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With 4 figures in the text

Studies on the life-cycle of *Himasthla militaris* (*Trematoda: Echinostomatidae*): influence of salinity and temperature on egg development and miracidial emergence

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SUMMARY

In this study we examined the influence of salinity and temperature on egg development and miracidial emergence of the digenean, *Himasthla militaris*. In the range 2·1 to 34‰ salinity the eggs developed normally (i.e. no significant difference in the percentage embryonation), and miracidial emergence was only markedly inhibited in water with a salinity of 34‰. At 12 °C embryonation ceased; at 20 and 30 °C egg development as well as miracidial emergence occurred, but at 20 °C both processes progressed at a slower rate than at 30 °C.

INTRODUCTION

In the life-cycle of many digenean trematodes the eggs are only beginning to segment at the time of laying and the miracidia are not ready for hatching until a relatively long period of incubation in the aquatic environment has elapsed. The physico-chemical characteristics of this environment, such as salinity, pH, osmotic pressure, oxygen tension and temperature are important factors which control this process of development.

The experiments described in the present paper were carried out in order to study the influence of salinity and temperature on the embryonation of the eggs and the emergence of the miracidia of the echinostome trematode, *Himasthla militaris*.

Himasthla militaris is an intestinal parasite of some sea birds, such as the herring-gull (*Larus argentatus*), the black-headed gull (*Larus ridibundus*) and the curlew (*Numenius arquata*). The 1st and 2nd intermediate host are respectively the snail, *Hydrobia ventrosa* (Montagu, 1803) and the polychaete, *Nereis diversicolor* (O. F. Müller).

Similar work on other *Himasthla* species has been reported by Miller & Northup (1926), Stunkard (1938, 1960), Adams & Martin (1963) and Loos-Frank (1967).

MATERIALS AND METHODS

Definitive hosts/infection

Black-headed gulls and herring-gulls were captured alive on a dumping ground with the aid of a net. The birds were kept in an aviary and given parasite-free food for 6–8 weeks during which time trematodes acquired by natural infection were eliminated. Experimental infection was carried out by mixing several heavily *Himasthla militaris* metacercariae-infected polychaetes, *Nereis diversicolor*, into the food.

Gravid trematodes/eggs

Gravid *H. militaris* trematodes were obtained from the intestine of the experimentally infected gulls which were killed 2–3 weeks post-infection. The trematodes were placed in Petri dishes (5/dish) filled with brackish water (17 % salinity) and the eggs were isolated by dilaceration of the tissue with fine dissection needles. Afterwards, the remnants of the worms were removed and the eggs were stored in a refrigerator.

Experiments

The eggs were pipetted into 5 ml fingerbowls (20–25 eggs/bowl) and water was added; the water was replaced daily in order to supply the eggs with oxygen.

The development of the egg to the miracidium stage and the emergence of the miracidia were observed under the following conditions. (1) In seawater (34‰) and seawater dilutions (17, 8·5, 4·2 and 2·1‰), at 20 °C. (2) At 12, 20 and 30 °C, in water with a salinity of 17‰.

The seawater dilutions were made by dilution of pure seawater (34‰) with distilled water. The temperature was kept constant by using either a water bath with a thermostat and cool-element (12 and 20 °C) or an incubator (30 °C).

Egg development was considered complete as soon as a continuously moving miracidium with eye-spot could be observed within the egg-shell under the binocular microscope. Miracidial emergence was determined by counting and pipetting off the empty operculate shells at regular time intervals.

RESULTS

Influence of salinity on the embryonation of H. militaris *eggs and the emergence of miracidia*

H. militaris eggs were incubated at 20 °C and miracidial emergence was followed in water with a salinity of 2·1, 4·2, 8·5, 17 and 34‰ with 100, 100, 105, 110 and 105 eggs respectively. The results of this experiment are represented in Figs 1 and 2.

The salinity of the water did not influence the embryonation of the eggs and it was found that 76, 75, 87, 82 and 73% of the eggs developed to fully grown miracidia in water with a salinity of 2·1, 4·2, 8·5, 17 and 34‰ respectively.

