

Crustáceos: Ecossistema, Classificação e Reprodução

Luciana do Nascimento Mendes
(Organizadora)



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Ano 2019

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APRESENTAÇÃO

O E-book *Crustáceos: Ecossistema, Classificação e Reprodução* é uma obra composta de um único volume que tem como foco principal a discussão científica por intermédio de trabalhos diversos que compõe seus capítulos. Cada capítulo abordará de forma categorizada e interdisciplinar trabalhos, pesquisas, relatos de casos e/ou revisões que transitam nos vários caminhos relacionados aos crustáceos de diferentes classes, famílias, gêneros e espécies.

Nesta obra, o objetivo central foi apresentar de forma categorizada e clara, estudos desenvolvidos em diferentes instituições de ensino e pesquisa do país. Em todos os trabalhos a linha condutora foi o aspecto relacionado à biologia, reprodução desenvolvimento larval, extensão pesqueira, hábitat natural, educação ambiental, e áreas correlatas. A conservação dos ambientes costeiros, sejam manguezais ou faixas litorâneas tem tido um olhar especial, uma vez que não só impacta os animais ali encontrados, mas outros ecossistemas fluvio-marinhos.

Temas diversos e interessantes são, deste modo, discutidos nesta obra com a proposta de fundamentar o conhecimento de acadêmicos, ligados não só à área ambiental, ecológica ou biológica, mas também a área humana, quando perpassa pela interação com os atores envolvidos nas práticas de captura de crustáceos, pessoas cujo conhecimento é de extrema importância para a compreensão da interrelação entre os animais e o meio onde vivem. Torna-se relevante a compilação de diferentes trabalhos sobre pesquisas com crustáceos, seja de forma laboratorial, ou através de dados coletados em campo, para fortalecer o estudo e difundir a importância desses animais tanto no meio acadêmico como social, de forma a proporcionar o equilíbrio entre pesquisa e extensão.

Deste modo, a obra *Crustáceos: Ecossistema, Classificação e Reprodução* apresenta os diferentes objetivos que culminaram nos resultados aqui apresentados que foram desenvolvidos por professores, juntamente com seus alunos e suas alunas, envolvendo também pessoas das comunidades pesqueiras, como forma de enaltecer não apenas a experiência laboratorial, mas a empírica desenvolvida de forma laboral nos ambientes de manguezais, dulcícolas e costeiros, que serão apresentados de maneira concisa, prática e didática. A divulgação científica se faz tão importante quanto o “saber fazer” daqueles que vivem da coleta de crustáceos, ou estão envolvidos em outras modalidades pesqueiras, mas que inúmeras vezes contribuem nas coletas de campo. Portanto, utilizar da estrutura da Atena Editora é uma oportunidade de oferecer uma plataforma consolidada e confiável para os diferentes pesquisadores apresentarem seus resultados à sociedade, para que esses dados possam servir de orientação e base para novas descobertas.

Luciana do Nascimento Mendes

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STRATEGY FOR THE HATCHING OF MICROCRUSTACEANS ENDEMIC TO INTERMITTENT ENVIRONMENTS ALONG ANNUAL HYDROPERIODS

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ABSTRACT: We followed the hatching strategy of *Branchinecta iheringi* and *Eulimnadia pampa* in different hydroperiods of the year: i) we verified the hatching strategies of the species, ii) we characterized the time of development and reproduction of the species, and iii) we analyzed the relevance of environmental factors (hydroperiod, temperature, and depth)

for hatching and reestablishment of the species. We followed four consecutive hydroperiods in three intermittent wetlands over one year; three short (SH1, SH2, and SH3), and one long (LH). *Branchinecta iheringi* hatched in all hydroperiods of the year (SH1: 4 ± 6.9 org; SH2: 7 ± 4.3 org; SH3: 7 ± 5.1 org; LH: 35.3 ± 43.3 org). *E. pampa*, however, hatched only in the long hydroperiod (LH: 51.6 ± 61.2 org). The hatching responses and life cycles of the two species were different. The water retention time and the temperature variation had a significant effect on the reestablishment of the species. Monitoring the hatching strategies of the two species allows us to demonstrate that they presented different responses to the annual hydroperiods. *Branchinecta iheringi* responded immediately to the hatching stimuli while *E. pampa* needed a longer time to initiate hatching. **KEYWORDS:** Anostraca; Diplostraca; dormancy; temporary ponds

