Studies on the monogenean, *Entobdella hippoglossi* Müller, 1776, parasitising a commercially important cultured fish, the Atlantic halibut, *Hippoglossus hippoglossus* Linnaeus, 1758

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BY

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DECLARATION

I hereby declare that this thesis has been composed by myself and is the result of my own investigation. It has neither been accepted, nor submitted for any other degrees. All the sources of information have been duly acknowledged.

GIL HA YOON

To My Mum with Love

ABSTRACT

The skin monogenean parasite, *Entobdella hippoglossi* Müller 1776 (family Capsalidae) has been found to commonly occur on broodstock halibut during the development of the culture of Atlantic halibut, *Hippoglossus hippoglossus* L., 1758. Due to the lack of information relating to its host's ecology and recent establishment of the halibut farming industry, research on *E. hippoglossi* is lacking. This study, therefore, was carried out to investigate the biological and pathological aspects of *E. hippoglossi*, on the skin of Atlantic halibut under culture conditions.

A comparison of the parasite populations from two different sites, Machrihanish and Ardtoe, showed that the parasite burden from Machrihanish was twice that of the parasite population on Ardtoe halibut (641 ± 233.3 and 307 ± 276.6, respectively). The mean length of parasites, however, from the Ardtoe halibut was longer than those collected from the Machrihanish halibut (10.6 ± 3.3 mm and 6.0 ± 2.4 mm, respectively) ($P< 0.05$). The mean intensity of the parasite from female halibut was 605 ± 244.7, while from male hosts it was 231 ± 226.6 ($P< 0.05$). Also the mean length of parasites from female fish (8.4 ± 3.6 mm) was significantly longer than from male hosts (7.9 ± 3.8 mm) ($P<0.05$). While the parasite burden from the dorsal and ventral surfaces was almost the same, there was a significant difference in the mean length of parasites. The mean length of the parasites from the ventral surface was 9.6 ± 3.7 mm while from the dorsal surface it was 6.9 ± 3.1 mm ($P<0.05$).

A comparison of the mean parasite length in four principal zones (front dorsal, middle dorsal, rear dorsal and ventral) on the fish revealed that the parasites from the ventral surface were significantly longer than those from the other zones (ventral: 9.7 ± 3.7 mm; front dorsal: 8.1 ± 3.3 mm; middle dorsal: 5.7 ± 1.9 mm and rear dorsal: 4.9 ± 1.8 mm) ($P<0.05$). On the dorsal surface, the mean parasite length from the front zone was significantly bigger than those from the middle and the rear zones ($P<0.05$).

The dominant length class of *E. hippoglossi* found from Atlantic halibut maintained at Machrihanish was 3-5 mm (30.9 %), whilst only 6.7 % of the parasites in the same size class were found on the fish at Ardtoe. In comparison, the majority of parasites in the Ardtoe population (26.1 %) measured from 11-13 mm. The same size class in the Machrihanish population was 3.6 %. The smallest size class (less than 3 mm) composed 0.98 % of the Ardtoe population whilst 6.6 % of the population were in this size class for Machrihanish halibut. The 5-7 mm group represented the dominant size class of parasites collected from the dorsal surface (27.7 %) whilst the majority of the parasites from the ventral surface belonged to the size class, 11-13 mm (20.9 %).

The eggs of *E. hippoglossi* are discharged from the uterus by a very powerful contraction of the anterior part of the parasite. Three kinds of egg laying methods were observed. In the first type the eggs were laid in a chain–like manner which grouped to form an egg bundle. The second style was that eggs were laid attached to an egg ball. From one to hundreds of eggs attached to the egg ball which was then anchored on the bottom of the experiment vessel. The third method found was that some parasites laid

eggs singly. The singly laid eggs were found to be sterile and did not develop any further.