In pure seawater (34‰) miracidial emergence was markedly inhibited (5% emergence) but in the range 2·1 to 17‰ emergence started at the 14–15th day of egg incubation and continued until the 65th day (90–95% emergence).

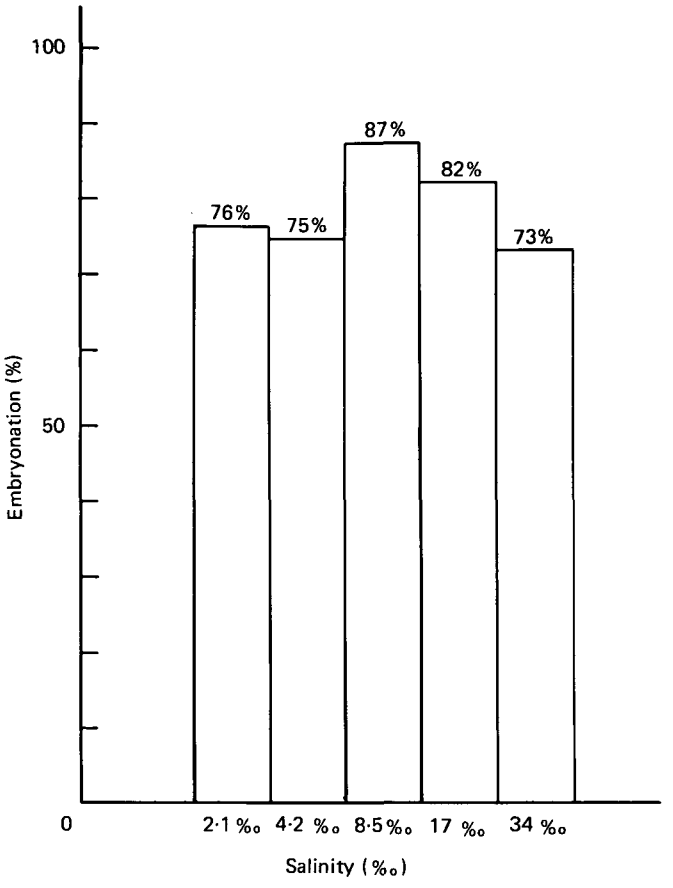

Fig. 1. Effect of salinity on egg embryonation in *Himasthla militaris*.

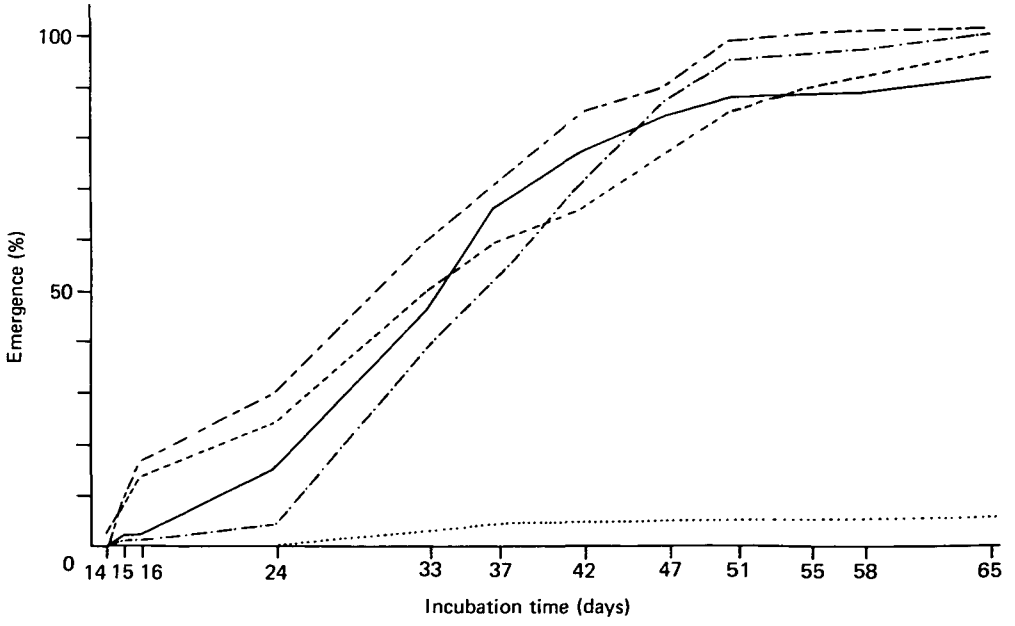

Fig. 2. Effect of salinity on miracidial emergence in *Himasthla militaris*. (····), 34‰; (– – –), 17‰; (— — —), 8·5‰; (——), 4·2‰; (—·—), 2·1 ‰ salinity.

