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Hatching of the eggs of *Eulimnadia colombiensis* (Branchiopoda) at different temperatures and regime of light

Túlio Paiva Chaves and Erminda da Conceição Guerreiro Couto

Post-Graduate Program in Applied Zoology - UESC; Benthic Ecology Laboratory of the Universidade Estadual de Santa Cruz (UESC), Departamento de Ciências Biológicas, Rod. Jorge Amado, km 16, 45662-900, Ilhéus, Bahia, Brazil. E-mail: (TPC) bio_tulio@yahoo.com.br

ABSTRACT - Clam shrimps are small crustaceans typical of temporary ponds. To survive in these highly variable and unpredictable environments, they produce resistant eggs. These settle on the bottom of pools where they remain dormant, forming a cyst bank. We investigated the effects of light and temperature, both of which break the dormancy of *Eulimnadia colombiensis* eggs. Dormancy was not broken below 25°C, irrespective of the effect of light. Hatching was highest (27.8% of eggs) at 30°C in continuous light. Light also influenced the development of eggs, and some nauplii hatched spontaneously without drought stress. Methods are suggested for the cultivation of this species in tropical areas.

Key words: Clam shrimps, dormancy, egg production, environmental influence

INTRODUCTION

Members of the Class Branchiopoda are common in temporary pools. These environments usually occur in hot, semi-arid, flat regions (Rabet and Thiery, 1996; Demeter, 2005). The temporary nature, abiotic factors, as well as the duration of the pools directly influence the life cycle of these animals (Marcus and Weeks, 1997).

The genus *Eulimnadia* Packard, 1874 (Family Liminadiidae) found in these microhabitats includes 48 species (Rabet, 2010) and has androdioic sexual system. This reproductive pattern is characterized by the presence of males and hermaphrodites genetically different, but phenotypically similar, differing only by the presence clasper (Sassaman and Weeks, 1993; Weeks *et al.*, 2009).

Survival of populations these genus in this variable environment, especially in the ephemeris, depends entirely on the production of resistant eggs, which are really cysts. The single-celled egg develops inside the adult and the embryo within its cyst (or egg-shell) is expelled of carapace. The embryo can remain dormant, awaiting favourable conditions, possibly for year one or more (Weeks *et al.*, 2002). During this stage the embryos are able to resist long periods of drought and, when rehydrated under favourable conditions of light, temperature and oxygen availability (Brendonck, 1996). However, even if the environment is favorable present, the hatch does not happen immediately. Dormancy breaking happens only when internal factors are activated, a mechanism known as "diapause" (Brendonck, 1996). This mechanism, linked to the "bet-hedging" strategy (asynchrony in hatching eggs of the same litter), can greatly reduce the chances of local extinctions, if the environment be altered abruptly, without the organisms have time to respond to changes (Seger and Brockman, 1987; Cáceres and Tessier, 2003; King and Masel, 2007; Benvenuto *et al.*, 2009).

Individual hermaphrodites of *Eulimnadia* can produce thousands of eggs (Weeks *et al.*, 1997), but as not all eggs hatch in the same instant (Benvenuto *et al.*, 2009) those, whose dormancy is not affected, accumulate in the sediment. The accumulation of these cysts, not hatched, and those of other species in the bottom sediment of water bodies form the so-called "cyst banks". These, in turn, can provide us with information ranging from the richness of species in ecosystems (Arnott *et al.*, 1999; Melo and Froehlich, 2001; Vandekerkhove *et al.*, 2005) to temporal and spatial aspects of community dynamics (Brendonck and De Meester, 2003).

Light and temperature are primary factors in the hatching of the cysts of numerous species of zooplankton (Hairston *et al.*, 1990; Fryer, 1996; Gyllstrom and Hansson, 2004). Large temperature variations are expected in temporary pools and studies have shown that this is a fundamental variable in the process of breaking the dormancy of cysts of many zooplankton species (May, 1987; Schwartz and Hebert, 1987; Vandekerkhove *et al.*, 2005).

Eulimnadia colombiensis was described by Roessler (1989), in temporary ponds around Villavicencio and San Juan de Arama (Meta, Colombia). The species is recorded for Venezuela (Pereira and Garcia, 2001), Brazil (Rabet *et al.*, 2012) and India (Weeks *et al.*, 2006). Unlike other species of the genus, little is known of its biology. Nonetheless, studies were not conducted on the factors that influence the hatching rate of the eggs of *Eulimnadia colombiensi*. Here we investigated the hatching rates under different conditions of temperature in the presence and absence of light.