ESTRATÉGIA DE ECLOSÃO DE MICROCRUSTÁCEOS ENDÊMICOS DE AMBIENTES INTERMITENTES AO LONGO DOS HIDROPERÍODOS ANUAIS

RESUMO: Nós acompanhamos a estratégia de eclosão de *Branchinecta iheringi* e *Eulimnadia pampa*, em diferentes hidroperíodos do ano. Para isso: i) verificamos as estratégias de eclosões das duas espécies; ii) caracterizamos

o tempo de desenvolvimento e reprodução das duas espécies; iii) analisamos a relevância dos fatores ambientais (hidroperíodo, temperatura e profundidade) para a eclosão e restabelecimento das duas espécies. Durante um ano acompanhamos quatro hidroperíodos consecutivos em três áreas úmidas intermitentes, três curtos (SH1: primavera; SH2: verão; e SH3: outono), e um longo (LH: inverno). *Branchinecta iheringi* eclodiu em todos os hidroperíodos do ano (SH1: $4 \pm 6,9$ org; SH2: $7 \pm 4,3$ org; SH3: $7 \pm 5,1$ org; LH: $35,3 \pm 43,3$ org). Por outro lado, *E. pampa* eclodiu somente no hidroperíodo longo (LH: $51,6 \pm 61,2$ org). A resposta de eclosão e ciclo de vida das duas espécies foi diferente. O tempo de permanência da água e a variação de temperatura tiveram efeito significativo no restabelecimento das espécies. Através do acompanhamento das estratégias de eclosão das duas espécies podemos destacar que elas apresentaram diferentes respostas aos hidroperíodos anuais. *Branchinecta iheringi* respondeu de imediato aos estímulos de eclosão enquanto *E. pampa* precisou de um tempo maior para iniciar as eclosões.

PALAVRAS-CHAVE: Anostraca, Diplostraca, dormência, ambientes temporários

1 | INTRODUCTION

The main characteristic of intermittent wetlands is the presence of water for a period during the year (MALTCHIK *et al.*, 2004). This is called a hydroperiod (VAN DER VALK, 2006). Aquatic organisms have developed adaptive mechanisms to remain in these ephemeral environments even after a period without water (BLAUSTEIN and SCHWARTZ, 2001). A dormancy strategy is one of the main mechanisms for resilience in temporary environments, used by many groups of invertebrates (BRENDONCK, 1996) and vertebrates (THOMPSON and ORT, 2016).

The main stimuli for the hatching of dormant eggs is variation in the temperature and light intensity between the seasons (ALEKSEEV *et al.*, 2007), but the effect is dependent on the hydration of the dormant eggs after a period without water (BRENDONCK, 1996). In the subtropical region, the hydroperiod has a marked seasonal variation of the flooded phase (MALTCHIK *et al.*, 2004), altering the length of hydration for dormant eggs. The southern winter (June to September) corresponds to the period of greatest rainfall in southern Brazil (ALVARES *et al.*, 2014), resulting in longer hydroperiods lasting weeks or months (MALTCHIK *et al.*, 2004). Precipitation is reduced in other seasons, but occasionally occurs, and water remains for a few days. The difference in the duration of hydroperiods may affect the frequency and intensity of species hatching throughout the year, since hatching stimuli may also vary (STENERT *et al.*, 2017). The long photoperiod stimuli and high temperatures that occur in the warmer months are related to the volume of precipitation, which can result in long periods without water in the summer months (ALVARES *et al.*, 2014).

In addition to the stimuli required for hatching, the depth of the intermittent environments is also important for the process of species reestablishment (BUCKUP

and BOND-BUCKUP, 1999; MARINONE *et al.*, 2016). Environments are more ephemeral in warmer seasons, with reduced water volume through evaporation, and thus less depth (MALTCHIK *et al.*, 2004; VAN DER VALK, 2006). The variation in depth over time may affect the time of permanence of the species (MARINONE *et al.*, 2016).

