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The effect of temperature and duration of incubation on the hatching of diapause eggs of *Centropages hamatus* (Copepoda, Calanoida)

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Introduction

A few studies have examined the hatching response of copepod diapause eggs to various factors (Ban and Minoda, 1991; Chen and Marcus, 1997). Marcus (1979) monitored the hatching of diapause eggs of *Labidocera aestiva* and suggested that diapause eggs of marine copepods undergo a refractory phase similar to other diapausing organisms. Eggs must complete this refractory phase before they can be induced to hatch. The eggs of species that experience cold winter temperatures (e.g. 5°C) in the wild while in the refractory phase e.g. *Labidocera aestiva* were shown to resume development when exposed to warmer (e.g. 25°C) temperatures. On the other hand the diapause eggs of species such as *Centropages hamatus* that experience warm summer temperatures (25°C) while completing the refractory phase only resume development when the temperature drops below a certain level (e.g. 20°C). Recently it has been suggested that copepod diapause eggs could be useful in aquaculture because they could provide a ready source of nauplii in the same way that brine shrimp and rotifer cysts provide a ready supply of those organisms. This study was undertaken to assess how exposure to different temperatures and for different periods of time could be used to facilitate the synchronous and rapid hatching of diapause eggs.

Materials and methods

Based on Chen and Marcus (1997), we chose two different temperatures, 15°C and 2 5°C, to stimulate the hatching of diapause eggs of *C. hamatus*. *C. hamatus* diapause eggs, that had been kept in 30 ml vials of deoxygenated seawater at 25°C in the dark for about eight months were used. The vials of eggs had been deoxygenated according to Lutz *et al.*, (1994). To initiate the present experiments the vials were opened and the contents were poured into 100 ml dishes so that the eggs could be transferred to polystyrene multi-well tissue culture plate containing GFF seawater. A micropipette was used to transfer the eggs. Ten sets of three replicates of 100 eggs or more were incubated for 10, 12, 14, 16, 20, 24, 28, 32, 36 and 40 hours at 15°C and 14h light:10 dark cycle. At the end of the respective incubation intervals the eggs were transferred to an incubator set at 25°C. A Control set of eggs with three replicates were incubated at a single temperature, 15°C. To determine the initial hatching day, daily hatch and cumulative hatch for each set, all of the eggs were checked once a day with a dissecting microscope until subsequent hatching would not occur anymore. During incubation, GFF water in the culture plate, if necessary, was added and hatched nauplii and dead eggs were removed.

Results and discussion

Hatching characteristics of the diapause eggs at 25°C after incubation at 15°C for different periods of time are summarized as follows: for controls, eggs began to hatch on the third day after starting incubation at 15°C. For the eggs incubated again at 25°C after being incubated at 15°C for 12 to 40 hrs, initial egg hatching occurred in one day after transfer from 15°C to 25°C while, for the eggs incubated at 15°C for less than 12 hrs, no hatching occurred.

Although there was a slight difference in day to 50% hatch between egg sources, each egg group reached 50% hatching success within 2 days and then stopped hatching in 4 days except for the controls which required 6 to 7 days. In addition, the greatest daily hatching success always occurred in 1 or 2 days after having been transferred from 15°C to 25°C while that in controls usually required 3 or 4 days.

Cumulative hatching success for the controls was relatively high, with average values ranging from 85.3% to 91.8%. This higher hatching success is comparable to that of Chen and Marcus (1997), who studied the cumulative hatching success of diapause eggs transferred to 15°C after incubation at 25°C

for 6 months.

On the other hand, the cumulative hatching success of diapause eggs (except for 10 hrs) incubated at 25°C after incubation at 15°C for different time ranged from 29.5% to 88.6%. However, the average cumulative hatching success of eggs incubated at 25°C after incubation at 15°C (less than 70%) was significantly lower than that of control (more than 85%) ($p=0.001$, t-test), but the hatching success of eggs incubated for 36 hrs to 40 hrs (more than 80%) was not significantly different from that of the control ($p=0.13$). And the cumulative hatching success for the eggs incubated at 25°C was affected by time periods during which the eggs were incubated at 15°C: higher hatching success was observed when a longer time of incubation at 15°C was applied.

In the northern Gulf of Mexico *C. hamatus* appears in the plankton as adults in late fall and disappears from the water column in the spring. It persists during the summer and early fall as diapause eggs in the bottom sediments (Marcus, 1989). Then when temperature falls below 20°C hatching occurs giving rise to a new population in the plankton. Chen and Marcus (1997) reported that the hatching of diapause eggs of *C. hamatus* is inhibited if eggs remain at 25°C (Chen and Marcus, 1997).

The present study supports these early findings that hatching is induced upon exposure to cooler temperatures. However, the present study also shows that exposure to cooler temperatures for a period of at least 12 hours is sufficient to stimulate the resumption of development such that development will continue if the eggs are returned to 25°C. Moreover, if the eggs are exposed to 15 oC for 36 to 40 hrs and then transferred to 25°C the cumulative hatching is comparable to the eggs kept at 15°C, but the time to achieve this level of hatching ranges from 60 to 64 hours vs 72 to 96 hours for eggs held continuously at 15°C. In this case high temperature accelerated development.

References

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