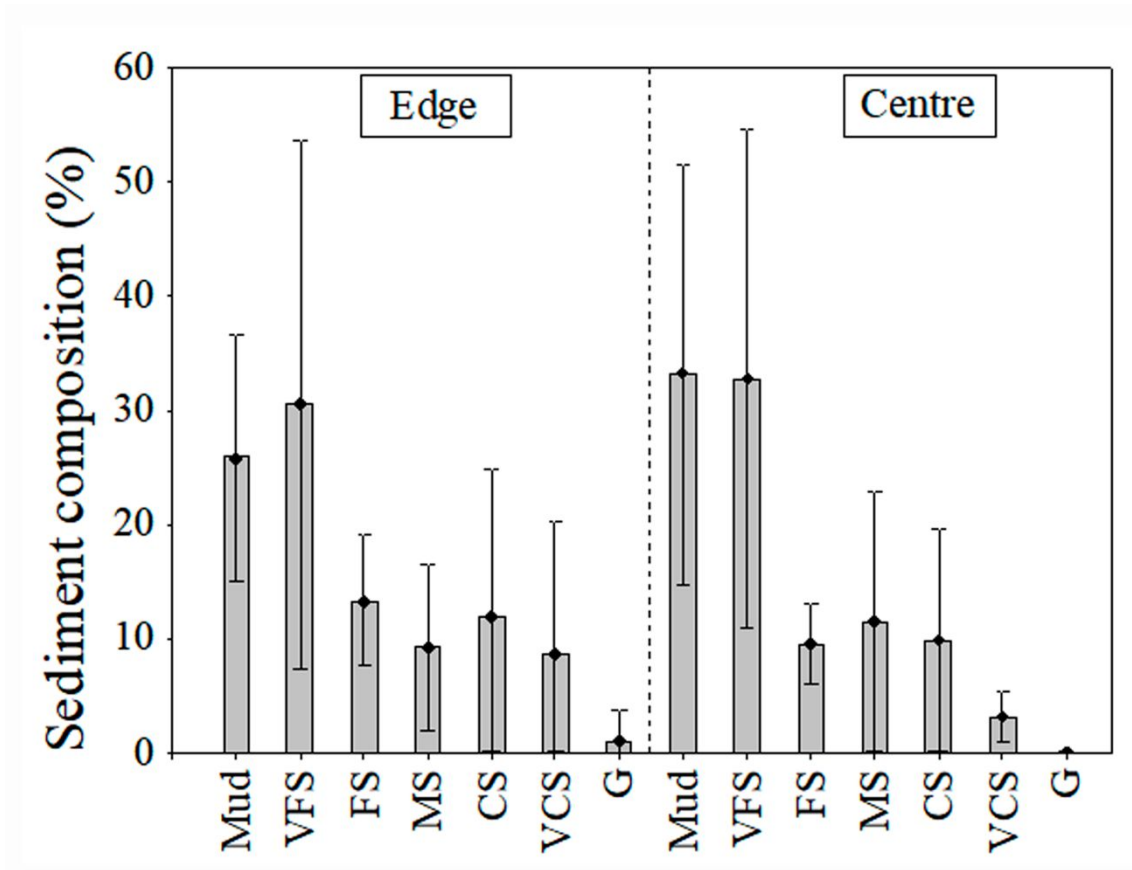


Figure 4

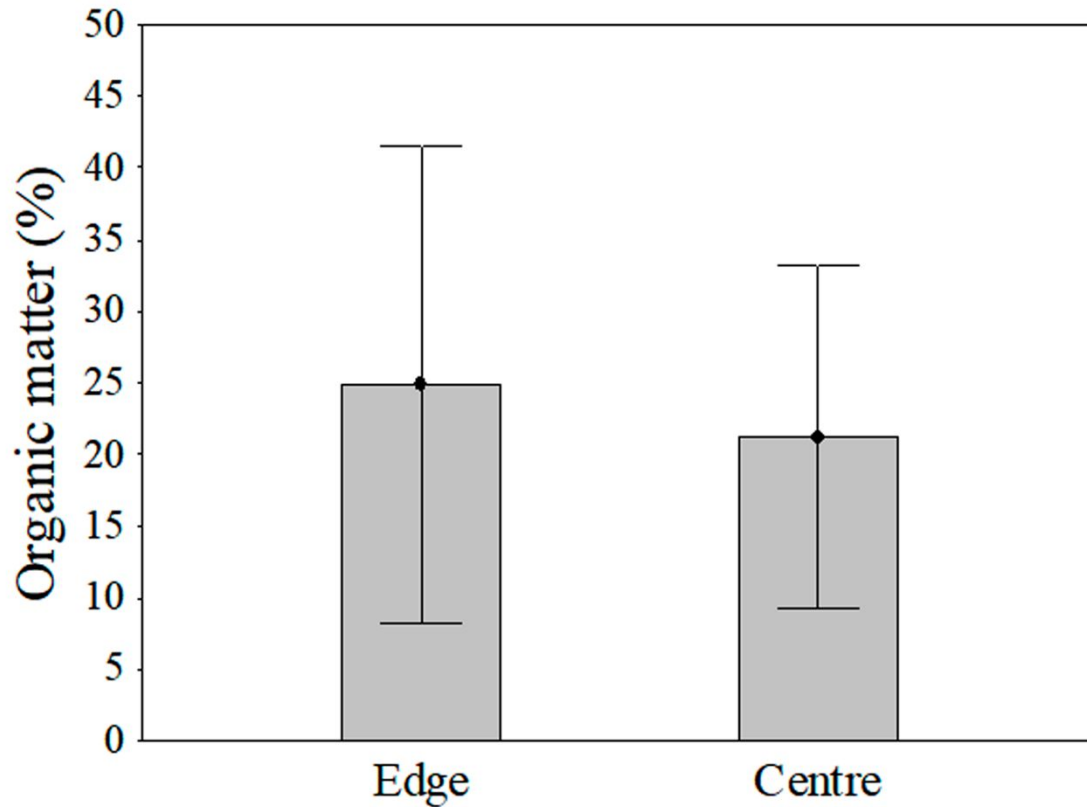


Figure 5

**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.”

