High temperatures and absence of light affect the hatching of resting eggs of *Daphnia* in the tropics

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ABSTRACT

Temperature and light are acknowledged as important factors for hatching of resting eggs. The knowledge of how they affect hatching rates of this type of egg is important for the comprehension of the consequences of warming waters in recolonization of aquatic ecosystems dependent on dormant populations. This study aimed at comparing the influence of different temperature and light conditions on hatching rates of *Daphnia ambigua* and *Daphnia laevis* resting eggs from tropical environments. The ephippia were collected in the sediment of three aquatic ecosystems, in southeastern Brazil. For each lake, the resting eggs were exposed to temperatures of 20, 24, 28 and 32 °C, under light (12 h photoperiod) and dark conditions. The results showed that the absence of light and high temperatures have a negative influence on the hatching rates. Statistical differences for hatching rates were also found when comparing the studied ecosystems (ranging from 0.6 to 31%), indicating the importance of local environmental factors for diapause and maintenance of active populations.

Key words: Cladocera, diapause, ephippium, light, temperature, tropical aquatic ecosystems.

INTRODUCTION

Throughout evolution, the aquatic organisms developed mechanisms by which they could survive the challenges imposed by the environment. For the zooplanktonic organisms, the diapause with production of resting eggs is a strategy that allows the recovery of populations and endurance of the species after periods of unfavorable conditions. Although this strategy is common in tropical and temperate environments, the knowledge of what factors induce the production and hatching of resting eggs in different Cladocera species is still not clear (Sarma et al. 2005).

In temperate regions, light and temperature have been considered the two most important stimuli for the hatching of zooplanktonic resting eggs (Stross 1966, Vandekerkhove et al. 2005) and more recently the presence of predators (Angeler 2005, Lass et al. 2005, La et al. 2009). However, in tropical regions, the importance of these factors for the termination of diapause in lakes has not been fully assessed yet. It is already known that rewetting of temporary ponds is an important stimulus for hatching in the tropics (Crispim et al.)
al. 2003), and that predators do not interfere with hatching of *Moina* resting eggs and salinity affects it negatively (Santangelo et al. 2010, 2013).

In tropical regions, the production of dormant stages has been studied mainly in temporary aquatic environments, in which the seasonal differences between the dry and wet periods strongly induce the beginning of the diapause process (Crispim and Watanabe 2001, Crispim et al. 2003, Palazzo et al. 2008, Paraneli et al. 2008, Araujo et al. 2013). For permanent lakes, the production of resting eggs due to anthropic action (Maia-Barbosa et al. 2003), seasonal effects (Brandão et al. 2012) and salinity (Santangelo et al. 2013) was reported. Most of these studies were conducted in order to understand population dynamics and to assess the difference between the active community in the water column and the egg bank in the sediments. Few studies experimentally investigated the factors that stimulate the formation and hatching of resting eggs (Santangelo et al. 2010, 2011), which may be relevant to the understanding of such effects.

Several adverse environmental alterations lead to the production of resting eggs and different factors may be involved in their hatching, which makes it even more difficult to evaluate and establish a pattern for diapausing mechanism. Among the factors that control diapause in Cladocera are photoperiod, temperature, population density, dessication and predation signs (Stross 1966, Crispim et al. 2003, Zadereev 2003, La et al. 2009).

Cladocera are relevant because they play an important role on energy transference through the food chain (Sarma et al. 2005). The use of *Daphnia* organisms is appropriate for biological and evolutionary studies, since the species reflect adaptations to different habitats, but their physiological properties and ecological function are very similar (Lampert 2006).

The different daphniid responses (metabolism, growth, reproduction, trophic interactions) to environmental changes depend on biotic (predator abundance and phytoplankton) and abiotic factors (hydrodynamics, thermal stratification intensity and duration, trophic state or geomorphology) of lakes, which are directly influenced by climate changes (Wojtal-Frankiewicz 2011). According to the IPCC (Intergovernmental Panel on Climate Change), there is a trend towards an increase on average temperature by the end of the 21st century (IPCC 2007). Thus, the effects of an increase in water temperature can directly influence the hatching of *Daphnia* ephippia and also be relevant for the understanding of the consequences of water warming for recolonization of the aquatic ecosystems dependent on dormant populations.

This study aimed at comparing, in laboratory, the influence of different light and temperature conditions on the hatching of *Daphnia ambiguа* and *Daphnia laevis* ephippia collected in permanent tropical aquatic ecosystems. The following hypothesis were formulated: a) higher temperatures and the presence of a photoperiod will induce higher hatching rates, b) resting eggs will hatch in a shorter period of time under higher temperature conditions and c) the environments studied will present differences on the hatching rates of resting eggs.