Some groups of organisms adjust their lifecycles according to the duration of the water in the environment, such as the microcrustaceans *Branchinecta iheringi* Lilljeborg, 1889 (Branchiopoda: Anostraca) and *Eulimnadia pampa* Marinone, Urcula and Rabet, 2016 (Branchiopoda: Diplostraca), which are endemic to intermittent environments and have adapted to different hydroperiods (BUCKUP and BOND-BUCKUP, 1999; MARINONE *et al.*, 2016). The two species use dormant eggs as a strategy to survive the waterless period (BUCKUP and BOND-BUCKUP, 1999; MARINONE *et al.*, 2016). *Branchinecta iheringi* was described with examples of intermittent areas in the state of Rio Grande do Sul (Southern Brazil), and was recorded only in that state (CÉSAR, 1990; YOUNG, 1999), but its distribution extends to Argentina and Uruguay (CÉSAR, 1990; COHEN, 1995). The literature on this species is focused on taxonomic resolution (CÉSAR, 1990; COHEN, 1993; COHEN, 1995; BELK and BRTEK, 1995; YOUNG, 1999; ROGERS, 2013), with reduced information on the life cycle and ecology. Information about *Eulimnadia pampa* is even more scarce, since there is only one work describing this species in the intermittent wetlands of Argentina (MARINONE *et al.*, 2016).

In the temporary wetlands of southern Brazil the hydroperiod has a duration of weeks and normally occurs in winter, but there are occasional short hydroperiods when it rains in other seasons (MALTCHIK *et al.*, 2004). During these annual hydroperiods, the duration of the water, the temperature variation and the depth of the environment seem to be determinant of the reestablishment of *B. iheringi* and *E. pampa* populations in the intermittent environments. These factors may change over the years, as the environments are suffering from anthropogenic pressure (STENERT *et al.*, 2017). The objective of this study was therefore to monitor how these species are responding to the different hydroperiods in the year, in intermittent wetlands in southern Brazil. For this: i) we verified the hatching strategies of the two species in the different hydroperiods; ii) we analyzed the environmental factors for hatching and the reestablishment of the two species; iii) we characterized the time of development and reproduction of the two species.

2 | MATERIALS AND METHODS

2.1 Sampling procedures

The study was conducted in three intermittent urban wetlands (area of 464 ± 276 m²), located in the city of Rio Grande, RS, Brazil (32 ° 04 '38.3 "S, 052 ° 10' 09.1" W). We followed four consecutive hydroperiods between 2016 and 2017. The first three were short hydroperiods (SH), of up to five days in duration. We sampled only

once in each short hydroperiod, four days after the precipitation. The first hydroperiod (SH1) occurred in spring (sampling on October 18, 2016), the second (SH2) in summer (sampling on March 13, 2017), and the third (SH3) in autumn (sampling on May 15, 2017). The fourth hydroperiod was longer (LH), in late fall and early winter (between May 22 and June 23, 2017). This hydroperiod had a duration of 34 days and we made collections every two days.

We examined biological (species) and abiotic (water temperature and depth) data to monitor how species respond to the four different hydroperiods of the year and analyzed the environmental factors. Composite sampling of organisms was used, with three subsamples. We collected 60 L of water (20 L/ sub-sample), which was filtered in a plankton net with 68 μm mesh opening. The samples were fixed with alcohol (concentration of 80%) and transferred to the Laboratory of the Limnology of Federal University of Rio Grande (FURG). In the laboratory, the samples were stained with Bengal Rose, sorted and identified. The temperature was measured with a multiparameter probe (U-5000/ Horiba®) and depth with a graduated ruler.

We quantified the organisms hatching and the different stages of development in each hydroperiod to characterize the time for the development and reproduction of the two species. The organisms were counted using a stereomicroscope (P45BI/ Precision®), and identified with an optical microscope (CX41/ Olympus®), and specialized literature (BUCKUP and BOND-BUCKUP, 1999; MARINONE *et al.*, 2016).

2.2 Data analysis

We tracked the two species through the hydroperiods, and analyzed the long hydroperiod separately from the short ones to identify the relevance of environmental factors in the hatching and reestablishment of the species. We performed a factorial ANOVA for the short hydroperiods (CRAWLEY, 2007), and compared the abundance of organisms (variable response) in the different hydroperiods (Factor 1), with their respective temperatures (Factor 2) and depths (Factor 3). A linear Pearson correlation was performed to verify whether there was a correlation between temperature and depth.

We performed three Covariance Analyses (ANCOVA) in the long hydroperiod, comparing abundances of species in the hydroperiod and the effect of the environmental conditions. The species were used as a factor in all ANCOVAs, and the hydroperiod time, temperature and depth were used as covariates (CRAWLEY, 2007). The abundance data was transformed ($\log + 1$) for the analyses in order to reach the normality assumption. All analyses were performed in Environment R, version 3.5.1 (R CORE TEAM, 2018).