The egg laying rates of parasites on male and female hosts were compared. There was no significant difference between the mean number of eggs produced by parasites from male halibut (26.9 ± 10.4 eggs) and eggs produced by parasites from female halibut (35.2 ± 9.9 eggs). There was however, a significant difference between the mean number of eggs produced by parasites collected from the dorsal (8.9 ± 2.8 eggs) and the ventral surface (53.2 ± 13.2 eggs). Newly laid eggs of *E. hippoglossi* were yellowish in colouration and tetrahedral in shape. The egg surface was pitted. Each side of the tetrahedron measured about 200 μm in length. An egg filament is attached to the proximal apex of the egg and is entwined at its free end within the egg bundle. The egg filament possesses buoy-like structures. These may contribute to eggs floating from the sea bed. The shape and size of these structures is totally different to the sticky droplets found from eggs of *E. soleae*. The operculum at one apex of the egg operates as a hatching gate for the emergent oncomiracidium.

The larvae began to hatch naturally, without any hatching stimuli during illuminated or dark conditions, after 27-30 days incubation at 12°C. The anterior region emerged first through the operculum of the egg, while the posterior region was captured by the gap between the egg and operculum. Whether the oncomiracidia were trapped or they simply paused there for a certain purpose is not clear. Once they were released from the gap, they actively and freely swam. The larvae had 3 zones of cilia corresponding to the anterior, the middle and the posterior regions of the body. The oncomiracidium had 4

eye-spots with pigmented cups and lenses. The haptor bore 3 pairs of medianly situated sclerites, the anterior hamuli, the posterior hamuli and the accessory sclerites, and fourteen marginally situated sclerites, the marginal hooklets.

The oncomiracidium of *E. hippoglossi* has a total of 64 epidermal ciliary plates, comprising 27 cells on the anterior region, 20 cells on the middle region and 17 cells on the posterior region. In comparison with those from the oncomiracidium of *E. soleae*, the anterior and the middle regions were the same in number but *E. soleae* had only 13 ciliary cells on the posterior region.

When the oncomiracidia were placed on the top of a 150 cm glass tube filled with sea water, they actively swam downwards. Of 20 parasites, 11 (52.3 %) reached their final destination within 8 minutes, four parasites (19 %) arrived around 11 minutes. Five parasites (23.8 %) swam actively but they changed their direction often before they reached the bottom (13-27 minutes), while one parasite did not move at all in a 30 minute period.

The average swimming speed of the oncomiracidium was 0.32 ± 0.1 cm/second (3.1 ± 1.8 sec/cm) throughout the experiments. The average swimming speed of parasites in the first 3 minutes as they headed downwards was 0.30 ± 0.12 cm/sec (3.3 ± 1.88 sec/cm) and that of the parasites after 3 to 6 minutes was 0.32 ± 0.12 cm/sec (3.1 ± 1.92 sec/cm). The average swimming speed of the parasites after 6 minutes was 0.37 ± 0.20 cm/sec (2.7 ± 2.2 sec/cm). The oncomiracidium showed an upward and downward movement continuously within 10 cm of the bottom of the experimental vessel, continuing until they became moribund. This behaviour seems to have a very important role in searching for its flat fish host. 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 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 out 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. When a test was carried out dividing 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.

Experimental infection of juvenile halibut with adult parasites was carried out. Almost all of the parasites were found to move to the head region of the ventral surface, whether they were placed on the dorsal surface or on the ventral surface. Some parasites settled there for at least 8 weeks whilst other parasites seemed to detach from the juvenile halibut. When the experiment was terminated, no oncomiracidia or juveniles were found on the juvenile halibut, although 8 weeks at 12°C was enough time to for this parasite to hatch.

Skin haemorrhages were found on experimentally infected juvenile halibut. A mass of sloughed necrotic epithelial cells mixed with mucus and debris was found on the surface of infected tissue. In the infected tissue, the mucous cells were irregularly

distributed in the epidermis. An SEM study revealed the sucking action of the opisthaptor, indentation by papillae of the parasite and lesions caused by the accessory sclerites on the juvenile halibut skin.