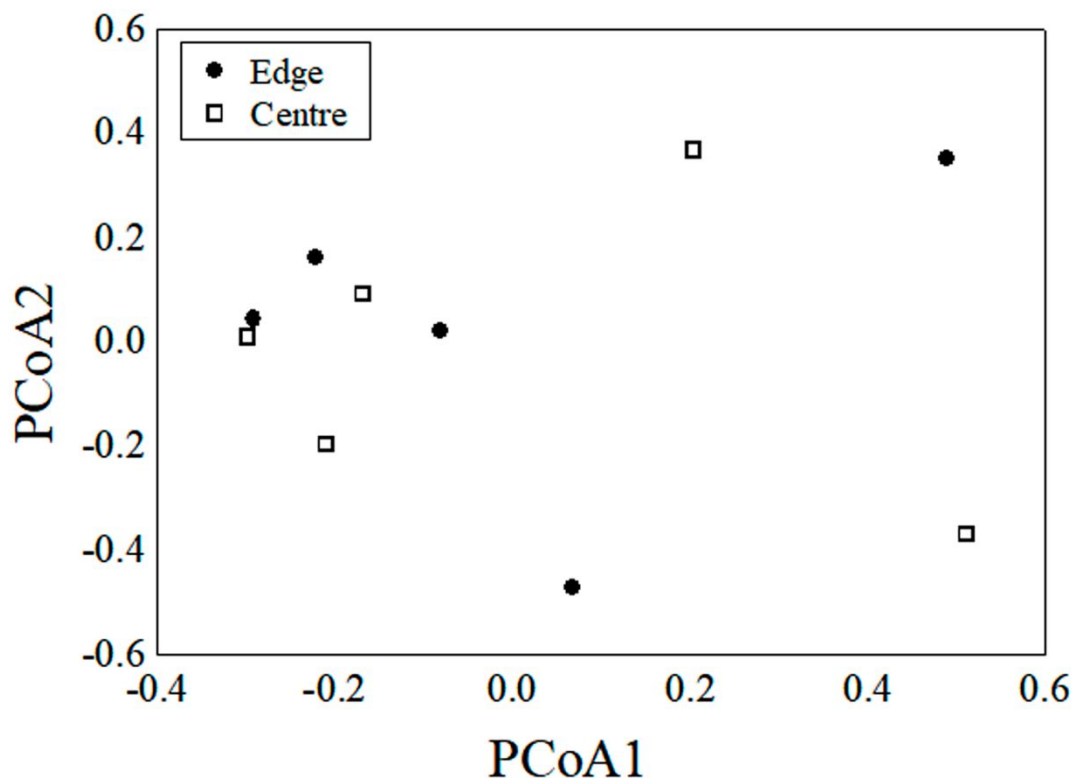


Figure 3

**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<sup>3</sup> 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.

