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Scanning Electronic Microscopy Examination for the Egg of Skin Parasite, *Entobdella hippoglossi* on the Commercially Important Culture Fish, the Atlantic Halibut, *Hippoglossus hippoglossi*

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The egg structure and shape of the skin parasite, *Entobdella hippoglossi* from the Atlantic halibut, *Hippoglossus hippoglossus* was examined using scanning electronic microscope. The newly produced eggs of *E. hippoglossi* were yellowish in color and tetrahedral in shape. The eggs were entwined together in a complicated chain-like fashion by a long filament. The appendage showed the presence of buoy-like structures. The buoy-like structures on the appendage of the eggs of *E. hippoglossi* in the present study were totally different from other sticky droplets. It is suggested that these structures are not sticky droplets but buoys for floating eggs which have entwined together on the bottom of the sea. It seems that these buoy-like structures may be needed for preventing the eggs from being covered by mud or particles and thus maintaining the eggs in an oxygenated environment.

Key words: *Entobdella hippoglossi*, Atlantic halibut, Skin parasite, Egg, SEM

Introduction

Monogeneans have a direct, single host life cycle and are usually well adapted to their fish hosts. The monogeneans often cause great losses, unlike most other parasitic platyhelminths, such as digeneans and cestodes. Whatever the cause or route of infection by monogeneans, health and growth of hosts are usually impaired by heavy infection (Thoney and Hargis, 1991). Infection by the monogenean skin parasite *Entobdella hippoglossi* appears to be a problem in the culture of the Atlantic halibut (Svendsen and Haug, 1991). However, due to the host's deep living behavior, research on *E. hippoglossi* is lacking.

Monogeans deserve special attention because they provide opportunity for easy observation of the egg assembly process and have variety of egg shapes and appendages. Usually, monogenean eggs have two main types of shape, one is the fusiform shape produced by polyopisthocotyleans and the other is the tetrahedral form produced by monopisthocotyleans. For instance, *Diclidophora luscae* had fusiform egg with hook-shaped appendages while *Entobdella soleae* were tetrahedral form with sticky material on the appendages (Kearn, 1986).

However, knowledge of the spawning behavior, egg production and the shape of *E. hippoglossi* have not been published yet. Therefore, the aim of the present study was to investigate the egg shape of *E. hippoglossi* on the Atlantic halibut from aquaculture systems.

Materials and Methods

The adults of *E. hippoglossi* were randomly harvested from the skin of Atlantic halibut which were originated from the nature and maintained in the broodstock tank for spawning. Individual parasites were maintained in plastic petri dishes measuring 5 cm in diameter by 1 cm in depth at 12 ℃. The water was changed daily using sterilised sea water (33 ppt) which was allowed to adjust to 12℃ before replacing the parasites. After 24 hours, eggs which had been released were counted using an Olympus binocular microscope (x 4). Eggs of *E. hippoglossi* were collected from egg laying chambers where they were held at 12℃.

The eggs were fixed in 1% glutaraldehyde at 4℃ for 3 hours. They were then transferred into 3% glutaraldehyde at 4℃ for 7 days. The egg material was then washed for 1 hour in 0.2 M cacodylate

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buffer, post - fixed in 1% buffered osmium tetraoxide for 2 hours at room temperature and rewashed in cacodylate buffer for an hour. The eggs were dehydrated through an ethanol series, where specimens were immersed in each concentration of ethanol (30%, 60%, and 90%) for 30 minutes, which was then followed by immersion in absolute ethanol for 1 hour (twice). The specimens were transferred directly to HMDS (Hexamethyldisilazane) for 5 minutes as an alternative to critical point drying. Specimens were left at room temperature to air dry and then mounted on aluminum stubs and sputter coated with gold palladium in a Polaron Edwards S150 B sputter - coater before SEM examination. The specimens were examined using a Philips scanning field emission microscope at an accelerating voltage of 12 kV.

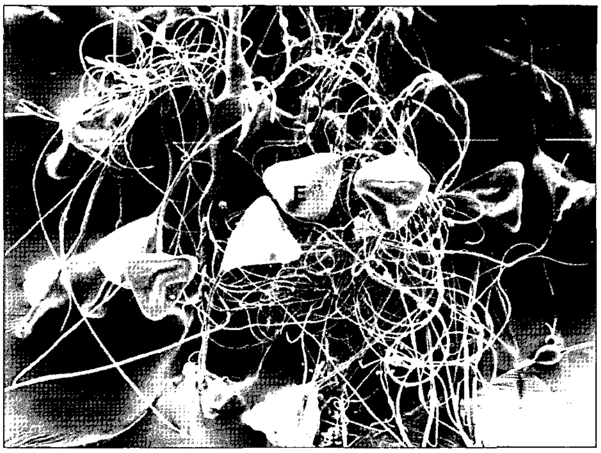

Fig. 1. Scanning electron micrograph showing the eggs entwined together by their long appendages. (E: egg, A: appendage). (Scale bar=100 μm).