It seems that the papillae might act as grips in attachment of the parasite. The arrangement of papillae showed quite a regular distribution on the ventral part of the haptor. The accessory sclerites of *E. hippoglossi* are quite different from those of *E. soleae*. The accessory sclerites of *E. hippoglossi* have a very sharp end compared to the smooth curved ending in *E. soleae*. Because of their deep penetration they may act as hooks rather than just acting as props as has been suggested for *E. soleae*.

Generally, the mucous cell size, epidermal thickness and mucous cell number of the front region of mature halibut skin, whether on the dorsal or ventral surface, were all greater than those of the other regions. On juvenile halibut, however, the mucous cell sizes from the rear and the middle regions were significantly bigger than those from the front region of the dorsal surface. The distribution and concentration of mucous cells on the halibut skin were clearly positively correlated with the parasite population on the halibut host.

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Chapter 1

GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

1.1 The Atlantic halibut, *Hippoglossus hippoglossus* L., 1758

1.1.1 Identification

The genus *Hippoglossus* is the largest of the flatfishes. The genus *Hippoglossus* belongs to the subfamily Pleuronectinae within the family Pleuronectidae. Listed below are synonyms of *H. hippoglossus*,

***Pleuronectes hippoglossus* L., 1758**

***Hippoglossus vulgaris* Flemming, 1828**

***Hippoglossus hippoglossus* Jordan and Everman, 1898**

***Hippoglossus americanus* Gill, 1864**

Andriyashev (1954) described the genus *Hippoglossus* as follows:
"Body elongate, covered with small cycloid scales and smaller supplementary scales. Lateral line with a steep bend above pectoral fin. Both eyes on the right side of head. Jaws large, symmetrical with large pointed teeth directed posteriorly; teeth on upper jaw in two rows and on lower jaw in one row. Vomer toothless. Infrapharyngeal teeth sharp, in two rows; the inner series with enlarged teeth. Anal spine present (in adult specimens overgrown by skin). Pectoral fin better developed on eye side. Caudal fin weakly emarginate. Vertebrae 49-53".

Usually Atlantic halibut is white in colouration on the blind – side and the eyed - side is grey brown. Haug and Fevolden (1986) gave the relative proportions of the

organs in relation to the body length as follows: head length 23.1-23.6 %; dorsal fin base 73.3-74.5 %; upper jaw 7.0-7.1 %; lower jaw 6.5-6.6 %. The number of fin rays are 91-109 in the dorsal fin, 64-80 in the anal fin, 11-17 in the pectoral fins and 5-6 in the pelvic fins.

1.1.2 Distribution

The Atlantic halibut can be found in the North Atlantic Ocean and the Barents Sea. It is particularly common along the Norwegian coast, off the Faeroes, all around Iceland and off the southern coast of Greenland. It may also be found in the North Sea and the Western Baltic Sea (Andriyashev, 1954).

Halibut of different ages occupy different habitats. Immature halibut appear in coastal areas at depths of 20-60 m, regions that represent nurseries for their first 4-6 years (Kohler, 1964; Haug and Sundby, 1987; Godø and Haug, 1988; Stobo, Neilson and Simpson, 1988). Larger halibut, which usually live at depths down to 2000 m, seem to migrate to coastal waters during the spring but move back to deeper waters in the autumn and early winter. In the winter, the sexually mature halibut gather together on deepwater spawning grounds and spawning occurs at great depths of between 100-700 m during December to April (Riis-Vestergaard, 1982). Out of the spawning season, the halibut leave the spawning grounds and may then be found in deep as well as shallow waters and in inshore as well as offshore waters (Haug and Tjemsland, 1986; Jakupsstovu and Haug, 1988).