**MATERIALS AND METHODS**

The resting eggs were collected from the sediments of three water bodies of southeastern Brazil. The characteristics of these environments are shown in Table I.

Sediment samples were collected at the limnetic region, with a corer-type sampler and the first 10 centimeters were used. The samples were placed in dark flasks and refrigerated at 4 °C, for two to ten days. Resting eggs were isolated from these sediments by means of the sugar flotation method (Onbé 1978). The ephippia were collected from the supernatant and placed in a recipient with a 50 µm net, where they were maintained in
a 2% hypochlorite solution for 20 min., allowing ephippium transparency, decapsulation and selection of the healthy-looking resting eggs. Afterwards, they were washed with 500 mL of distilled water (T.A.S.V. Paes et al., unpublished data).

For the tests, resting eggs of *Daphnia laevis* found at Pampulha and Jacaré lakes and *Daphnia ambiguа* from Nado lake were used. An experimental design was performed manipulating light (absence x presence) and temperature (20, 24, 28 and 32 °C), providing a total of 8 treatments (2 light conditions x 4 temperature conditions) for each lake system. For each environment, healthy resting eggs were selected and transferred to a culture plate containing water from the respective environment, filtered with a 50 µm net. Each treatment was performed in triplicate (3 x 60 resting eggs), providing a total of 1,440 resting eggs/environment. Hatching was monitored daily for 20 days. The temperatures used in the experiments were selected from values obtained in field.

The significance of the predictors (i.e. temperature, light and environment) in relation to the response variable (hatching rate) was tested using general linear models which can adequately handle non-normal and heteroscedastic data. Due to the presence of overdispersion (i.e. residual deviation larger than the residual degrees of freedom), the regression models were fit by quasi-likelihood estimation for the binomial family (quasibinomial) (Venables and Ripley 2002). Overdispersion may be caused by lack of independence among samples, or parameter heterogeneity, and it can be modeled by the use of quasi-likelihood estimation (Anderson 2008). This approach inflates the estimated covariance matrix of the predictors, thus reducing the probability of selecting spurious relations (type-1 error) due to violations of the two assumptions previously described (i.e. independence of samples and parameter homogeneity).

### RESULTS

For the samples of Jacaré lake, hatching occurred from the fourth to the seventh day; for Pampulha lake, the highest hatching rates occurred on the fifth day, for the treatment at 24 °C with photoperiod (22%) and also on the fifth day for the treatment at 20 °C with photoperiod (16%). For this environment, the hatchings were not synchronized; they were detected throughout the whole assessment period. Resting eggs collected at Nado lake hatched faster than in the other environments (third and fourth days) and were more synchronized. The higher hatching rates were observed at 20 °C and in the light condition, on the fourth day (78.3%), followed by the treatment at this same temperature, in the dark condition (42%) (Fig. 1).

Among the analyzed factors, temperature was the most important for the hatching of *Daphnia* resting eggs (31%). However, its importance varied in relation to the environment, as indicated by the significant interaction between temperature and aquatic ecosystem (11%) (Table I). The environmental characteristics (aquatic ecosystem) of Pampulha, Nado and Jacaré lakes are shown in Table I.

**Table I**

<table>
<thead>
<tr>
<th>Aquatic ecosystems</th>
<th>Coordinates</th>
<th>Area (ha)</th>
<th>Maximum depth (m)</th>
<th>Circulation pattern</th>
<th>Water temperature (°C):</th>
<th>DO (mg.L⁻¹):</th>
<th>Secchi (m):</th>
<th>Trophic state</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pampulha</td>
<td>S 19°55'09&quot; W 43°56'47&quot;</td>
<td>196.8</td>
<td>15.1</td>
<td>-</td>
<td>Epi: 19.6 - 27.4, Hipo: 18.7 - 24.1</td>
<td>Epi: &gt; 8.0, Hipo: 0.0</td>
<td>0.8</td>
<td>eutrophic</td>
<td>Pinto-Coelho et al. 2003</td>
</tr>
<tr>
<td>Nado</td>
<td>S 19°49’50&quot; W 43°57’34&quot;</td>
<td>1.5</td>
<td>7.6</td>
<td>monomitic</td>
<td>Epi: 22.8 - 27.5, Hipo: 18.9 - 22.3</td>
<td>Epi: 8.0, Hipo: 0.0</td>
<td>0.3 - 1.4</td>
<td>mesotrophic</td>
<td>Bezerra-Neto et al. 2007</td>
</tr>
<tr>
<td>Jacaré</td>
<td>S 19°48’28.8&quot; W 42°38’55.5&quot;</td>
<td>109.0</td>
<td>9.8</td>
<td>monomitic</td>
<td>Epi: 28.5 - 33.1, Hipo: 20.0 - 22.0</td>
<td>Epi: 8.5, Hipo: 0.0</td>
<td>1.0 - 4.5</td>
<td>oligo-mesotrophic</td>
<td>Brandão et al. 2012</td>
</tr>
</tbody>
</table>

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also influenced the hatching, indicating that there was a significant difference on the mean hatching rates of the resting eggs between Pampulha lake (26 ± 5.6%) and Nado lake (31 ± 8.2%), which presented higher hatching rates than Jacaré lake (0.6 ± 0.2%). Photoperiod was the third most important factor (25%) and the hatching rates were always lower in the dark condition.