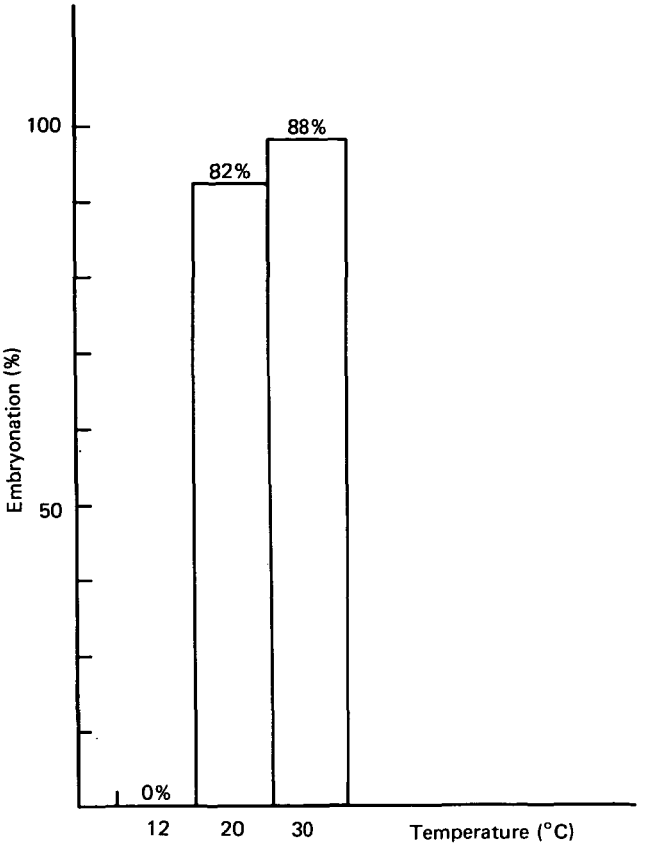

Fig. 3. Effect of temperature on egg embryonation in *Himasthla militaris*.

Influence of temperature on the embryonation of H. militaris *eggs and the emergence of miracidia*

H. militaris eggs were incubated in water with a salinity of 17‰ and miracidial emergence was followed at 12, 20 and 30 °C with 100, 110 and 190 eggs respectively. The results of this experiment are represented in Figs 3 and 4.

Egg development was inhibited at 12 °C; at 20 and 30 °C, 82 and 88 % of the eggs embryonated, respectively after 14 and 5 days.

No emergence occurred at 12 °C. At 20 °C, 90–95 % of the miracidia emerged after 65 days of egg incubation. However, after 11 days at 30 °C, the same percentage of larvae had already left the egg.

DISCUSSION

Previous work on the influence of abiotic factors on egg development and/or miracidial emergence for flukes belonging to the genus *Himasthla* is very scarce.

Adams & Martin (1963) followed the embryonation of *H. rhigedana* eggs and found that the developmental period was 17 days at room temperature. Miller &