1.1.3 Life – cycle of Halibut

Halibut spawning in the northern – most waters of Norway peaked at the end of January and beginning of February (Kjørsvik, Haug and Tjemsland, 1987; Haug and Gulliksen, 1988). Halibut spawn over soft clay or mud bottoms at depths of 300 – 700 m in Norwegian coastal waters (Kjørsvik *et al.*, 1987). During the spawning period, the temperature and salinity are between 5-7 °C and 34.5 – 34.9 ppt, respectively on the bottom of the spawning area (Kjørsvik *et al.*, 1987).

After spawning, the fertilized eggs move upwards. The halibut egg is among the largest planktonic fish eggs, the diameter of eggs being between 3.06 and 3.49 mm (Haug, Kjørsvik and Solemdal, 1984; Jakupsstovu and Haug, 1989). The incubation time is strongly dependent on temperature. Lønning, Kjørsvik, Haug and Gulliksen (1982) found that hatching occurred after 18 days at 5°C and Blaxter, Danielssen, Moksness, and Øiestad (1983) reported that hatching of 50 % of the eggs took 20, 18 and 13 days at 4.7, 5 and 7°C respectively.

The newly hatched larva is about 6-7 mm in length (Blaxter *et al.*, 1983). The larva has a very large yolk sac, functional eyes and a pigmented body. The yolk is absorbed around 50 days post-hatching, when the larvae are 11.5 – 13.0 mm long (Blaxter *et al.*, 1983; Pittman, Berg and Naas, 1987; Bjørnsson and Tryggvadottir, 1996).

The gills of the halibut appear to be fully developed around 60 days after hatching. The left eye starts migrating at about 80 days at a length of 16.3 mm and by 130 days the eye has migrated halfway around the head when larvae are 29.5 mm in length (Pittman *et al.*, 1987).

Berg and Øiestad (1986) observed that on day 75 post-hatching, most larvae were in an early stage of metamorphosis and most of them were able to rest on the bottom on day 90. The settling on the bottom occurs before the final completion of pigmentation, eye movement and formation of the fins. The transformation to real flatfish seems to take place on the bottom when fish are between 34 mm and 47 mm in length (Vedel-Tåning, 1936).

Juvenile halibut stay in nursery grounds around coastal areas that are 20 – 60 m deep with a sandy bottom. The temperature of the nursery ground is 2.5 – 8°C (Haug and Sundby, 1987; Stobo *et al.*, 1988; Godø and Haug, 1988). Although their feeding stops at around 2°C, they can survive in water below freezing (Goff and Lall, 1989). Halibut emigrate from nursery areas when the fish are 3-4 years old (Haug and Sundby, 1987; Godø and Haug, 1988). Male and female halibut reach sexual maturity at different ages and lengths. In males, they appear on spawning grounds at an age of 4-5 years, and at a length of 60 - 70 cm, while females become mature at an age of 7 years, at mainly over 100 cm in length (Jakupsstovu and Haug, 1988).

1.2 Halibut culture

Halibut has always been an attractive species for European fishermen and North American fishermen because of its excellent taste. According to Nickerson (1978), about 4500 tonnes of halibut were captured annually from the northwest Atlantic Ocean in the middle 1880's and between 3000 and 6000-9000 tonnes in the 1920's and 1930's, respectively. However, the total world catch of the Atlantic

halibut has been steadily declining from yearly catches of 10,000 tonnes in the years before 1970 to less than 4000 tonnes/year in recent years (Haug, 1990).

The Atlantic halibut fishery has been traditionally important for the UK, Norway, Iceland and North America. Since the world catch has been declining and the market price remains high, interest in the farming of Atlantic halibut has increased.