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**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."

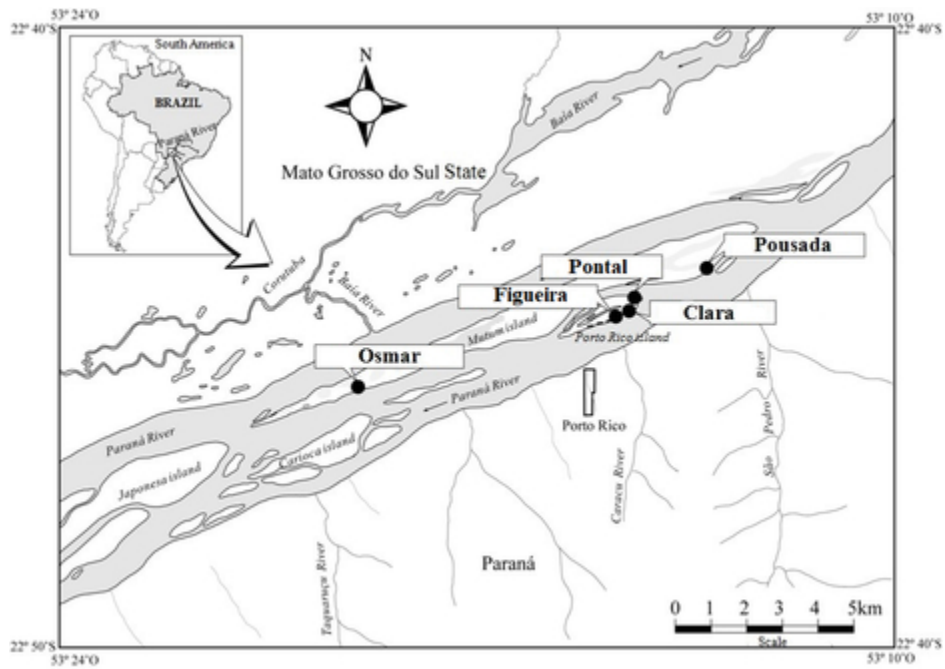


Figure 1. Location of the temporary lakes of the Upper Paraná River floodplain. Flow direction is from right to left.

39x28mm (300 x 300 DPI)

