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Calanoid Copepod Eggs in Sea-Bottom Muds. III. Effects of Temperature, Salinity and Other Factors on the Hatching of Resting Eggs of *Tortanus forcipatus*

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Abstract

The "resting" eggs of a marine neritic copepod, *Tortanus forcipatus* Giesbrecht, recovered from sea-bottom sediment were hatched in the laboratory. Hatching occurred at temperatures of 13° to 30°C, no eggs hatched at 10°C. Temperatures around 25°C were found to be optimal for hatching, although the range of optimal temperatures for hatching was approximately 5C° lower in eggs stored for 14 to 15 months than in those stored for 1 to 2 months. A wide range of salinity, from 18 to 54‰S, was favourable for hachting. Eggs failed to hatch within the sediment mud, which suggests that they are in a state of dormancy in the mud. Hatching was successful under both light and dark conditions.

Introduction

Since the discovery of abundant numbers of "resting" eggs of marine copepods in the sediment of the sea bottom (Kasahara *et al.*, 1974), we have continued to study the distribution and ecology of these eggs in the neritic waters of the Inland Sea of Japan. The purpose of the study is to determine their role in the succession of copepod populations and to develop an effective cultivation technique for resting eggs.

This paper, comprising the third report, mainly deals with the effects of varying temperatures and salinities upon the hatching of resting eggs of *Tortanus forcipatus* in the laboratory.

Materials and Methods

The eggs used in the present study were isolated from sea-bottom mud collected on 19 November, 1971 and 25 December, 1972 at a station off Tomo Fisheries Laboratory, Hiroshima University, Fukuyama (Japan). The station and the methods of collection and isolation were the same as described previously (Kasahara *et al.*, 1974).

In *Tortanus forcipatus* eggs, 4 morphological types have been found in the mud; Type B, the disc of which is partially torn or folded, is the most numerous. Type B eggs were used throughout the present study. All the experiments were carried out during January and February, 1973. Damp bottom mud, collected in November, 1971 was put in hermetically closed polyethylene bags, and placed under the eaves of the laboratory for about 6 months, to the end of May, 1972 (1° to 19°C); it was then stored in an electric refrigerator at 2.4° to 4.0°C. The eggs isolated from this mud are termed Sample 1. Mud collected in December, 1972 was held at room temperature (11° to 16°C) in polyethylene bags. The eggs obtained from this mud are called Sample 2. Therefore, Samples 1 and 2 were kept under the above laboratory conditions for 14 to 15 and 1 to 2 months, respectively, before experimentation.

Small plastic vials of 20 ml capacity, containing 10 ml of membrane (0.45 µm)-filtered sea water were used throughout the hatching experiments. Hatching was inspected by pipetting all the sea water from each vial under a dissecting microscope; hatched nauplii were removed as soon as observed. More detailed procedures will be described in the following section.

Results

Effect of Temperature upon Hatching

Ten eggs were placed into each vial containing filtered sea water of 30.41‰S. The vials were then placed in constant-temperature water-baths ranging between 10° and 30°C (in a preliminary experiment, see below, the range used was 13° to 30°C) at intervals of about 5C°.

Preliminary Experiment (9-24 January, 1973).

In order to determine whether hatching could be induced by merely placing the eggs in sea water at any temperature, a preliminary experiment was performed using both Samples 1 and 2. The results are summarized in Table 1.

From Sample 1, the highest hatching rate of 90% was achieved at 20°C, followed by 80% at 25°C and 70% at 15°C, after an incubation period of 12 days. At the lowest temperature (13°C) half the eggs hatched, and at the highest temperature (30°C) only 30% hatched. All unhatched eggs at each temperature were subjected to 30°C after 12 days, and additional hatches of 2 eggs (20%) each were obtained 2 days later from those incubated previously at 15° and 13°C.

The eggs of Sample 2 behaved rather differently from those of Sample 1. After 14 days incubation, it became clear that hatching rate increases with increasing temperature. At 30° and 25°C, 100% hatching was achieved, followed by 70% at 20°C, 50% at 15°C and 30% at 13°C. After 14 days incubation at 13° to 20°C the unhatched eggs were also exposed to 30°C, and all hatched within 1 to 2 days.

Experiments I and II (27 January-13 February, 1973)

As it had been demonstrated that hatching could occur at each temperature tested in the preliminary experiment, the above experiments were repeated, checking hatching at daily intervals during the first 4 days and at intervals of 2 days or more, thereafter. The results are shown in Fig. 1. In Sample 1, at temperatures of 25° and 20°C, all eggs hatched within 2 to 6 days; at 15°C, 80% hatched within 11 days; and at 30°C, 70% within 4 days of incubation. No eggs hatched at 10°C throughout the experimental period of 17 days.

In Sample 2, the results were much the same as in the preliminary experiment (Fig. 1B). At high temperatures of 30° and 25°C, 100% of the eggs hatched within 2 and 4 days, respectively. At 20°C, 80% hatched within 8 days, followed by 20% at 15°C. Again, no egg hatched at the lowest temperature of 10°C.