There has been considerable interest in the farming of Atlantic halibut due to its relatively fast growth rate and high market price (Bolla and Holmefjord, 1988; Kjørsvik and Holmefjord, 1995). Initial interest in the early stages of halibut hatchery experimentation began at the Trondheim Biological Station in Norway, by Rollefsen, in 1934. He succeeded in keeping the larvae alive for ten days post hatching (Finn, Fyhn and Evjen, 1991). After that, no hatching experiments with halibut were conducted until the 1970's, when interest in halibut farming rose again. Since 1985, the interest in halibut farming has increased dramatically and considerable work has been carried out, mostly focusing on the early life stages of the halibut. At the present time, experiments with halibut farming are carried out in Norway, Scotland, Iceland, Canada and the Faeroe Islands (Bjørnsson, 1995). Commercial halibut farming has been undertaken in Norway with thousands of fry being produced every year since 1988 (Harboe, Huse and Öie, 1994; Lein, 1996). Production in Norway has increased rapidly from 30,000 juveniles in 1992 to 350,000 in 1994. Within the United Kingdom, research has been undertaken since 1983 at the Marine Farming Unit (MFU) of Seafish Aquaculture and commercial interest is increasing with companies such as Otter Ferry based at Tighnabruaich in Scotland (Holmyard, 1996). According to Shields (1998, Personal communication), there are some problems of production of halibut fry due to lack

of knowledge of the early life history of halibut in the wild, especially the provision of proper nutrition for early larvae and the occurrence of some known and unknown diseases of larval stages of halibut. Despite those problems, Atlantic halibut culture production has been increasing with improved cultivation techniques. In 1997, around 30,000 fry and over 60,000 fry of Atlantic halibut from the UK and Iceland were produced, respectively (Shields, 1998, personal communication).

For cultivation of Atlantic halibut, the quality and quantity of gametes stripped from newly caught fish on the spawning grounds were not satisfactory. Also the lack of stable broodstock was not conducive to further development and progress in the cultivation experiments. Therefore, broodstock halibut were cultivated to provide a constant supply of gametes for halibut farming (Figure 1.1 and 1.2).

1.3 *Entobdella hippoglossi* Müller, 1776 infection

The monogenean skin parasite *Entobdella hippoglossi* Müller, 1776 has been found very commonly on captive broodstock halibut (Figure 1.3). The Sea Fish Industry Authority based on the west coast of Scotland has been holding captive broodstock halibut since 1983 for the purpose of culture as well as research. Throughout this time, *E. hippoglossi* appeared on the halibut brood stock. Fish were treated for 20 minutes in a bath of freshwater that was 99 % effective in removing the parasites, but within a few weeks the parasites had recovered. Figures 1.4 and 1.5 show the live and stained parasite, *E. hippoglossi* from heavily infected Atlantic halibut.

Entobdella hippoglossi Müller, 1776 was categorized by Yamaguti (1963) and described by Price (1939) as below:

Monogenea

Monopisthocotylea Odhner, 1912

Capsaloidea Price, 1936

Capsalidae Baird, 1853

Capsalinae Johnston, 1929

Entobdella Blainville in Lamarck, 1818

***Entobdella hippoglossi* Müller, 1776**

Description – "Body elliptical, 13 to 18 mm long by 3.6 to 4.8 mm wide (up to 24 mm long and up to 11 mm wide, according to various authors); cephalic lobe set off from rest of body by slight marginal constrictions. Anterior haptors in form of 2 elongate, slightly depressed, glandular areas, 1 on each side of median line near anterior margin of cephalic lobe. Posterior haptor sucker-like, 3.6 to 4.8 mm in diameter, surrounded by marginal membrane about 170 μm wide; ventral surface concave, the posterior half covered with radiating rows of more or less prominent papillae, and armed with 3 pairs of large hooks and 14 marginal hooklets. Hooks of first pair spearhead shaped, median margin slightly curved, 510 to 640 μm long; hooks of second pair slender, 815 to 935 μm long, tips recurved; hooks of third pair 95 to 122 μm aperture ventral, at level of lateral constrictions of body. Pharynx 680 to 850 μm long by 850 μm to 1.1 mm wide. Intestine as in species of *Benedenia*. Common genital aperture at left marginal constriction. Cirrus pouch club shaped, its base to right of margin of ovary. Testes globular, 1.7 to 2 mm in

diameter, equatorial. Ovary transversely oval, 595 to 695 µm long by 850 µm to 1 mm wide, pretesticular, separated from anterior margins of testes by wide band of vitelline follicles. Vitelline follicles occupying almost entire body from level of oral aperture to posterior end of body proper. Vagina slender, opening postero-median of common genital aperture. Ootype oval, lying in median line posterior to cirrus pouch; metraterm slender. Egg tetrahedral, about 228 µm wide with long, slender filament".

