Hatching rates of resting eggs of 'Cladocera' (Crustacea; Branchiopoda) at a tropical bay, Brazil

Mugrabe, G, Barros, S., Marazzo, A. and Valentin, JL.*

Laboratório de Zooplâncton, Departamento de Biologia Marinha, Instituto de Biologia,
Universidade Federal do Rio de Janeiro – UFRJ,
Ilha do Fundão, Cidade Universitária, CEP 21949-900, Rio de Janeiro, RJ, Brazil

*e-mail: glenda@biologia.ufrj.br

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(With 5 figures)

Abstract

The aim of this study was to determine the development time of embryos and to estimate the hatching rates of resting eggs of cladocerans found in the sediment of Guanabara Bay, Rio de Janeiro, Brazil, under experimental conditions. Eggs were sorted by species (*Penilia avirostris* – Sididae; *Pleopis polyphemoides* and *Pseudevadne tergestina* – Podonidae) and incubated at a temperature of 25 °C, salinity 35 and photoperiod 12 hours light/ 12 hours dark. Hatching rates were about 38% for *Pseudevadne tergestina* and 28% for *Pleopis polyphemoides.* Embryos of resting eggs of *Penilia avirostris* developed comparatively slowly (hatching after 86 days of incubation), with a hatching rate of only 5%. It was observed that development and hatching of resting eggs of marine cladocerans suggest that pulses of recruitment may exist, thus contributing to the rapid appearance and maintenance of planktonic populations of these crustaceans in Guanabara Bay.

Keywords: Cladocera, resting eggs, hatching, Guanabara Bay, Brazil.

Taxas de eclosão de ovos de resistência de 'Cladocera' (Crustacea; Branchiopoda) em uma baía tropical, Brasil

Resumo

O objetivo do presente estudo foi determinar o tempo de desenvolvimento de embriões e estimar as taxas de eclosão de ovos de resistência de cladóceros encontrados no sedimento da baía de Guanabara, Rio de Janeiro, Brasil, sob condições experimentais. Os ovos foram separados por espécie (*Penilia avirostris* – Sididae; *Pleopis polyphemoides* e *Pseudevadne tergestina* – Podonidae) e incubados a 25 °C, salinidade 35 e fotoperíodo 12 horas claro/ 12 horas escuro. As taxas de eclosão foram de aproximadamente 38% para *Pseudevadne tergestina* e de 28% para *Pleopis polyphemoides*. Os embriões dos ovos de resistência de *Penilia avirostris* desenvolveram-se de forma relativamente lenta (eclodindo apenas 86 dias após o início da incubação), com uma taxa de eclosão de apenas 5%. Foi observado que o desenvolvimento e a eclosão dos ovos de resistência de cladóceros marinhos sugerem que podem ocorrer em pulsos, contribuindo assim para o rápido aparecimento e manutenção destes crustáceos na baía de Guanabara.

Palavras-chave: Cladocera, ovos de resistência, eclosão, baía de Guanabara, Brasil.

1. Introduction

Marine cladocerans are seasonally abundant microcrustaceans, widely distributed in estuarine and continental shelf waters and the open sea, where they comprise a significant portion of the mesozooplankton community. Some authors (e.g., Egloff et al., 1997; Onbé, 1999) have suggested that gamogenesis (or sexual reproduction) is initiated under unfavorable environmental conditions. Females produce eggs that give rise to male and female individuals. After copula and fertilization, the female produces, in general, a single, larger egg, with a resistant outer membrane and full of vitellus, which is called a resting egg. After releasing, the egg sinks towards the sea bottom until environmental conditions become favorable again. When the egg hatches, a female individual is born to restart parthenogenic reproduction. Normally, a sharp decrease of the planktonic population is observed, as well as increased production of resting eggs (Onbé, 1978a, 1985; Egloff et al., 1997).

The benthic phase of cladocerans, in which they remain in the environment as resting eggs, is considered critical for the perpetuation of the species in the plankton. These eggs can remain viable for several years on the sea bottom. Hatching success depends on many factors: for example, a rise in temperature is considered a key factor for egg hatching (Barros et al., 2002).