Results

The newly produced eggs of *E. hippoglossi* were yellowish in colour and tetrahedral in shape. The edges of each side of the tetrahedron measured 175.3 ± 9.2 μm (n=40) in size. The eggs were entwined together in a complicated chain-like fashion by a long filament or appendage as shown in Fig. 1. The appendage attached to one corner of the egg (proximal apex) and entwined at its free end within the egg bundle. Closer examination of the appendage showed the presence of buoy-like structures as shown in Fig. 2. These structures were not a regular size and were not spaced regularly. Individual eggs had an operculum formed at an apex opposing that bearing the appendage (distal apex) (Fig. 3). Fig. 4 shows the operculum on one apex of the egg and that the egg surface is pitted. The function of the operculum was clearly evident, operating as a hatching gate for the emerging oncomiracidium (Fig. 5). The empty egg with the operculum removed following hatching by the oncomiracidium is shown in Fig. 6.

Fig. 2. Scanning electron micrograph showing the buoy-like structures on the appendages of eggs. Note these structures were not a regular size and were not spaced regularly. (A: appendage, B: buoy like structure). (Scale bar=10 μm).

Discussion

Kearn (1986) observed that the tetrahedral shape of the eggs of *E. soleae* was due to the shape of the ootype chamber in which they are made. It seems likely that those of *E. hippoglossi* are moulded in the same way. The long chains of eggs produced by certain hexabothriids, in which eggs are joined together by fusion of the opercular and abopercular appendages, may become entangled on the sea bottom. According to Guberlet (1933), chains of between 10 and 65 eggs were produced by *Squalonchocotyle catenulata* and egg chains were observed by Dillon and Hargis (1968) in *Erpocotyle callorhynchi*. Similar egg chains have been reported from *Microcotyle caudata*. Bovet (1959) observed that the coiled appendage of *Diplozoon paradoxum* during laying eggs and became entangled with the appendages of other eggs so that most of the eggs were held off the bottom. He suggested that this

Fig. 3. Scanning electron micrograph showing an egg has an operculum forming an apex opposing that of the one bearing the appendage (distal apex) (A: appendage, O: operculum). (Scale bar=10 μm).

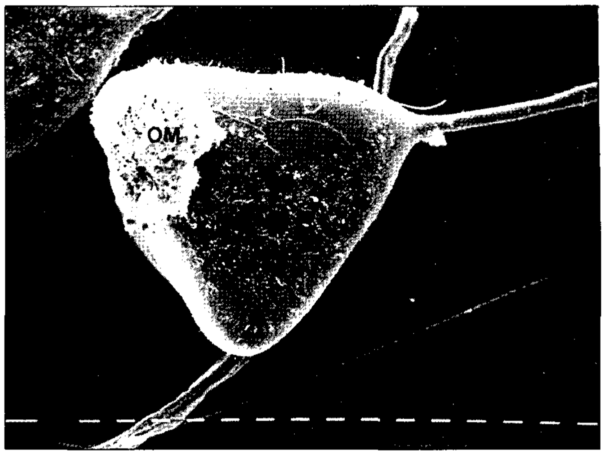

Fig. 5. Scanning electron micrograph showing the function of the operculum. Note the oncomiracidium emerging through the operculum as a hatching gate. (E: egg, OM: oncomiracidium). (Scale bar=10 μm).

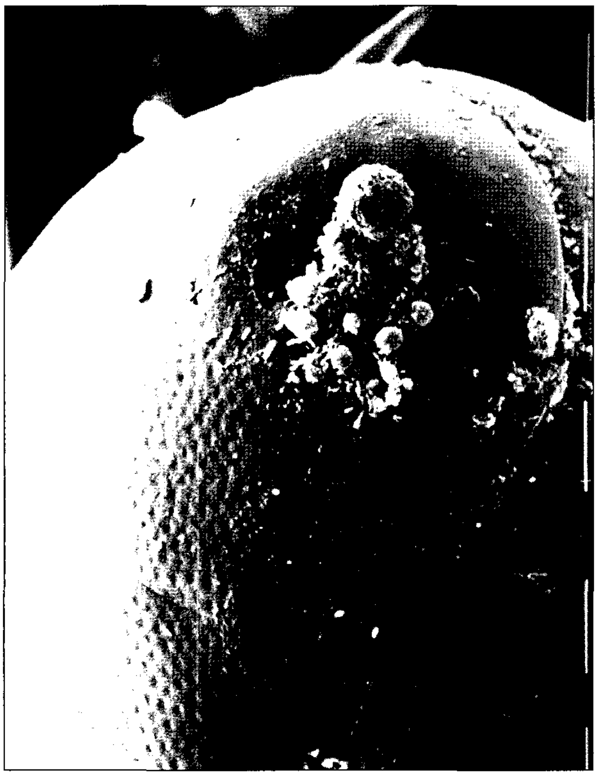

Fig. 4. Closer examination of scanning electron micrograph showing the operculum and the pitted surface of the egg. (Scale bar=10 μm).