Thus, the temperature effect was high for Nado lake, was average for Pampulha lake and had no effect on Jacaré lake. The temperature effect was significant for all treatments at Nado lake, but more important for the light condition. It was significant only for the light treatment at Pampulha and it was not significant for both treatments at Jacaré. The temperature effect was less pronounced for the dark treatment (Fig. 2).

Figure 1 - Daily percentage of hatching of resting eggs at temperatures of 20 °C, 24 °C, 28 °C and 32 °C in the light (12 h photoperiod) and dark conditions. Pampulha lake (a), Nado lake (b) and Jacaré lake (c).

Table II - Analysis of the residual deviation for each of the assessed predictors

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Degrees of freedom</th>
<th>Deviation</th>
<th>Residual degree of freedom</th>
<th>Residual deviance (%)</th>
<th>Drop in residual deviance (%)</th>
<th>P value for inclusion of a new predictor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null model</td>
<td>71</td>
<td>1889.46</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>581.87</td>
<td>70</td>
<td>1307.59</td>
<td>69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aquatic ecosystem</td>
<td>2</td>
<td>572.46</td>
<td>68</td>
<td>735.13</td>
<td>39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>1</td>
<td>259.03</td>
<td>67</td>
<td>476.1</td>
<td>25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aquatic ecosystem:Temperature</td>
<td>2</td>
<td>207.6</td>
<td>65</td>
<td>268.5</td>
<td>14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temperature:Photoperiod</td>
<td>1</td>
<td>11.91</td>
<td>64</td>
<td>256.59</td>
<td>14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aquatic ecosystem:Photoperiod</td>
<td>2</td>
<td>2.88</td>
<td>62</td>
<td>253.72</td>
<td>13</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Figure 2 - Adjusted models for the hatching rate of resting eggs of *Daphnia ambiguа* (b) and *Daphnia laevis* (a and c) in each environment, according to the treatments (grey line: light and black line: dark). The dotted lines represent the reliability intervals. Pampulha lake (a), Nado lake (b) and Jacaré lake (c).
DISCUSSION

Our results indicate differences between the Daphnia spp. hatching rates comparing the assessed aquatic ecosystems. Temperature and light were important factors for the hatching of the resting eggs. Furthermore, the dynamics of the hatching of the resting eggs were also different between the environments, which evidences the importance of other factors that were not measured.

The third hypothesis of this study was corroborated. The hatchings were significantly different when comparing the water bodies, demonstrating the importance of the environment’s peculiar characteristics for the diapause mechanism. Similar results were obtained by Cáceres and Tessier (2003) in temperate lakes. These authors observed that the differences between the resting eggs hatching rates can be more strongly related to specific characteristics of the aquatic environment, especially to the seasonal risks to which the ephippia are submitted. The environments considered in the present study show different trophic conditions, thermal and chemical stratification, which may influence the hatching of resting eggs, in different ways.

Biological interactions, such as the presence (kairomones) of vertebrate and invertebrate predators, could also influence the dynamics of the dormant stages. Bozelli et al. (2008) showed hatching delays related to fish predation signals in tropical aquatic ecosystems. Their findings could explain the production and low hatching of resting eggs of D. laevis in Jacaré lake. However, experiments being conducted in our laboratory did not confirm this hypothesis (T.A.S.V. Paes et al., unpublished data). Similar information was found by Santangelo et al. (2010), showing that effects of vertebrate kairomones may not always influence the hatching rate and the time to hatching of ephippia in tropical aquatic ecosystems. Thus, the factors that induce the beginning and termination of the diapause can be more intimately related to the local environmental characteristics to which the organism is subjected, such as seasonality (Brandão et al. 2014, Cáceres and Tessier 2003), endogenous aspects of the eggs, such as egg age or maternal effects (Zadereev 2003) and calcium availability (Dumont et al. 1992). Moreover, the eggs belonged to two different species, which could also influence the outbreak.