Resting eggs of cladocerans occur in the sediment of Guanabara Bay (Barros et al., 2000). The viability of these eggs has not yet been tested. In this tropical bay, located in Rio de Janeiro State, intense eutrophication from high inputs of organic matter (Paranhos et al., 1995) may have become a determinant factor in the development and hatching of these eggs.

We followed the development of embryos and estimated hatching rates of resting eggs of marine cladocerans in the sediment of Guanabara Bay in order to estimate whether these may serve as a recruitment resource for planktonic populations in this highly impacted tropical bay.

2. Material and Methods

Sediment samples were taken by means of an Ekman grab at a fixed 15 m depth station in Guanabara Bay, where muddy sediment predominates. In the laboratory, resting eggs were sorted from sediment samples, adapting the method proposed by Onbé (1978b) which consists of washing the samples through a 100 µm mesh. The residue remaining on the mesh was transferred to centrifuge tubes with a dense solution composed of 1 kg sucrose in 1 liter of water. Samples were centrifuged 3 times at 3500 rpm for 3 minutes. The supernatant was collected after each centrifugation, and then analyzed under stereoscopic microscopy to sort resting eggs. The eggs were identified following the descriptions by Onbé (1991).

The resting eggs belonged to the genera *Penilia* (*Penilia avirostris* Dana, 1852), *Pleopis (Pleopis polyphemoides* Leuckart, 1859*) and Pseudevadne* (*Pseudevadne tergestina* (Claus, 1877)). Immediately after sorting, 20 eggs of *P. avirostris*, 72 *P. polyphemoides* and 8 *P. tergestina* were placed separately in 25 ml flasks containing filtered water collected from the bay. The eggs were incubated under constant temperature (25 °C) and salinity (35) conditions, similar to the water of the bay at the collecting point. The water in the flasks was changed daily in order to maintain constant salinity. Dissolved oxygen was at a saturated level (5-6 $ml.L^{-1}$) and photoperiod was 12 hours light/12 hours dark. Since the main objective of this first study was not to test the influence of water conditions, but just to observe the viability of the resting eggs in the Guanabara Bay and due to the small volume of the flasks, we did not measure other indicator parameters of water quality, such as ammonia. However, it is known (Paranhos, 2002) that this nutrient is lower (<5.0 µM) at the entrance o f the bay when salinity is high (35), due to the coastal water input under the influence of the tide. Eggs from each flask were transferred by pipette to a Petri dish, and observed in a stereoscopic microscope to follow the embryo development.

3. Results

Resting eggs of Podonidae are spherical, with a mean diameter of 200 µm, and may have ornamentation on the outer membrane (*Pleopis*), or be smooth (*Pseudevadne*). The first signs of embryonic development in resting eggs of *P. polyphemoides* occurred after 11 days of incubation. The vitelline content, which occupied the entire inner space of the egg, formed a concentrated mass, creating a space between the vitellus and the outer membrane (Figure 1a). One side of the vitelline mass curved inwards (Figure 1b). After about two days it was possible to see the whole formation of the embryo. The presence of red pigment in the eye corresponds to the final phase of embryonic development, when the egg is about to hatch. Before hatching, it was possible to observe that the egg was divided into two distinct parts. The outer membrane was then broken at the line site (Figure 1c), followed by a rupture of the inner membrane and hatching of the embryo (Figure 1d).

The development of resting eggs of *P. tergestina* is similar to *P. polyphemoides*. The only difference is the eye pigment, which is black (Figure 2b).

Resting eggs of *P. avirostris* are ovoid and flattened dorsal-ventrally, with a mean length of 250 µm and width of 192 µm (Figure 3). Their development follows a similar pattern to *Pleopis polyphemoides*. The black eye pigment appears only at the end of development.

3.1. Hatching rate and frequency

About 38% of the resting eggs of *P. tergestina* from Guanabara Bay hatched between day 20 and 31 after incubation, whereas for *P. polyphemoides*, hatching rates were about 28% (Figures 4 and 5). The embryonic development of such eggs was completed only 2 days after observation of the first signs of development.

Penilia avirostris had a single hatching egg (5%), after 86 days of incubation (Figure 5).