MATERIAL AND METHODS

Eulimnadia colombiensis adults were randomly collected from one fish pond at CEPLAC (Comissão Executiva do Plano da Lavoura Cacaueira) near Itabuna, Bahia, Brazil (14º46'51.51"S, 39º13'29.11"W). They were taken to the laboratory and cultured in aquaria using low aeration (after Weeks *et al.*, 1997) and exposed to ambient light and temperature. After the adults had died, the water was drained and filtered in a fine mesh sieve (80μm), which retained the deposited cysts. The latter were allowed to dry naturally in Petri dishes and stored in 7mL glass flasks.

After 45 days, simulation of time of interval average as that the tank remain dry, fourty two samples were taken of 30 eggs each, randomly selected, totalling 1,260 eggs of different hermaphrodites. Each sample was placed in Petri dishes and 40mL of purified water was added to each Petri dish, 24 hours afterward further 10mL were added.

Each series with three samples (= 90 eggs) was submitted to different combinations of temperature and light for 48 hours as suggested by Weeks *et al.* (1997). The thermal amplitude recorded for local water ponds varied between 21° (could months) e 29° C (hot months) (Tejedo *et al.*, 2012). However, the environment studied by these authors differs from our local collection by presenting a lower volume of water and constant shade conditions. Preliminary tests were conducted at 15°C e 20°C and no hatching was observed.

A total of 14 treatments were tested at seven temperatures (25°, 26°, 27°, 28°, 29°, 30° and 35°C) in an incubator in the presence or absence of light. The temperature of water of each sample was monitored and maintained the same during time proposed. After this were period removed from the incubator and, with the aid of a stereomicroscope, hatched *nauplii* were pipetted and counted.

Finality, "bi-factorial ANOVA" test of, a 95% significance level, was used to analyse the existence of significant differences between the number of hatchings at different temperatures, and in the absence and presence of light, for each replica. The a posteriori Tukey test was used to determine the significance of potential differences.

RESULTS

Light strongly influenced the hatch of the *nauplii*. The highest hatching rates (17.8% and 27.8%) were obtained at 25°C and 30°C, respectively, in the cultures under constant light. Under this light condition, the difference between hatching rates at intermediate

Table 1. Summary of results of Bi-factorial ANOVA for temperature range in the presence and absence of light.

Source of variation	df	Residue	Variance	F	P
Temperature (T)	6	56.667	9.111	9.225	<0,001
Presence of light (L)	1	113.357	113.357	110.721	<0,001
TxL Interation	6	69.143	11.524	11.256	<0,001
Residual	28	28.667	1,024		
Total	41	267.833	6.533		

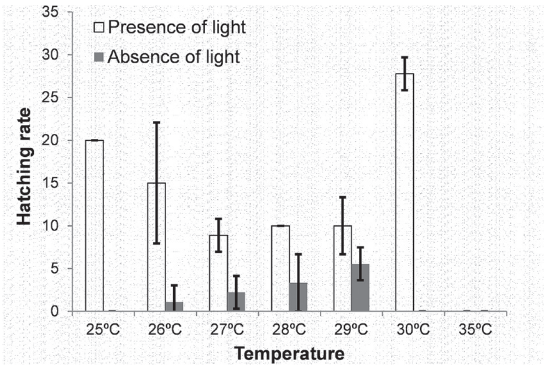

Figure 1. Hatching rate of *nauplii* of *Eulimnadia colombiensis* in the absence and presence of light, and at different temperatures.

temperatures (26ºC, 27°C, 28°C and 29°C) did not show a clear pattern. However, when eggs underwent these same temperatures in the absence of light, there was a gradual increase in hatching rate from 27°C to 29°C (Fig. 1). None of the eggs hatched at 35ºC disrespected of the light regime.

The results showed that there is interaction between temperature and light on the hatching rates, the presence of light being fundamental in increasing the number of hatched *nauplii* (Tab. 1).

With respect to the different paired temperatures, the *a posterior* Tukey test showed significant differences between the samples in the presence of light of the pair 25°C X 27°C and in all the combinations with 30°C. In the absence of light no significant differences between pairs of temperatures were observed.

In addition to the above findings, we also observed that light positively affects the development of individuals after hatching. In the presence of light, the *nauplii* were shown to be active, free-natant and with no trace of egg connected to the body, contrary to what was observed in the absence of light. Dead *nauplii* were found among the set great of eggs where were extracted the samples for the study, suggesting the occurrence of hatching without water stress.