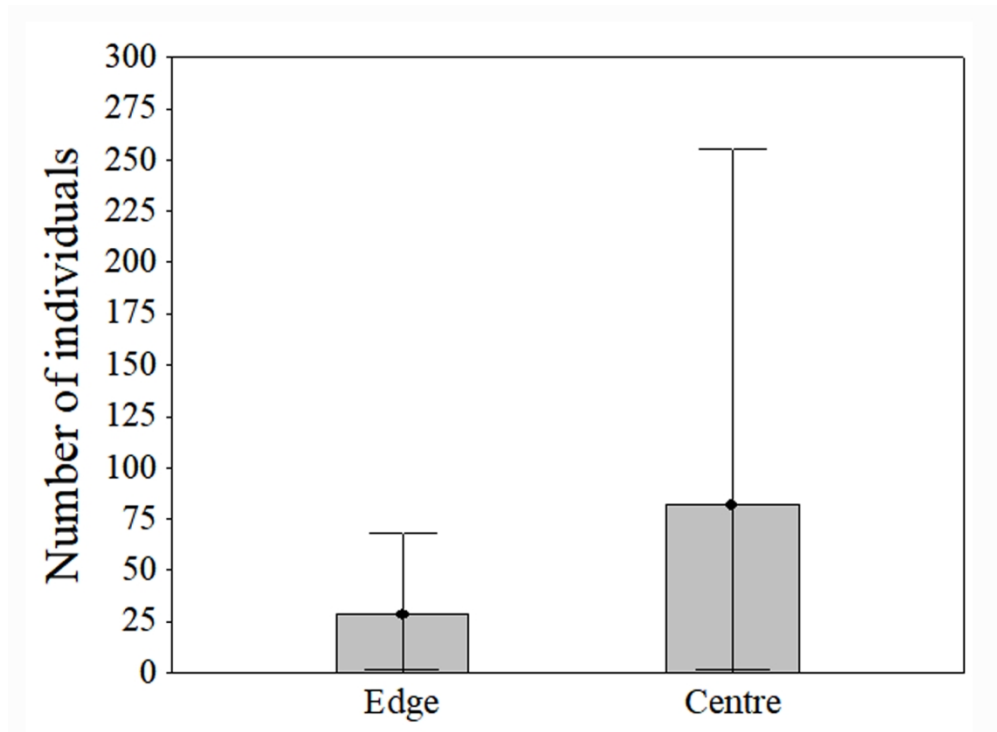


Figure 2. Mean values and standard error of abundance of ostracod hatched from egg banks at the edge and in the centre of the temporary lakes.

165x120mm (300 x 300 DPI)

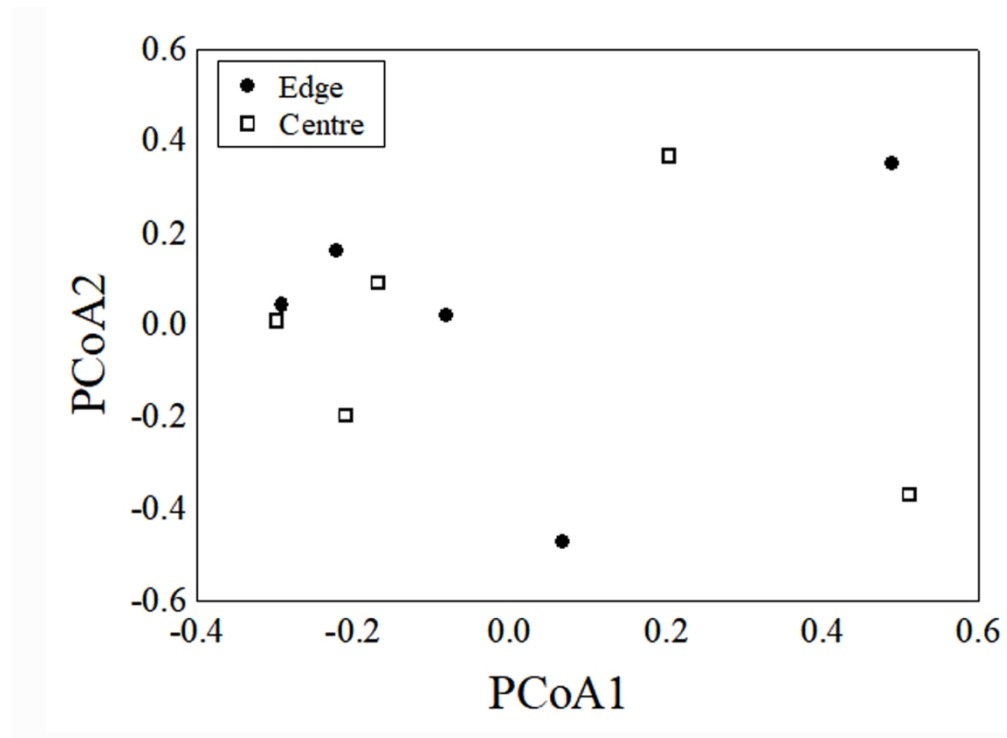


Figure 3. Ordination diagram of the principal coordinate analysis of the passive ostracod communities at the edge (dots) and in the centre (squares) of the five temporary lakes.

168x124mm (300 x 300 DPI)