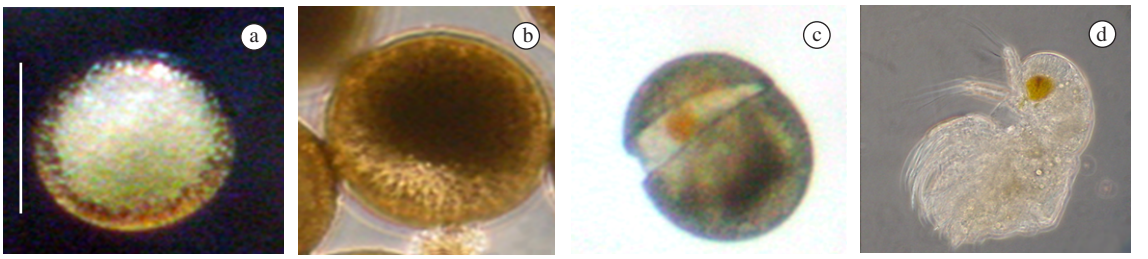

Figure 1. *Pleopis polyphemoides*. Resting egg development. Bar size: 200 µm.

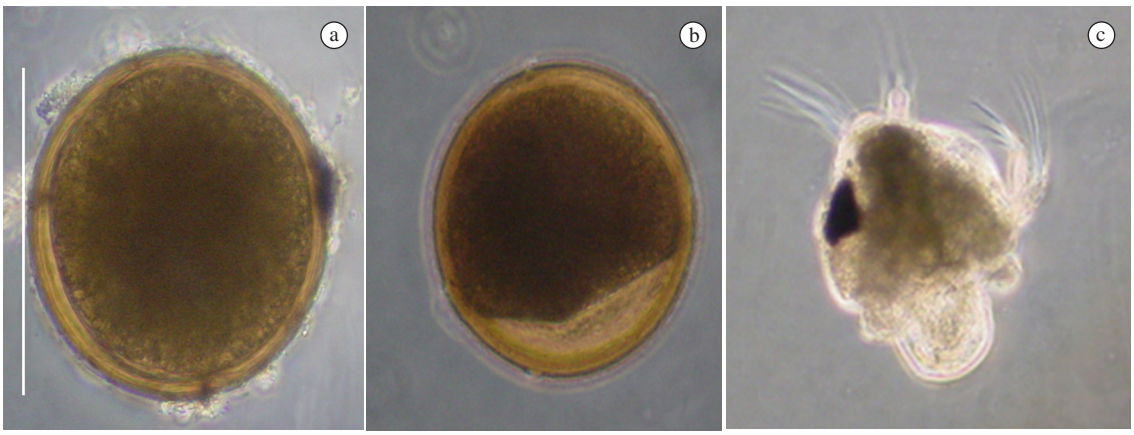

Figure 2. *Pseudevadne tergestina*. Resting egg and embryo developed. Bar size: 200 µm.

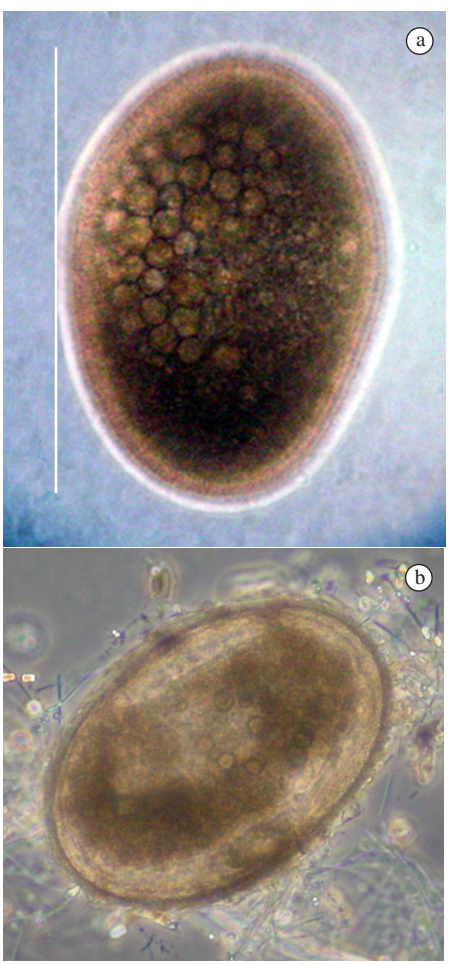

Figure 3. *Penilia avirostris*. Embryonic development of the resting egg. Bar size: 250 µm.