3 | RESULTS

The two species responded differently to the duration of the water. *Branchinecta iheringi* hatched in both short and long hydroperiods, while *Eulimnadia pampa* hatched only in the long hydroperiod. There was a significant correlation between temperature and depth ($R = 0.76$) in the SH. The highest temperatures and the lowest depths were recorded in SH2, corresponding to summer, followed by SH3 (autumn) and SH1 (spring) (Table 1). In short hydroperiods (SH1, SH2, and SH3) we only recorded the presence of *Branchinecta iheringi* in the larvae stage (Figure 1). There was no significant difference in larval abundance among the SHs (Table 2, Figure 1).

	Temperature (°C)	Depth (cm)
SH1 (Spring)	15.51 ± 0.13	30.6 ± 4.7
SH2 (Summer)	27.92 ± 1.67	21.6 ± 7.3
SH3 (Autumn)	16.47 ± 0.97	25.0 ± 21.0

Table 1: Water temperature and depth data (mean ± standard deviation) for the short hydroperiods of spring (SH1), summer (SH2) and autumn (SH3).

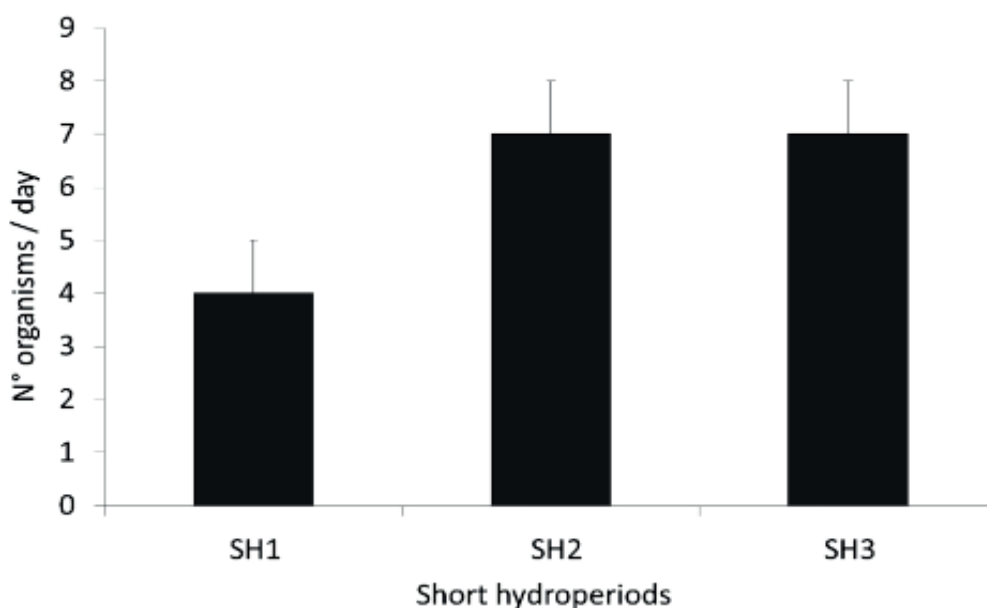


Figure 1: Number of *Branchinecta iheringi* organisms (mean ± standard error) by sampling in short spring (SH1), summer (SH2) and autumn (SH3) hydroperiods.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
ANOVA					
Hidroperiods	2	18.00	9.00	0.200	0.827
Temperature	1	6.47	6.47	0.144	0.724
Depth	1	1.35	1.35	0.030	0.871
Residuals	4	180.18	45.04		
ANCOVA 1					
Species	1	3.16	3.155	4.310	0.041*
Time of hidroperiod	1	7.96	7.962	10.875	0.001*

Species : time of hidroperiod	1	4.02	4.016	5.485	0.021*
Residuals	98	71.75	0.732		
ANCOVA 2					
Species	1	3.16	3.155	4.514	0.036*
Temperature	1	15.07	15.074	21.564	<0.001*
Species : temperature	1	0.15	0.146	0.209	0.648
Residuals	98	68.50	0.699		
ANCOVA 3					
Species	1	3.16	3.1551	3.877	0.052
Depht	1	1.81	1.8060	2.219	0.139
Species : depth	1	2.16	2.1641	2.659	0.106
Residuals	98	79.75	0.8138		
TukeyHSD (Species)					
		diff	lwr	upr	p adj
<i>B.iheringi</i> – <i>E.pampa</i>		0.351	0.007	0.711	0.055

Table 2: Results of factorial ANOVA for *Branchinecta iheringi* abundance in the short hydroperiods, and of the three ANCOVAs for the abundance of *Branchinecta iheringi* and *Eulimnadia pampa* and the environmental conditions in the long hydroperiod. The abundance of *B. iheringi* in the three short hydroperiods was considered in the ANOVA. In ANCOVA 1 the two species were considered as factors and the hydroperiod time as a covariate. In ANCOVA 2 the two species were considered as factors and temperature as a covariate. In ANCOVA 3 the two species were considered as factors and depth as a covariate. Indicator of statistical data significative (*).