These experiments showed that there seem to be slight differences in the temperature effect upon hatching between the two samples of eggs. The highest rates of hatching were obtained at 20° and 25°C in Sample 1 and at 25° and 30°C in Sample 2. In Sample 1, 70 to 80% hatched at 15°C, while in Sample 2 only 20 to 50% hatched at that temperature.

Effect of Salinity upon Hatching

Each vial contained 10 eggs, and sea water of different salinities ranging from 0 to 72‰. Normal sea water of 33.80‰ was condensed about 3 times on a rotary evaporator and filtered through a 0.45-µm membrane filter. To this was added distilled fresh water to obtain each desired concentration. All the experiments were run for 7 consecutive days at a constant temperature of 25°C.

Experiment I

Experiments were carried out at 6 different salinities ranging from 18.1 to 36.1‰. The experiment with Sample 1 was made between 18-26 January, and for Sample 2 between 17-25 January, 1973. As shown in Fig. 2A, hatching occurred at all salinity levels tested. In Sample 1, the hatching rate was high at higher salinity concentrations (28.9‰ or more), while in salinities lower than 25.3‰ the hatching rate was significantly lower (between 50 and 60%). In Sample 2, a high hatching rate of 80% or over occurred at all levels of salinity.

Experiment II

This time a wider range of salinity, between 0 and 90‰, was tested. The experiments with Samples 1 and 2 were carried out between 5-13 and 2-10 February, 1973, respectively. As shown in Fig. 2B, hatching occurred over a wide range of salinities (between 9 and 63‰) in both Samples 1 and 2. At salinities between 18 and 54‰, high hatching rates were attained: 60 to 80%

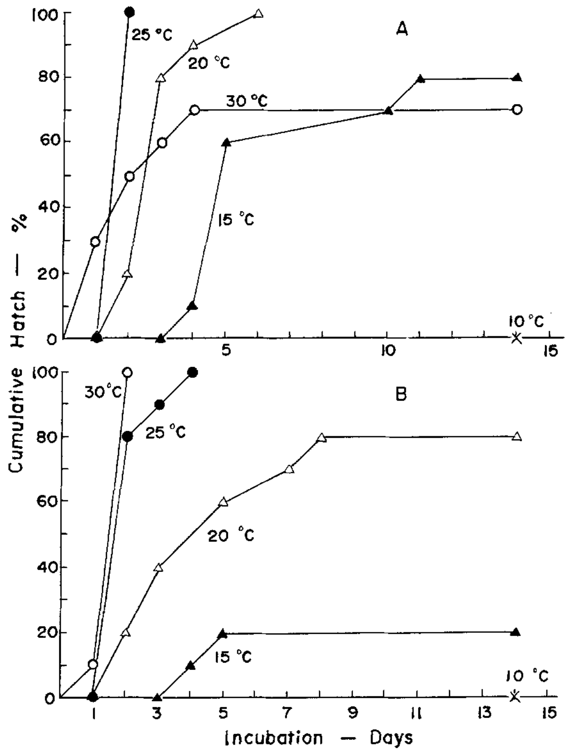

Fig. 1. *Tortanus forcipatus*. Hatching of resting eggs incubated at different temperatures. (A) Experiment I, Sample 1; (B) Experiment II, Sample 2

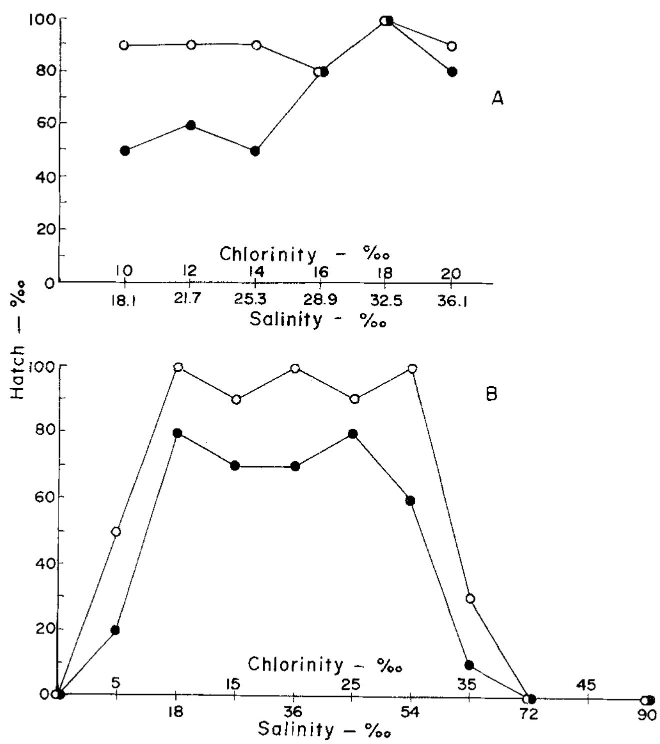

Fig. 2. *Tortanus forcipatus*. Hatching of resting eggs incubated at different salinities at 25°C. (A) Experiment I; (B) Experiment II. Filled circles: Sample 1; open circles: Sample 2