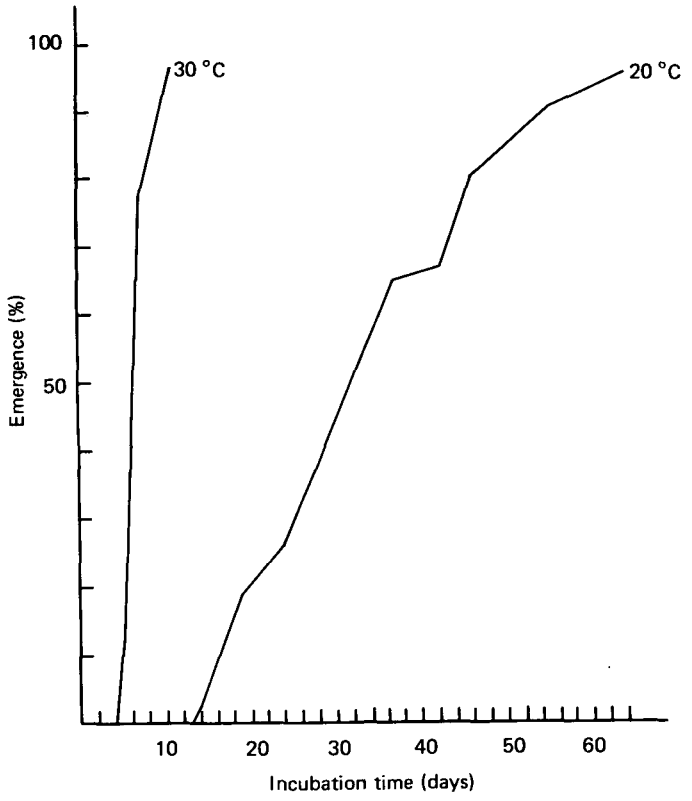

Fig. 4. Effect of temperature on miracidial emergence in *Himasthla militaris*.

Northup (1926) and Stunkard (1938) studied the life-cycle of *H. quissetensis*; the latter author wrote: 'the work was done during the summer ... and development of the eggs was so slow that hatching was not obtained before the end of the season', which is an indication for the very long embryonation-time for the species. Stunkard (1960) also examined some stages in the life-cycle of *H. compacta* and found that the miracidium needed 4 weeks to ripen. Loos-Frank (1967) showed that in pure seawater 65 % of the eggs of *H. continua* and *H. interrupta* developed within 12–15 days and in 25 % seawater only 10 % developed, whereas in freshwater the process was completely inhibited.

In this study we found that temperature and salinity were two important factors controlling egg embryonation as well as miracidial emergence of *H. militaris*.

At 20 and 30 °C the eggs embryonate normally (80–90 % embryonation), but at a significantly different rate (Q_{10} = 2·8), and the period of miracidial emergence is spread over respectively 65 and 11 days; at 12 °C both processes are inhibited.

In water with a salinity of 2·1, 4·2, 8·5, 17 and 34‰, 70–85 % of the eggs develop to fully grown miracidia, and within this salinity range (between 2 and 17‰) the miracidia emerge spontaneously while in pure seawater (34‰) the larvae apparently are prevented from leaving the egg.

Hydrobia ventrosa, the 1st intermediate host of *H. militaris* is also very tolerant with regard to salinity changes; the limiting values, cited in the literature, vary considerably according to the locality (Nicol, 1936; Muus, 1967; Fenchel, 1975; Hylleberg, 1975).

The euryhalinity of the miracidium, snail host and polychaete worm is undoubtedly a factor which enhances the chances of transmission of the parasite's larval stages.

Other extensive studies in this respect are mostly limited to flukes of economic importance. Rowcliffe & Ollerenshaw (1960) found that at 9·5 °C the development of the eggs of *Fasciola hepatica* was inhibited, while at 10 and 30 °C the process lasts, respectively, 161 and 8 days.

Styczynska (1964) examined the influence of salinity on this process; between 0 and 5‰ salinity the fasciolid eggs embryonated normally and miracidial emergence occurred, while in the range 5 to 19‰ hatching eggs only developed to undifferentiated miracidia and above 19‰ embryonation was inhibited.

Magath & Mathieson (1946) observed the emergence of *Schistosoma japonicum* miracidia; at 28 °C the larvae emerged spontaneously, at 4 and 37 °C the miracidia did not leave the eggs; miracidial emergence was also inhibited in a solution of 0·6 % NaCl, but when placed in 0·1 % NaCl the miracidia were liberated *en mass*.

Say (1972) studied the embryonation of *Paramphistomum daubneyi* eggs and the emergence of the miracidia; the development of the eggs lasted 10–25 days at 27 °C, the emergence of the miracidia started at the 11th day and on the 15th day 70–80 % of the larvae had emerged.

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