During the LH the temperature reached a maximum of 23 ± 1.7 °C on the fourth day and a minimum of 11 ± 0.6 °C on the thirtieth day (Figure 2). The depth varied as a function of the precipitation in the hydroperiod, to a maximum of 38 ± 0.03 cm on the sixth day, and a minimum of 11 ± 0.05 cm on the thirty-fourth day (Figure 3). The temperature was significantly correlated with depth ($R = 0.72$). The two species co-occurred during LH and the responses in terms of predominance were different (Figure 4). *Branchinecta iheringi* predominated at the beginning of the hydroperiod until the tenth day, and then *E. pampa* predominated until the environments completely dried up (Figure 4).

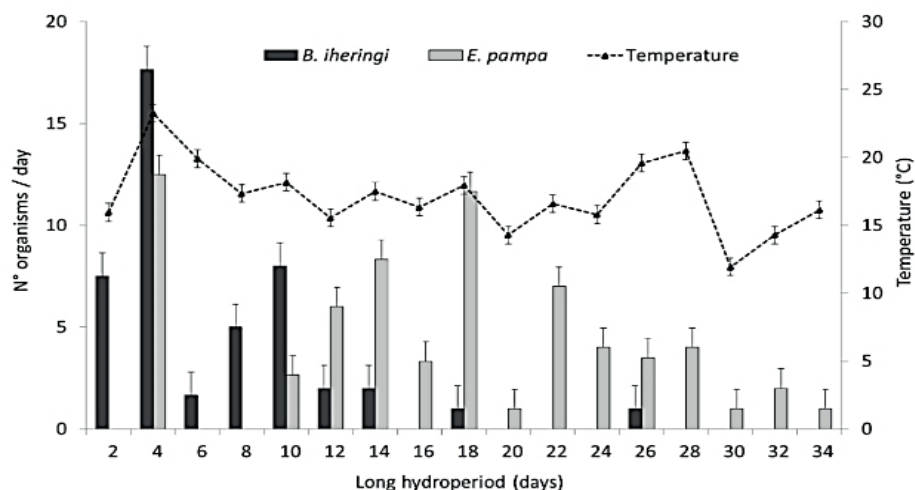


Figure 2: Variation in the temperature and number of organisms of *Branchinecta iheringi* and *Eulimnadia pampa* in the winter hydroperiod (mean \pm standard deviation).

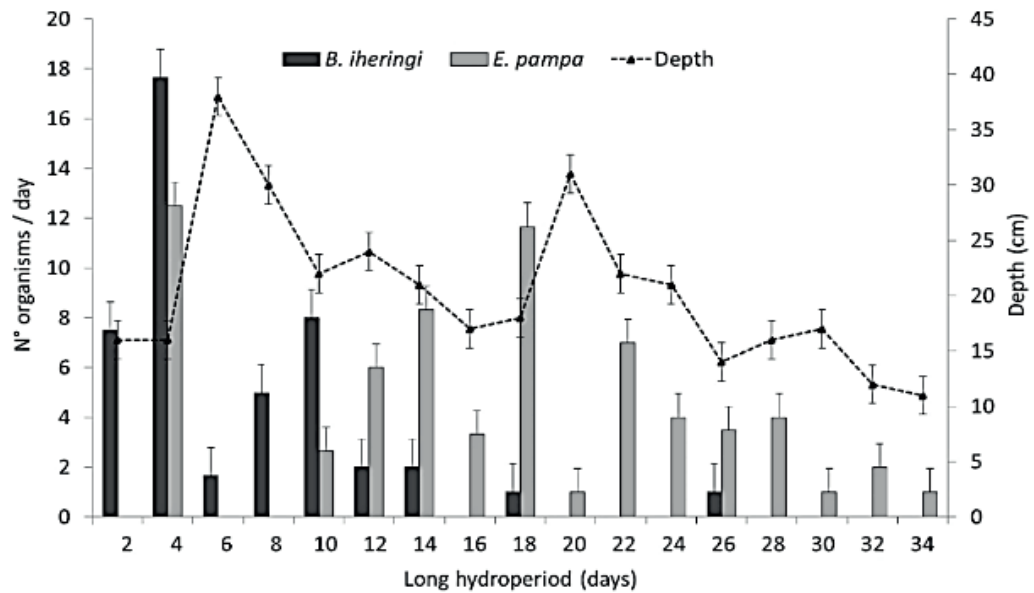


Figure 3: Variation in the depth and number of organisms of *Branchinecta iheringi* and *Eulimnadia pampa* in the winter hydroperiod (mean \pm standard deviation).

