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# Host Finding Behavior of Oncomiracidium of Monogenean Parasite *Entobdella hippoglossi* from the Atlantic Halibut

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The host finding behavior of *Entobdella hippoglossi* oncomiracidium, skin parasite of the Atlantic halibut was investigated. Almost of the parasite swam downward from the top to the bottom of the 150 cm glass tube within 30 minutes. The average swimming speed of oncomiracidium was $0.32 \pm 0.10$ cm/second throughout the experiment. When the parasites arrived on the bottom of the glass tube, they moved upwards and downwards continuously within the 10 cm of the bottom. This behavior would suggest that it may be one of the essential methods for host searching of the parasite. When the oncomiracidia was exposed different stimuli, they responded positively light and halibut mucus.

Key words: *Entobdella*, Oncomiracidium, Atlantic halibut, Behavior

## Introduction

Parasites must able to consistently reproduce themselves, and their offspring must find and infect a host. Great interest has been shown in the behavior of the miracidium and cercaria of larval digeneans. The behavioral patterns of miracidia, particularly their photo-, geo- and chemotactic responses, have been extensively studied (Erasmus, 1972). In comparison, relatively little is known about the behaviour of the oncomiracidia. Kearn (1967a) has investigated how the larvae of *E. soleae* find their flatfish host, *Solea solea*, using a chemical substances. Kearn (1980) also studied the response of *E. soleae* to light and gravity. However, information on the oncomiracidia of *E. hippoglossi* is lacking. Therefore, this study set out to investigate the host finding behaviour of the oncomiracidia of *E. hippoglossi* based on laboratory experiments.

## Materials and Methods

A glass tube, 150 cm in length and 1 cm in diameter was sealed at one end with paraffin tape. Sea water at 12℃ was used to fill the glass tube. Newly hatched (within 30 minutes) oncomiracidia which were actively moving were carefully collected by pipette from the hatching chamber under the microscope. An individual was carefully placed on the top surface of the water in the glass tube. All the oncomiracidia used were collected from same batch of the hatching chamber. The geotactic response and swimming speed of the oncomiracidium from the top of the tube to the bottom was checked and determined every 3 minutes under ambient light conditions (laboratory).

Observations on the swimming speed of the oncomiracidia were taken by observing their direction and speed against a black background with a hand lens. This allowed the larvae to be seen as white specks moving up or down through the glass tube. Movement appeared to be an active downward swimming behavior. After completely monitoring one individual parasite. the next parasite was checked using the same method. The experiment was repeated 20 times (n=20). Every experiment used a different parasite and water at the same temperature.

The chemotactic behavior of *E. hippoglossi* oncomiracidia was investigated using a two-armed-chamber based upon the design utilized by Bell (1995). The two-armed-chamber (Fig. 1) was used to quantify the chemotactic behavior of *E. hippoglossi* oncomiracidia in response to differing stimuli. The system was soaked in natural sea water for 10 days before being using for these experiments, to avoid any unexpected materials having an effect on the parasite. After one experiment, the chamber was washed and the control arm and test arm were swapped. Ten oncomiracidia were placed in the isolated central chamber. Following a 3 minute acclimation period, the closure ring was turned to the open position, thereby connecting the main chamber with the side arms. After 5 minutes, the closure ring was turned back to the closed position and the number of oncomiracia in the test and control arm was recorded. Three different experiments were tested as shown in Table 1. Every experiment was repeated 5 times. The results obtained from the experiments were statistically analyzed using Wilcoxons two-related-samples test for non-parametric data. Findings were considered significant at values of $P<0.05$.

## Results

Prior to carrying out the experiment, trials were conducted in the dark and under ambient light conditions (repeated 5 times). From these results it was found that the behavior of the oncomiracidia did not differ between light and dark. All parasite swam downward in the glass tube. Therefore, this experiment was carried under ambient laboratory illuminated conditions. When, however, parasites were first put onto the top of the 150 cm glass tube, the parasite stayed at the top of the water, spun around for about a minute and then swam actively for the bottom of the glass tube. After they had fixed their chosen direction, the parasites moved very actively as shown in Fig. 2. Eleven parasites (52.3%) swam directly almost to the bottom of the glass tube reaching their final destination within 8 minutes. Four parasites (19%) swam actively for approximately half of the 150 cm distance and then they reduced their speed arriving after around 11 minutes. Five parasites (23.8%) also swam actively but they often changed directions, upward then downward, and reached the bottom at over 13 minutes (13~27 minutes). One oncomiracidium (4.8 %) did not move at all over a 30 minute period.

The average swimming speed of oncomiracidia was $0.32 \pm 0.10$ cm/sec. ($3.1 \pm 1.8$ sec/cm) (Mean ± SD) throughout the experiments. The average swimming speed of parasites in the first 3 minutes as they headed downwards was $0.30 \pm 0.12$ cm/sec ($3.3 \pm 1.88$ sec/cm) and that of the parasites between 3 and 6 minutes was $0.32 \pm 0.12$ cm/sec ($3.1 \pm 1.92$ sec/cm). The average swimming speed of the parasites after 6 min. was $0.37 \pm 0.20$ cm/sec ($2.7 \pm 2.2$ sec/cm) (Fig. 2).