DISCUSSION

Both temperature and light regime are critical in the breaking of dormancy of *Eulimnadia colombiensis* cysts. The rates of hatching varied greatly as these environmental factors changed. The highest hatching rates were at 25°C and 30°C in the presence of constant light. However, at small temperature intervals (±1°C) and in absence of light, the gradual increase in the hatching rate strongly suggests that temperature is an important stimulus in breaking dormancy.

The temperature variation in local temporary ponds is very small (Tejedo *et al.*, 2012), with daily values recorded between 21°C and 25°C (Tejedo, pers. comm.). However, our data show that hatching did not occur below 25°C after 48 hours of incubation in either the presence or absence of light so it may be necessary to extend the incubation period. It is likely that under natural conditions the hatching eggs need a period exceeding 48 hours. The situation is less clear at higher temperatures. None of the eggs hatched at 35°C, which was unexpected in tropical species that living in areas where the temperature of the water exceeds these values into higher irradiation times. This result suggests that this species of the genus *Eulimnadia*, despite the large phenotypic plasticity (Rabet, 2010), the temperature variation can be an important factor in establishing the limits of their biogeographical distribution.

The light influences not only the number of cysts that will hatch but also the subsequent development of *nauplii*. *Nauplii* that hatched in the presence of light proved to be active and with no trace of residual egg. Cysts incubated in the dark remained a *pre-nauplius*, inactive and the residual of the egg remained connected to the body, suggesting that light plays an important role in the species development.

For populations of *E. colombiensis* from tropical regions with high rainfall, we suggest that cultures should be made at 30°C, under constant presence of cold light. This value is higher than suggested by previous authors (Weeks *et al*., 1997, 1999, 2001; Rogers *et al*., 2010) for other species of the same genus. However, the consulted works dealt with populations of species from regions with different climatic characteristics. Thus, information about the place of origin of the specimens is key to the guidelines of an efficient cultivation for scientific reasons.

When comparing the egg production between *E. colombiensis* and *E. texana*, the difference is apparent. The average initial egg production by *E. colombiensis*, originating from the same area of study in question is 345 eggs.ind-1.d-1 (Chaves, 2014). Much higher than its congener *E. texana*, studied by Weeks *et al*. (1997), in which the initial production was ~ 50 eggs.ind-1.d-1. When we compare the contribution to the bank of eggs between coexisting genres, other observations arise. In one experiment (Chaves - unpublished data), comparing as absolute number of eggs of the coexistents *Eulimnadia* (Conchostraca) and *Dendrocephalus* Daday 1908 (Anostraca), in 100g of dry sediment from small puddles (~ 1m^2) and large (~ 400m^2), it was observed that smaller pools have a greater number of *Eulimnadia* eggs while larger pools have a higher number of *Dendrocephalus*. This difference in colonizing potential can be reinforced by the difference in the life cycles of the two genera (*Eulimnadia* ~ 16 days, personal observation, and *Dendrocephalus* ~ 15-75 days, see Lopes *et al*., 2011; Rabet and Thiery, 1996; Pereira and Gonzalez, 1994). Thus, individuals of *Eulimnadia* reach adulthood and reproduce faster, increasing its power to colonize ephemeral environments. Thus, in case of competition between these groups, *E. colombiensis* has an advantage in the initial colonization. Further studies are needed to test this hypothesis.

Just as in the natural environment, the variations found between hatching plates subjected to the same conditions, and the existence of percentages of hatching without a clear pattern, may be explained by the so-called *bet-hedging* mechanism. This strategy can be understood as a moment of hatching asynchrony in individuals originating from the same brood, which prevents local extinctions if the environment is change abruptly, without the bodies have time to adjust to changes (Seger and Brockman, 1987; Cáceres and Tessier, 2003; King and Masel 2007; Benvenuto *et al*., 2009). Several groups of microcrustaceans (cladocerans, calanoid copepods and anostracans) present in ephemeral environments use this strategy as a way to ensure success in this type of environment (Saiah and Perrin, 1990; Hairston *et al*., 1995. Simovich and Hathaway, 1997; Cáceres and Tessier, 2003; Gyllstrom and Hansson, 2004).

The presence of dry *nauplii* in the material from which the eggs were extracted for the experiment was never reported for this genus. This record shows that *nauplii* hatch without necessarily going through a period of desiccation, but possibly some other snapshot unidentified environmental factor capable of modifying the dormant stage or avoid it as observed in other species of Branchiopoda (Fryer, 1996). This record suggests that maintaining environmental conditions favourable for longer favours lineages that have sequential development without the need to water stress.

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