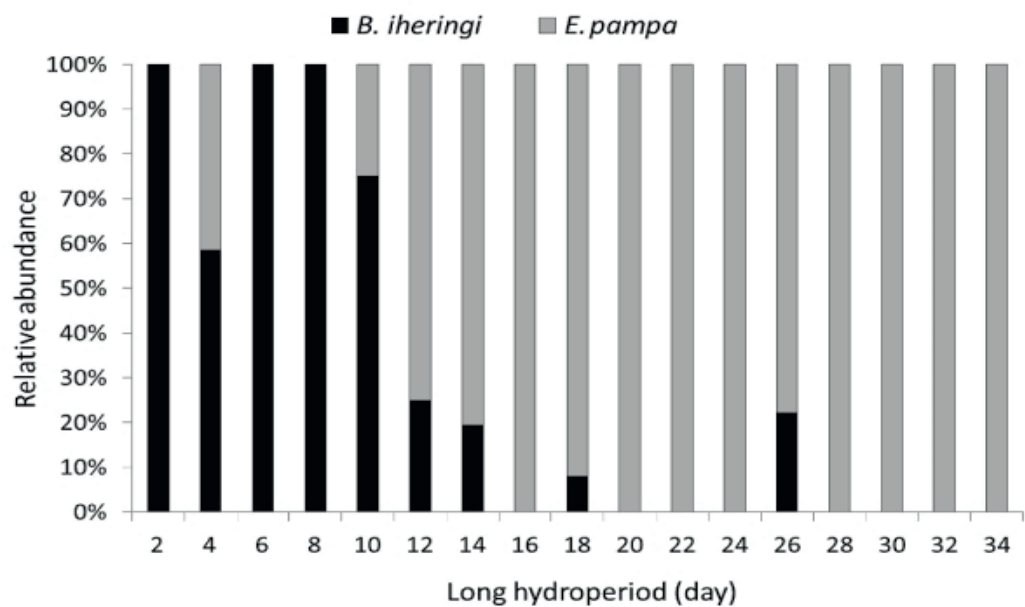


Figure 4: Relative abundance of the species *Branchinecta iheringi* and *Eulimnadia pampa* over the 34 days of the winter hydroperiod.

When we evaluated the relevance of environmental conditions to the abundance of species in the LH, we observed a significant effect on the interaction of the species and hydroperiod duration (Table 2, ANCOVA 1: $F_{1-98} = 5.5$, $p = 0.021$). There was a significant effect on abundance in relation to temperature variation (Table 2, ANCOVA 2: $F_{1-98} = 21.6$, $p < 0.001$, Figure 2). There was no significant effect of depth on species abundance (Table 2, ANCOVA 3: $F_{1-98} = 2.2$, $p = 0.139$, Figure 3). When comparing the abundance of the species in the hydroperiod we found that there was no significant difference between them (Table 2, Tukey HSD: $p = 0.055$).

We characterized the time of development and reproduction for the two species

and recorded the stages of development of *B. iheringi* and *E. pampa*. The larvae of *B. iheringi* were recorded from the second to the tenth day of the hydroperiod (Figure 5). The young of *B. iheringi* were recorded on the fourth and eighth day, the males on the twelfth and fourteenth day, and the females with eggs on the twelfth, eighteenth and twenty-sixth days (Figure 5). Only the young and female adults were recorded for *E. pampa*. The young were recorded from the fourth to the twenty-fourth day of the hydroperiod (Figure 5). Females were recorded from the tenth day until the end of the hydroperiod, and the first females with eggs were found from the twenty-second day (Figure 5).