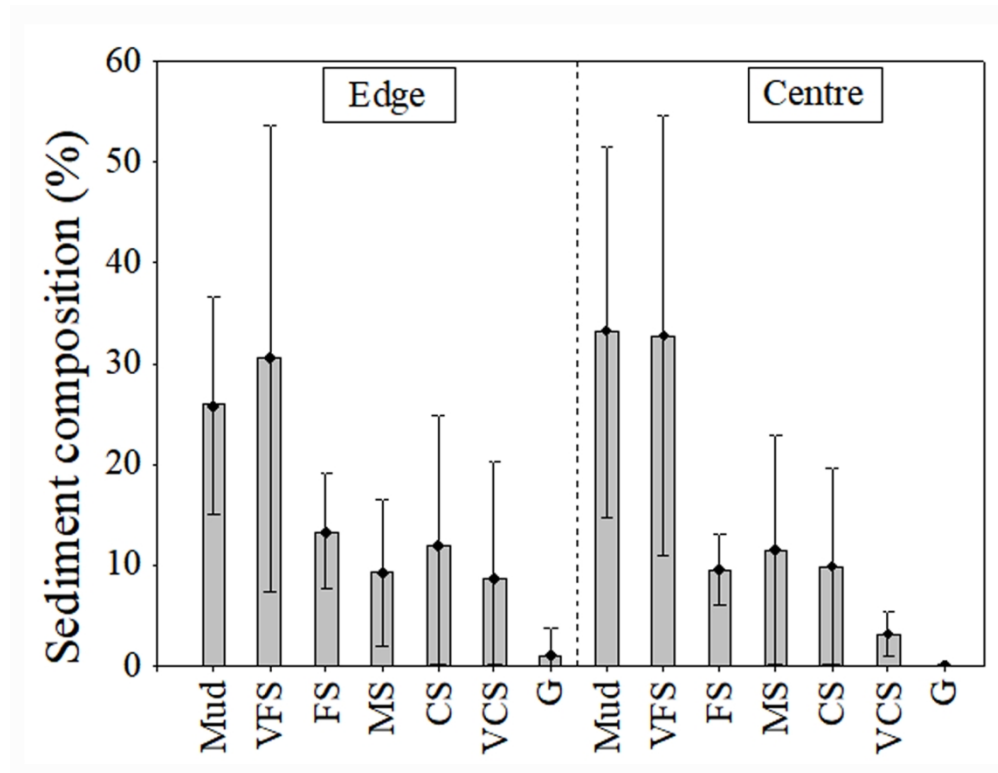


Figure 4. Mean values and standard error of sediment composition at the edge and in the centre of the temporary lakes. P = Pontal, C = Clara, F = Figueira, Ps = Pousada, O = Osmar, Mud = Silt and clay, VFS = Very fine Sand, FS = Fine Sand, MS = Medium Sand, CS = Coarse Sand, VCS = Very Coarse Sand, G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

159x122mm (300 x 300 DPI)

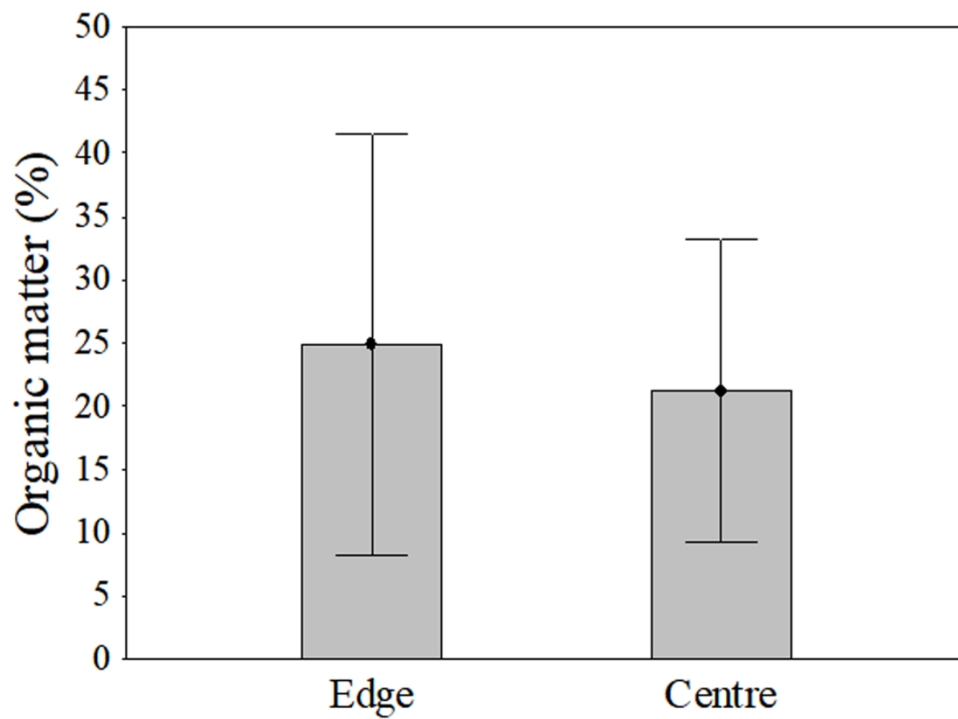


Figure 5. Mean values and standard error of organic matter of the sediment at the edge and in the centre of the temporary lakes.

161x118mm (300 x 300 DPI)