Fig. 6. Scanning electron micrograph showing the empty eggs after hatching. (Scale bar=10 μm).

had an advantage: a high proportion of eggs in such tangled heaps develop normally compared with eggs without appendages which rest on the bottom and become rapidly covered with micro-organisms or detritus. His suggestion might be applicable to *E. hippoglossi* eggs.

In general, the egg of *E. hippoglossi* is similar to that of *E. soleae* in shape and size. Also it was found that *E. hippoglossi* has a tanned eggshell as does *E. soleae*.

In the present study, the eggs of *E. hippoglossi* were shown to possess a long appendage on a corner of the egg. The appendages carried irregular sized, buoy-like structures. The eggs of the skin parasite *E. soleae, Acanthocotyle lobianchi* (Kearn, 1967) and *Entobdella australis* (Kearn, 1978) have appendages with sticky droplets, and *Calicotyle kröyeri* has this structure on the surface of eggs (Kearn, 1970). All of these parasites are found on bottom living, marine teleost or elasmobranch flat-fishes, and sticky material may be of great importance for such parasites since attached sand particles would prevent the eggs being carried upwards. All these structures are sticky, flat and fitted to adhesive on the bottom of the sea bed. However, the buoy-like structures on the appendage of the eggs of *E. hippoglossi* in the present study are totally different from other sticky droplets. They were not sticky or fitted for attaching some of bottom materials. It is suggested that these structures are not sticky droplets but buoys for floating eggs which have entwined together on the bottom of the sea. Therefore the structures found on the appendage of the eggs in the present study, may be explained as follows. When the eggs are expelled from parasites then they entwine together on the bottom of the deep sea. The deep sea, where Atlantic halibut spawning, is very calm and there are no strong currents, so the sticky droplet is not needed but some buoy-like structures are needed for preventing the eggs from being covered by mud or particles and thus maintaining the eggs in an oxygenated environment. However, more studies will be needed for proving the role of these buoy-like structures for egg of *E. hippoglossi.*

References

Bovet, J. 1959. Observations sur l'oeuf et l'oncomiracidium de *Diplozoon paradoxum* von Nordmann, 1832. Bulletin de la Société Neuchâteloise des Sciences Naturelles, 82, 231~245.

Dillion, W. A. and W. J. Hargis. 1968. Monogenetic trematodes from the southern Pacific Ocean. Part IV. Polyopisthocotylids from New Zealand fishes: the families Mazocraeidae, Diclidophoridae and Hexabothriidae. Proceedings of the Biological Society of Washington, 81, 351~366.

Guberlet, J. E. 1933. Notes on some Onchocotylinae from Naples with a description of a new species. Pubblicazioni della Stazione zoologica di Napoli, 12, 323~336.

Kearn, G. C. 1967. The life-cycles and larval development of some acanthocotylid (Monogenea) from Plymouth rays. Parasitology, 57, 157~167

Kearn, G. C. 1970. The oncomiracidia of the monocotylid monogeneans *Dictyocotyle coeliaca* and *Calicotyle kröyeri.* Parasitology, 61, 153~160.

Kearn G. C. 1978. *Entobdella australis,* sp.nov., a skin-parasitic monogenean from the Queensland stingrays, *Taeniura lymma* and *Amphotistius kuhlii.* Australian Journal of Zoology, 26, 207~214.

Kearn, G. C. 1986. The Eggs of Monogeneans. Advances in Parasitology, 25, 175~262.

Svendsen, Y. S. and T. Haug. 1991. Effectiveness of formalin, benzocaine and hypo and hyper saline exposures against adults and eggs of *Entobdella hippoglossi* (Müller), an ectoparasite on Atlantic halibut (*Hippoglossus hippoglossus* L.). Laboratory studies. Aquaculture, 94, 279~289.

Thoney, D. A. and W. J. J. R. Hargis. 1991. Monogenea (Plathyhelminthes) as hazards for fish in confinement. Annual Review of Fish Diseases, 1, 133 ~153.