Table 1. *Tortanus forcipatus*. Hatching of resting eggs incubated at different temperatures; 10 eggs tested in each case

Temperature (°C)	Number of hatched eggs	
	Sample 1, 12 days incubation	Sample 2, 14 days incubation
30	3	10
25	8	10
20	9	7
15	7	5
13	5	3

Table 2. *Tortanus forcipatus*. Effect of sediment mud upon hatching of resting eggs incubated for 3 days at 25°C

Treatment of eggs	Percent hatched
Centrifuged and buried in mud	0
Settled on top of mud	80
Control, without mud; centrifuged	90
Control, without mud; untreated	90

in Sample 1 and 90 to 100% in Sample 2. No egg hatched in fresh water nor in salinities higher than 72‰. At all levels of salinity in which hatching occurred, Sample 2 exhibited a significantly higher rate of hatching than Sample 1.

Effect of Presence of Sediment Mud upon Hatching

The effect of sea-bottom mud upon hatching was examined on Sample 2 using a glass centrifugal tube of 10-ml capacity, containing 5 ml of membrane-filtered sea water of 30.41‰S. A small amount of the sea-bottom mud from which the eggs had been removed, was put into one tube, and centrifuged at 1000 revs/min for 5 min. Ten eggs were gently introduced into the tube with a fine capillary pipette, and allowed to settle on the top of the mud. Ten eggs were placed in a second tube and centrifuged. The mud was then added, and the tube centrifuged again so as to settle the mud on the eggs. Two tubes without mud were provided as controls; one with untreated eggs and the other with centrifuged eggs. All 4 tubes were incubated at 25°C for 3 days, and the newly hatched nauplii and unhatched eggs counted. The results are presented in Table 2.(See page 33)

None of the eggs buried in the mud hatched within 3 days of incubation, while 80% of those on top of the mud hatched. In both untreated and centrifuged controls without mud, a 90% hatch was obtained. All the unhatched eggs that had been buried in the mud hatched successfully within 3 days when incubated again in normal sea water. From this experiment it can be recognized that the eggs of *Tortanus forcipatus* are in a state of rest, even at an optimal temperature for hatching, when buried in mud.

The effect of light on hatching of the eggs was tested but no significant effect was noted, a 100% hatch being achieved both in complete darkness and in natural room light (maximum ca. 1000 lux) after 3 days of incubation at 25°C.

Discussion

The eggs used in the present study were first discovered among copepod eggs in the sea-bottom sediment in late autumn, 1971 and identified later as those of *Tortanus forcipatus* (Kasahara *et al.*, 1974). In the present study, the eggs in the mud were collected during periods when the planktonic population of this species disappeared from the waters of this area (Kasahara *et al.*, 1975), and then stored in polyethylene bags under laboratory conditions for several months. They therefore could be defined as so-called "resting" eggs.

The mechanism of resting of copepod eggs is interesting from both biological and ecological points of view. There have been several studies on resting eggs of fresh-water copepods (cf. Brewer, 1964; Cooley, 1971), but little was known about those of marine copepods until recently. Zillioux and Gonzalez (1972) have proved the existence of temperature-dependent dormancy in the eggs of *Acartia tonsa* which were shed singly and deposited rapidly on the bottom. These authors suggest that a winter "resting" egg may be reasonable for the spring resurgence of this species following its normal mid-winter disappearance from boreal waters of North America. For *Tortanus forcipatus*, the present study revealed that, at temperatures lower than 10°C, the eggs remain in resting state, or at least hatching is inhibited. The most favourable temperature for hatching was around 25°C, although in the present study the range of optimal temperature was approximately 5C° lower in Sample 1 than in Sample 2. Consequently, temperature is considered to be the most important factor affecting hatching of the resting eggs of this species. The "resting" state in eggs of this species may be essentially temperature-dependent dormancy as described by Zillioux and Gonzalez (1972) for *A. tonsa;* further studies, however, are necessary to determine if the eggs display a diapause, as described by Cooley (1971) for the resting eggs of *Diaptomus oregonensis*, a fresh-water copepod.

Unpublished data of ours show that lack of oxygen in water completely inhibits hatching of the resting eggs studied, as observed for resting eggs of the fresh-water calanoid *Epischura lacustris* by Main (1962). The inhibitory effect of mud on hatching (Table 2) is probably due in part to oxygen deficiency. This chemical factor, in addition to the above-mentioned temperature effects, would be closely related to the natural fluctuation in abundance of the eggs of this species in the sea bottom. The resting eggs hatched equally well when incubated in the light or in the dark. Thus, in this species, there is no effect of light stimulation such as described by Stross (1966) on diapause release in resting eggs of *Daphnia pulex*.

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