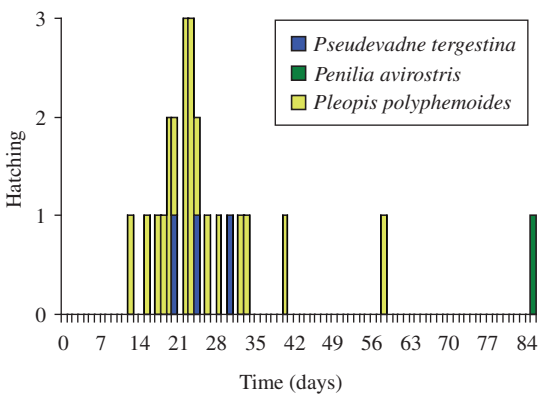

Figure 4. Number of hatched resting eggs during the incubation period.

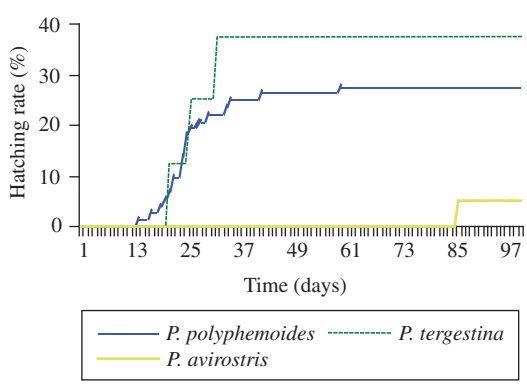

Figure 5. Cumulative frequency of hatched resting eggs during the incubation period.

4. Discussion

Hatching synchronism may be a rule among marine cladocerans, as related by Madhupratap et al. (1996), who reported the same behavior. Observations of the embryonic developments of resting eggs made during this study agree with those of Onbé (1991), who observed that 50% of the eggs collected in the Inland Sea of Japan hatched in 15-17 days; while Madhupratap

et al. (1996) observed a maximum hatching rate during days 20-30 at 15 °C.

Onbé (1991), on the other hand, observed hatching resting eggs at lower temperatures (20 °C) after about 4 days following the first noticeable signs of embryonic development. New experiments are necessary to explain these differences and elucidate the environmental factors which may influence embryonic development time and induce the hatching of resting eggs of *Penilia avirostris* in Guanabara Bay.

Various hypotheses could be suggested to explain the non-hatching of the majority of the resting eggs collected during this study. The unknown date of when the eggs were released into the water column is the main obstacle for such an attempt. We do not know if they became unviable because of a long time spent in the sea bottom, or if they have a longer period of latency, which can vary with environmental conditions. According to Onbé (1985), resting eggs can lose their viability because of environmental deterioration in the sediment, such as deoxygenation, as well as a production of ammonia, sulfide gas and other toxic substances. Onbé (1985) suggested that in polluted locations these eggs can be buried in deeper layers in the sediment, due to increased sedimentation rates, and this burial can eventually cause the death of the embryos because of the toxic conditions in the sediment.

This preliminary investigation allowed us to reach certain conclusions about the viability of cladoceran resting eggs in Guanabara Bay. Embryonic development phases and egg hatching follow the same pattern as other areas in the world. Resting eggs of *P. tergestina* and *P. polyphemoides* from Guanabara Bay hatched earlier under incubation conditions and had a higher cumulative hatching success than the eggs of *P. avirostris*. A better understanding of the ideal conditions for the incubation and maintenance of *P. avirostris*, as well as the environmental factors favorable to its embryonic development is needed. The development and hatching synchronism of resting eggs of marine cladocerans suggest that pulses of recruitment may exist, thus contributing to the rapid appearance and maintenance of planktonic populations of these crustaceans in Guanabara Bay.

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