When the parasites arrived at the bottom of the glass tube, they moved upwards and downwards continuously within 10 cm of the bottom until they became moribund. A total of ten parasites were observed closely. The maximum vertical movement of oncomiracidia was 8 cm while the minimum movement was 1.5 cm. The average swimming distance of up and downward movement from the bottom was $5.2 \pm 1.9$ cm. The average swimming speed upwards was faster, $0.46 \pm 0.11$ cm/sec ($2.2 \pm 0.82$ sec/cm) than downward swimming speed, $0.37 \pm 0.20$ cm/sec ($2.7 \pm 2.2$ sec/cm) within the bottom 10 cm of the vessel. The response of the oncomiracidia of *E. hippoglossi* to the different stimuli is given in Table 1. A positive photo response was observed in oncomiracidia of *E. hippoglossi*. The responses of 50 larvae were recorded, 43 of them (86%) were photo-positive ($P<0.05$). Four larvae were found to be photo-negative and 5 others showed no clear cut directional

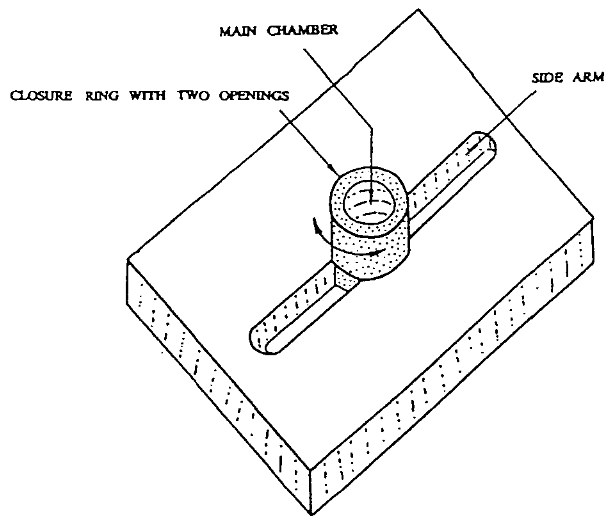


Fig. 1. Two - arm - chamber for qualification of the oncomiracidial host finding behavior (after Bell, 1995).

**Table 1. The responses of the oncomiracidia of *E. hippoglossi* to various stimuli**

| Test arm | Central reservoir | Control arm | Total |
|---|---|---|---|
| Light | | Dark | |
| 43 (86%) | 5 (10%) | 2 ( 4%) | 50 (100%) |
| Halibut mucus | | Dark | |
| 24 (48%) | 21 (42%) | 5 (10%) | 50 (100%) |
| Mucus | | Light | |
| 7 (14%) | 3 ( 6%) | 40 (80%) | 50 (100%) |

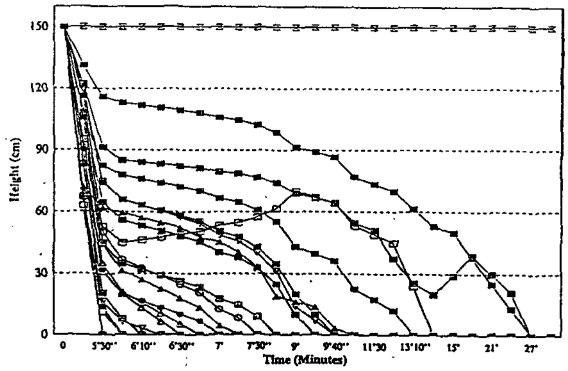


Fig. 2. Swimming pattern and geotactic response of newly hatched oncomiracidia.

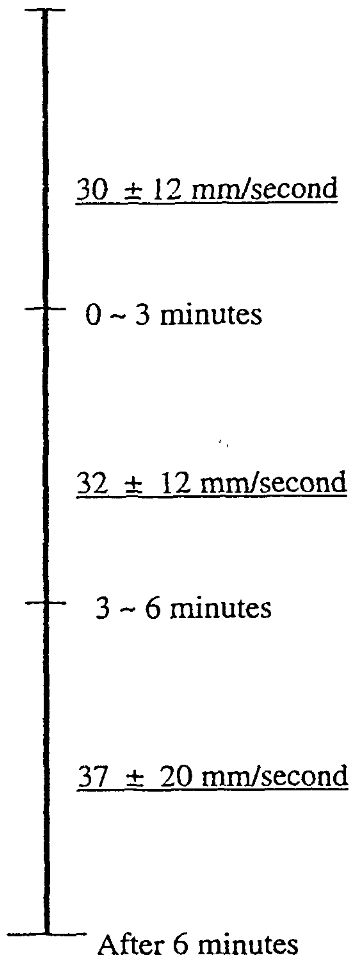


Fig. 3. Average swimming speed of oncomiracidia at different times after introduction to the 150 cm glass tube.

responses. In a second test, using halibut mucus taken from a mature halibut as a stimulus, the responses of 50 freshly hatched larvae were recorded. Twenty four of a total of 50 oncomiracidia (48%) responded positively to halibut mucus ($P<0.05$), while 21 of the larvae (42%) did not respond clearly and 10% of these showed a negative selection for halibut mucus. The last test was with mucus and light as different stimuli. Eighty percent of the parasites moved towards the light ($P<0.05$) and 14% of the parasites went towards the mucus side arm, while 6% of the parasites did not show a clear response to either mucus or light.