Several experiments have shown the importance of temperature, photoperiod and luminosity for the termination of diapause in temperate lakes (Dupuis and Hann 2009, Pancella and Stross 1963, Vandekerkhove et al. 2005), since, in these regions, the most evident alterations of temperature and photoperiod can be especially critical for the zooplankton that depend on these signals to exit dormancy (Dupuis and Hann 2009). In tropical regions, other factors, could work as stimuli for this process, however, for some tropical aquatic environments, temperature and photoperiod strongly influence hatching rates, as observed in the present study for D. laevis and D. ambiguа, at Pampulha lake and Nado lake, respectively. Thus, such studies should be expanded to other locations in the tropical region in order to verify the possible regional responses.

The first hypothesis, that higher temperatures would induce a faster hatching of the eggs, was not corroborated. The results obtained suggest that higher temperatures have a negative effect on the hatching of D. laevis (Pampulha lake) and D. ambiguа (Nado lake) resting eggs. Similar results were obtained in the tropical region for Moina micrura ephippia originated from fish culture tanks (Rojas et al. 2001).

Dupuis and Hann (2009) demonstrated, by modeling, that climate changes can have negative consequences for the zooplanktonic populations of a temperate region, since it can affect important environmental stimuli which would stimulate the hatching of resting eggs, leading to dormancy.
rescission. The consequent temperature increase could also inhibit the hatching of resting eggs in some tropical environments. Thus, the recolonization of aquatic environments dependent on dormant populations would be affected by the low hatching rate of resting eggs. The absence of active species in the water column would affect the trophic interactions and the functional processes exercised by them.

The results obtained in this study evidenced the importance of the association of appropriate light and temperature conditions (photoperiod) for the hatching of *D. laevis* and *D. ambigua* ephippia collected at Pampulha and Nado lakes. Photoperiod was even more relevant at Pampulha lake. Thus, low light incidence in eutrophic environments such as Pampulha lake, where the photic zone is established at less than one meter, could negatively affect the hatching of resting eggs. On the other hand, while floating on superficial waters (Cáceres et al. 2007, Ślusarczyk and Pietrzak 2008), the effect of water warming can negatively affect the hatching, even in the presence of light, as observed for Nado and Pampulha lakes.

The difference between the hatching rates and dynamics of the resting eggs between the environments, together with the observed high residual deviation, evidences the importance of other environmental factors that were not measured, which could be related to the low hatching rate at Jacaré lake. Previous studies (Brandão et al. 2012) have shown that, for this environment, temperature was an important factor, responsible for the density fluctuations of *D. laevis* on the water column. However, under laboratory conditions, this factor, associated with the photoperiod, was not sufficient to induce the hatching of these species’ resting eggs, suggesting the influence of other environmental variables. In tropical ecosystems, the stratification and destratification events, which are related to temperature variation, promote several alterations on the physical, chemical and biological characteristics (refuge, reproductive rate, competition, predation, nutrient availability, levels of oxygen and food resources) of the environment.

Due to the difficulties in obtaining significant hatching rates at Jacaré lake, studies related to the interactions between abiotic and biotic factors are being conducted in order to better understand the causing factors of the beginning and termination of diapause for this *Daphnia laevis* population.

**CONCLUSIONS**

Temperature and photoperiod were important factors for the hatching of resting eggs on the permanent tropical aquatic environments studied. However, the difference between hatching rates and dynamics of these water bodies evidenced the importance of local factors for the diapause process. Temperature increase and absence of light negatively affected the hatching of *Daphnia* resting eggs. However, the reestablishment of populations are not exclusively depend on hatching rates, since daphnids are organisms with a large ability to adapt to environmental changes. Although temperature and photoperiod are relevant to hatching, we can not ignore the effects of trophic conditions and thermal and chemical stratification of the studied lakes for the maintenance of active populations.

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RESUMO

Temperatura e luz são reconhecidos como fatores importantes para eclosão de ovos de resistência. O conhecimento de como eles afetam taxas de eclosão deste tipo de ovo é importante para a compreensão das consequências do aquecimento das águas para a recolonização dos ecossistemas aquáticos dependentes das populações dormientes. Este estudo teve como objetivo comparar a influência de diferentes condições de temperatura e luz sobre as taxas de eclosão de ovos de resistência de *Daphnia ambigu* e *Daphnia laevis* de ambientes tropicais. Os efípios foram coletados no sedimento de três ecossistemas aquáticos, no sudeste do Brasil. Para cada lago, os ovos de resistência foram expostos a temperaturas de 20, 24, 28 e 32 ºC, à luz (12 h de fotoperíodo) e total ausência de luz. Os resultados mostraram que a ausência de luz e altas temperaturas influenciaram negativamente as taxas de eclosão. Foram verificadas diferenças estaticisticamente significativas para as taxas de eclosão dos ovos de resistência entre os ecossistemas estudados (média de 0,6 a 31%), indicando a importância de fatores ambientais locais na diapausa e manutenção de populações ativas.

**Palavras-chave:** Cladocera, diapausa, efípio, luz, temperatura, ecossistemas aquáticos tropicais.

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