Price (1939) gave the synonyms of *E. hippoglossi* as follow;

Synonyms – ***Hirudo hippoglossi* Müller, 1776**

***Phylline hippoglossi* (Müller, 1776) Oken, 1815**

***Epibdella hippoglossi* (Müller, 1776) Blainville, 1828**

***Tristoma hamatum* Rathke, 1843**

***Nitzschia hippoglossi* (Müller, 1776) Taschenberg, 1878**

***Phyllonella hippogloss* (Müller) Goto. 1899**

***Epibdella bumpusii* Cnavan, 1934.**

1.4 Capsalid parasites on commercially important fishes

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 infections (Thoney and Hargis, 1991).

Because of the commercial importance of the host fishes, parasites belonging to the Capsalidae have been investigated by many researchers. Ernst and Whittington (1996) investigated hatching pattern and hatching conditions of *Benedenia lutjani* Ernst and Whittington, 1996 from the skin and *B. rohdei* Ernst and Whittington, 1996 from the gills of *Lutjanus carponotatus* Richardson, 1842. Ogawa, Bondad-Reantaso and Wakabayashi (1995a) redescribed *B. epinepheli* (Yamaguti, 1937) Meserve, 1938 from the fins, eyes and body surface of 12 cultured marine fishes in Japan. They suggested that heavy infection of this parasite is able to kill cultured fishes due to heavy infection.

Ogawa, Bondad-Reantaso, Fukudome & Wakabayashi (1995b) reported that *Neobenedenia girellae* (Hargis, 1955) Yamaguti, 1963 infected cultured marine fishes and it was able to kill fish in Japan. The oncomiracidium of *Benedenia rohdei* from the gills of *Lutjanus carponotaus* was described by Whittington, Kearn and Beverly-Burton (1994). Kearn (1992) reported mating behaviour of *Benedenia seriolae* Yamaguti, 1934, and Kearn, Ogawa and Maeno (1992a) described its egg laying rate and larval development from the skin of the yellowtail, *Seriola quinqueradiata*, Temminck & Schlegel which is one of the most important marine culture species in Japan.

In the genus *Entobdella*, *Entobdella soleae* (van Beneden and Hesse 1864) Johnston, 1929 of the common sole, *Solea solea* L. has been well investigated from many aspects. Numerous works have been done by Kearn: the life cycle of the parasite (Kearn, 1963a), the egg productivity (1985), hatching mode and hatching rhythm (1973, 1975), hatching pattern (1974a), larval development (1963b), host finding behaviour of the oncomiracidium (1967b, 1980), feeding behaviour (1963d) and movement (1984, 1988).