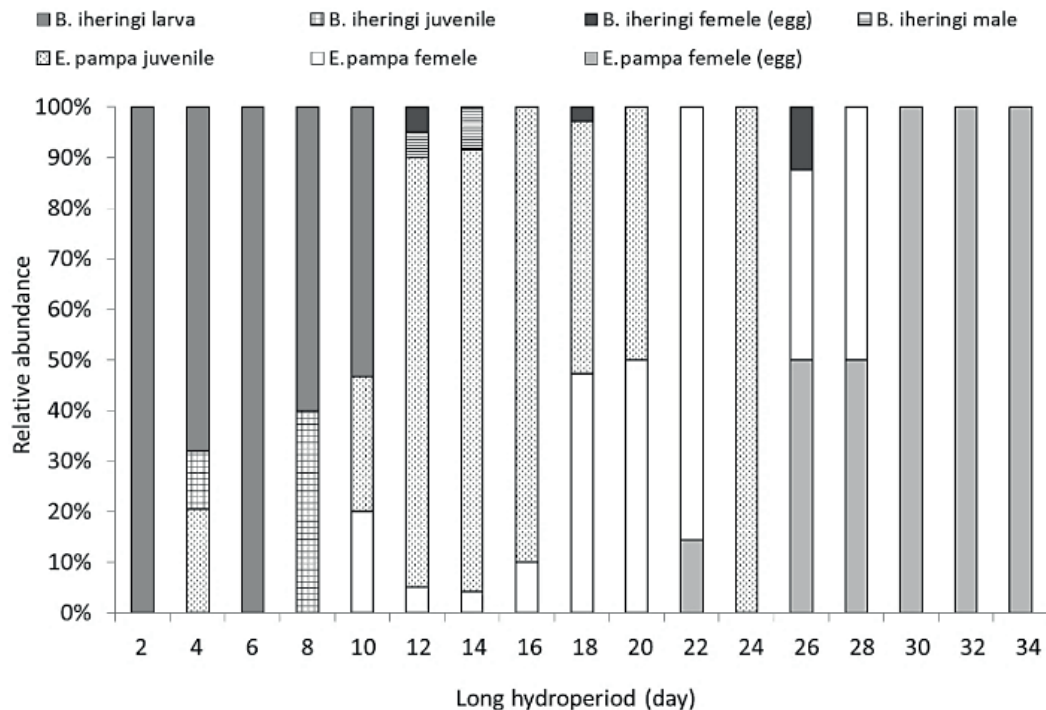


Figure 5: Relative abundance of the stages of development of *Branchinecta iheringi* and *Eulimnadia pampa* in the long winter hydroperiod.

4 | DISCUSSION

4.1 Strategies for species hatching in different hydroperiods

Both *Branchinecta iheringi* and *Eulimnadia pampa* were resilient regarding the recolonization of temporary environments after a period of desiccation, indicating the importance of dormant eggs for the reestablishment of organisms during the hydroperiods. The two species responded differently to the duration of the water, as *B. iheringi* hatched in all hydroperiods of the year and *E. pampa* only in the long hydroperiod.

The long hydroperiod was fundamental to verifying the recolonization process of the two species. It was possible to see that there was a difference in species permanence in the hydroperiod, since *B. iheringi* was predominant at the beginning and only afterward *E. pampa* predominated. Many authors describe the importance of

this variation in the permanence of the species for equilibrium in the reestablishment of communities of intermittent environments (BRENDONCK, 1996; WANG *et al.*, 2012; PINCEEL *et al.*, 2017; STENERT *et al.*, 2017). This variation can be affected by competition for food or space for reproduction. Establishing themselves at different times minimizes competition for food and enables the success of organisms throughout hydroperiods. This was demonstrated by Wang *et al.* (2012), who recognized that large branchiopods synchronize their life cycles and use space at different times to reduce competitive pressure in intermittent environments.

4.2 The relevance of environmental factors for hatching and reestablishment of the species

The *B. iheringi* species showed a rapid response to hatching stimuli in all the hydroperiods monitored. The temperature appeared to be of little relevance in the hatching process of this species because there was great temperature variation during the year which did not significantly affect the hatching abundance of the organisms in the short hydroperiods. Hatching in the long hydroperiod started when the temperature was around 15 °C, however, but the highest hatching number was recorded when the temperature increased to 24 °C. The optimum temperature for egg hatching for other species of the same genus as those evaluated in our study is reported as 15 °C for *Branchinecta lindahli* and 20 °C for *Limnadia stanleyana* (BRENDONCK, 1996). Similarly, *Branchinecta sandiegonensis* will hatch in the range of 10 to 20 °C (HATHAWAY and SIMOVICH, 1996). This may indicate that an increase in temperature accelerates the hatching process, but is not necessarily the main stimulus for *B. iheringi*. This observation has already been noted for other groups, such as Paes *et al.* (2016), who found that other factors such as the absence of light can affect the hatching process of dormant Daphnia eggs.

