Effects of Water Temperature on the Egg-laying of the Monogenean *Neoheterobothrium hirame*

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ABSTRACT—The egg-laying rates of the monogenean *Neoheterobothrium hirame*, a parasite of Japanese flounder, were measured at different water temperatures. The daily egg-laying rates per worm were 203, 578, 781 and 651 eggs at 10, 15, 20 and 25°C, respectively. Thus the egg-laying rate increases progressively with temperature from 10 to 20°C, but no significant difference was detected between values at 20 and 25°C or between 15 and 25°C. While all the eggs obtained below 20°C appeared normal with straight appendages at both ends, 1.4% of the eggs obtained at 25°C appeared abnormal, suggesting that 25°C is at or near the upper limit for the normal egg-laying of this parasite.

Key words: *Neoheterobothrium hirame*, egg-laying, Monogenea, temperature effect, *Paralichthys olivaceus*

The monogenean parasite *Neoheterobothrium hirame* Ogawa, 1999 occurs on wild Japanese flounder (*Paralichthys olivaceus*) in Japanese coastal waters^{1–4}. This parasite infection causes anemia of the host fish^{5–9}, which may have a negative impact on wild flounder populations. However, knowledge of the parasite's biology is limited. To understand the ecology of the parasite and to evaluate its impact on wild flounder populations, we examined the egg-laying of *N. hirame* in relation to water temperature to determine its reproductive potential.

Materials and Methods

Wild Japanese flounder infected with *N. hirame*, were caught in Miyako Bay, Iwate Prefecture, and were used as the parasite egg source. They were transported to Kamiura Station of Japan Sea-Farming Association and maintained in a 500 L tank with constant water flow at 20°C. Eggs for the experimental infection of fishes were collected by filtering water draining from the tank containing infected fishes through nylon mesh with a mesh size of 50 µm. Previous tests had shown that all eggs were retained by mesh of this size.

For the experimental infection, 20 uninfected flounder (30±1 cm in total length) were placed together with about 1200 parasite eggs in a recirculating tank (500 L) equipped with a biological filter system. The fish were maintained in the tank at 20°C and fed dry pellets once a day for longer than 2 months. Some of the experimentally infected fish that were seemingly not anemic with normal gill color were used for measurement of the egg-laying rates of the parasite. Each fish was placed in a recirculating tank (25 L) at a different time post-infection and gradually acclimated over a 3 day period to the fixed experimental temperature (10, 15, 20 or 25°C). Subsequently, each acclimated fish was transferred to another 20 L tank, the water temperature of which was controlled in a water bath, and the water in the tank was changed every day for 3 days. From 1 week after the transfer, the water was filtered through 50 µm mesh every day for 3 days to measure the egg-laying rate; eggs trapped by the filter were collected and counted under a stereomicroscope. After the measurement, the fish was sacrificed to count infecting parasites and the daily egg-laying rate per worm was calculated. During the whole period after the transfer to the acclimation tank, the fish were kept without feeding. Two separate trials were conducted at each temperature.

Results

The mean daily egg-laying rates at different water temperatures are shown in Table 1. As no significant difference was detected between the two trials at any temperature, the data were combined for statistical analysis. As shown in the table, the egg-laying rates increased with increasing temperature from 10 to 20°C, whilst no significant difference was detected between egg-laying rates at 15 and 25°C or at 20 and 25°C. All the eggs obtained at or below 20°C appeared normal, with spindle-shaped capsules and straight appendages at both ends. Their dimensions, including the appendages, were 493–750 µm (mean 602 µm) in length and 50–70 µm (mean 60 µm) in width (n=50). However, 1.4% of the parasite eggs obtained at 25°C appeared abnormal, with deformed shells and appendages deformed, short or absent (Fig. 1).

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Table 1. Effect of water temperature on the egg-laying rate of *Neoheterobothrium hirame*

Water temperature (°C)	1st trial				2nd trial				Total	
	Days post infection	No. of worms	Egg-laying rate (eggs/worm/day)		Days post infection	No. of worms	Egg-laying rate (eggs/worm/day)		Egg-laying rate (eggs/worm/day)	
			Mean*	SD			Mean*	SD	Mean**	SD
10	78–80	1	185	40	108–110	3	221	83	203 ^a	52
15	88–90	1	561	99	118–120	3	593	71	577 ^{bc}	58
20	68–70	2	785	51	128–130	3	776	202	781 ^d	96
25	98–100	1	643	117	138–140	8	658	174	651 ^{cd}	109

* No significant difference was detected in the mean values between the 1st and 2nd trials at any temperature (Student's t-test, $P>0.05$).

** Values in the 1st and 2nd trials were combined for statistical analysis, because no difference was detected between the trials. Values with the same superscript were not significantly different (Scheffe's multiple comparison, $P>0.05$).

Fig. 1. The eggs of *Neoheterobothrium hirame* obtained at 25°C. A normal egg (a) and abnormally shaped eggs (b and c). Bar = 200 µm.

Discussion

The egg-laying rate of *N. hirame* is likely to be affected by the age of the parasite and the hematological condition of the host as well as by water temperature. Although the corresponding egg-laying rates at each temperature were measured at different times post-infection in the two trials, no significant difference was detected in the rates between the 1st and 2nd trials at any temperature, suggesting that the obtained values were for fully mature worms. Moreover, the measurements were carried out using non-anemic flounder i.e. with normal gill color, suggesting that all the worms had an adequate food supply. Therefore, the values obtained in this study probably represent natural egg-laying rates for the parasite.

Egg-laying rates increased with temperature between 10 and 20°C and all of those eggs appeared normal, with dimensions consistent with those previously reported by Yoshinaga *et al.*⁵⁾ However, there was no significant difference between egg output at 20 and 25°C

or between values at 15 and 25°C, and 1.4% of eggs produced at 25°C were abnormal. This indicates that 25°C is at or near the upper limit for normal egg-laying in this parasite.

There have been a few reports concerning egg-laying rates of monogeneans. The rates of *Entobdella solea*, *Benedenia seriola*, *Neobenedenia girellae*, *N. melleni*, all of which are capsalid monogeneans infecting the skin and fins of marine fishes, were 30–60, 648 (at 20°C), 293–850 (at 27–30°C) and 144–720 eggs per worm a day (e/w/d), respectively^{10–13)}. The rate of *Discocotyle sagittata*, which is a discocotyloid monogenean infecting the gill of rainbow trout (*Oncorhynchus mykiss*) was 12.2 e/w/d at 18°C¹⁴⁾. The rate of *Polystoma integrum*, which is a polystomatid monogenean infecting the urinary bladder of the African clawed toad (*Xenopus laevis*) was 2500 e/w/d¹⁵⁾. The maximum egg-laying rate of *N. hirame*, which infects the buccal cavity wall of Japanese flounder, demonstrated in the present study was 780 e/w/d at 20°C. Generally, the egg-laying rates of monogeneans are expected to be related to the biology or the survival strategy of each species. However, the rates greatly varied among species, and any relation was not found between the egg-laying rates and either of the habitats or classification. More data should be compiled to clarify the relation between egg-laying rates and the survival strategies of monogeneans.

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