## Discussion

In order to survive, parasitic organisms must find a suitable host during their free swimming period. Therefore, the parasite has evolved very efficient host finding mechanisms. Due to its strong positively phototactic response (Wootten et al., 1982; Bron et al., 1993), the copepodids of the salmon louse, *Lepeophtherius salmonis* distributed near the surface of water during the day and spread out into deeper layers at night (Heuch et al., 1995). It seemed that they might have a better chance of locating and infecting a suitable host. In the present study, the newly hatched oncomiracidia of *E. hippoglossi* swam downward in a 150 cm glass tube with a speed of 3.2 mm/sec. Almost all of the parasites swam towards the bottom of the glass tube. It suggests that the oncomiracidia of *E. hippoglossi* have a very strong geotactic response. Comparison of the swimming speed during the downward travel showed that swimming in the first 3 minutes was slower than the remaining period. This may be for two reasons. First, when the oncomiracidia were put on the top of the glass tube, they stayed a short time on the top prior to selection of a direction. Second, the water pressure in the lower part of the vessel was greater than that in the higher part so that during the downward swim. Within 10 cm of the bottom of the glass tube, the oncomiracidia swam up and down continuously until they became moribund. The average swimming speed was 4.6 mm/second and 3.7 mm/second upward and downward, respectively. When comparing the swimming speed of the two phases, upward and downward, the swimming speed of the upward phase was surprisingly faster than that of the downward phase. It seemed that oncomiracidia could control their swimming speed. It might be an advantage for the downward swimming speed to be slower than the upward speed because, when they are searching for the host, they might have a longer searching time (staying time), conserving energy and thereby increasing longevity.

In the choice chamber, the oncomiracidium of *E. hippoglossi* were positively phototactic and positively chemotactic. However, it was found that the oncomiracidia were more attracted by light than by halibut mucus. This suggests that, when oncomiracidia are exposured to multiple stimuli, they respond to the strongest stimulus which, in this case, was light which may not be advantageous. On the other hand, it may be that the light used was too intense. There is danger, therefore, in extrapolating results from such *in vitro* tests to the natural environment.

Kearn (1967b) found that the oncomiracidium of *E. soleae* swam about 5 mm/second at 20C, however the larvae did not appear to rotate on their longitudinal body axis when swimming. Whittington and Kearn (1989) found that the oncomiracidium of *Plectanocotyle gurnardi* swam upwards at speeds of 3~4 mm/ second and the swimming speed between ascending and descending larvae was not different. Their finding was different from that of the larvae of *Diclidophora* spp which descended passively (Macdonald, 1974). In the present study, the larvae of *E. hippoglossi* swam more actively upwards but there was no evidence of the larvae were sinking passively.

Mackerel, *Scomber scombrus* gill parasites, *Kuhnia scombri* and *K. sprostonae* showed a strong photo positive response in 30 minutes after hatching but about 1-2 h after hatching, there was no evidence of a photo positive response. The larvae of both species continuously swam upwards and downwards (Whittington and Kearn, 1990). Age differences were not addressed in this study and all the test animals were the same age. Kearn (1980) found that most of the newly hatched oncomiracidia of *E. soleae* were photopositive. The larvae responded immediately, turning towards the light path after hatching, but 12 h after hatching most larvae showed a photonegative response. He suggested that the changing behaviour of the parasite larvae might relate to the capability of host finding by the oncomiracidium. In the present study it was found that the larvae of *E. hippoglossi* had not such a strong response to light as shown by the larvae of *E. soleae.*

The question is raised as to why the larvae were very strongly attracted by the light source when the larvae were horizontally exposed in the choice chamber, but why the same aged larvae did not follow the light source when the light was given in the 150 cm glass tube.

This may suggest that geotactic behavior has a stronger influence than phototactic behavior on the oncomiracidium when they were simultaneous. From these findings from the above experiments, an hypothesis might be made as to how the oncomiracidium of *E. hippoglossi* finds its host, the Atlantic halibut. First, newly hatched oncomiracidia keep swimming upward and downward within about 10 cm height from the sea bed until they find a host. Because the host rests on the sea bed, so the oncomiracidia does not need to swim higher. Secondly, when they find the host, at a very close distance, the larvae recognize their specific host using chemotactic cues which are released from the host skin.

However the host finding and behaviour of oncomiracidium is not only related to one cue or only one single factor in the environment. Therefore, more precise studies are needed for future research.

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