As well as the temperature, the duration of the water was also a significant factor in the long hydroperiod. *B. iheringi* dormant eggs hatch soon after they are hydrated, regardless of the temperature to which they are exposed. This may suggest that hydration time is more important than temperature for *B. iheringi* hatching, because hatching occurred in all the hydroperiods of the year, regardless of the duration of the water (short or long period), or the temperature that was recorded. The duration of the water was also an important factor in the reestablishment of other crustaceans, such as Cladocera (FLORENCIO *et al.*, 2015; STENERT *et al.*, 2017) and Copepoda (FLORENCIO *et al.*, 2015) in intermittent environments. Different adaptive responses by large branchiopods, regarding the duration of the water have been demonstrated for several species of fairy shrimps: for example, in experiments with three species, Dararat *et al.* (2011) demonstrated that *Branchinella* and *Streptocephalus* hatch after three to four days of egg hydration.

For *E. pampa*, the response was the reverse as that recorded for the first species,

as it seems that the temperature is as important as the duration of the water since hatching only occurred in the long winter hydroperiod. The temperature increase caused hatching on the fourth day of the hydroperiod for this species, indicating the importance of this factor for hatching of *E. pampa*. This increase may act as an initial trigger for the hatching, because even when the temperature was reduced the hatching continued. The need for a specific temperature as the initial trigger in hatching is very common in cladocerans (ALEKSEEV *et al.*, 2007; PAES *et al.*, 2016), and it may be that this strategy is also adopted by *E. pampa*. Marcus and Weeks (1997) tested the effect of the duration of the water in *Eulimnadia texana* and recorded that high temperatures can affect the onset of hatchings.

4.3 Characterization of the time of development and reproduction of the species

Observation of the time of development and reproduction for the two species demonstrated that, even though *B. iheringi* had more stages of life (egg, larva, young and adult), the development of these stages was faster, with a 12-day interval between larval hatching and recording of adult males and females with eggs. This *B. iheringi* cycle occurred at the beginning of the hydroperiod when instability in the water temperature was recorded. *Eulimnadia pampa* has direct development, hatching on the fourth day of the hydroperiod, and predominated only after temperature stability, and there was an interval of 18 days between the hatching of dormant eggs and the recording of females with eggs. The maturation time of *E. pampa* appears to be somewhat slower than *B. iheringi* since we recorded adult females on the tenth day of the hydroperiod, but only observed females with eggs after 12 days. Marcus and Weeks (1997) observed that the duration of the water altered the maturation time of *Eulimnadia texana*, where shorter hydroperiods accelerated the maturation time. Brendonk (1996) reported that in other subtropical regions the difference in maturation time for *Branchinecta* and *Eulimnadia* species favors the maintenance and viability of the dormant egg bank.

Depth and temperature were important for the beginning of production of the *E. pampa* eggs since we registered females with eggs from the twenty-second day. There was an increase in the temperature and a reduction in the depth of the environment, causing acceleration in the production of the eggs. This may be indicative of the timing of the species in realizing that the environment is becoming unfavorable, and egg production is a response to the final hydroperiod condition. This is also well documented for Cladocera, in which species can perceive environmental stimuli, and produce dormant eggs to ensure the next generation in the next hydroperiod (ALEKSEEV *et al.*, 2007). Although the two species predominate at different times in the hydroperiod, we observed that after the beginning of the production of both eggs, there was no record of larvae or newborns. This may suggest that in intermittent environments these organisms are investing primarily in the production of dormant eggs. This finding emphasizes the importance of duration of the water of these environments, to maintain the dormant egg bank in the sediment.

5 | CONCLUSION

In conclusion, monitoring the hatching strategies of the dormant eggs of the two species, allows us to demonstrate that they presented different responses to the annual hydroperiods of the intermittent wetlands. *Branchinecta iheringi* responds immediately to hatching stimuli, and *E. pampa* needs a trigger to initiate hatching. Both species have a different maturation time and this may be an indication that there is no competitive pressure between them. Even when they appear in the same hydroperiod, each predominates at different times of the duration of the water. In intermittent environments, these species are apparently investing mainly in the production of dormant eggs, strengthening egg bank maintenance, which proves to be fundamental for the recolonization and dynamics of these aquatic environments.

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