1.5 The aims and objectives of the present study

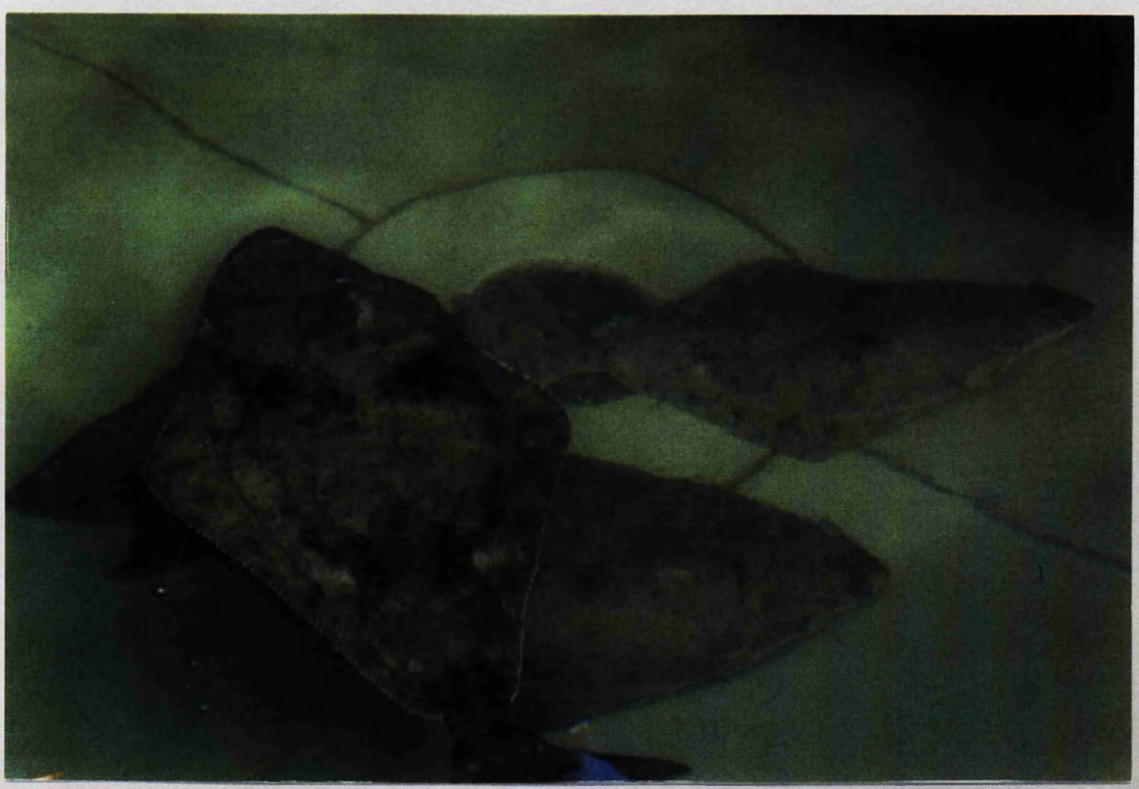
Infection by the monogenean skin parasite *Entobdella hippoglossi* appears to be a problem in the culture of the Atlantic halibut (Svendsen and Haug, 1991). However, due to the host behaviour and the establishment of the halibut farming industry, research on *E. hippoglossi* is lacking. Kearn (1974a) had established the nocturnal hatching pattern of *E. hippoglossi*, and compared glandular and excretory systems of the oncomiracidium of this parasite with that of *E. soleae* and *E. diadema* Monticelli, 1902 (Kearn, 1974b). In the laboratory, Svendsen and Haug (1991) showed that treatment against the adults and eggs of *E. hippoglossi* using formalin, benzocaine and both hypo and hypersaline methods were effective in detaching adult parasites and affecting egg hatching rate.

This parasite can represent a problem and heavy infections have been observed on broodstock during cultivation. More knowledge on this halibut parasite and its host-parasite relationship under culture conditions is needed.

The present studies, therefore, were carried out to investigate the biological aspects of the parasite, *E. hippoglossi* such as their population structure related with host sex, site specificity on the host (dorsal and ventral surface), the egg development, structure and hatching pattern and the host finding behaviour of the oncomiracidium and also its pathological effects on Atlantic halibut, *H. hippoglossus* under artificial culture conditions.

Figure 1.1 Land based culture tank (4 m diameter) for broodstock Atlantic halibut, *H. hippoglossus* at The Marine Environmental Research Laboratory (Stirling University), Machrihanish, Argyll, Scotland.

Figure1.2 Broodstock Atlantic halibut, *H. hippoglossi* held in land based culture tank for providing a constant supply of gametes for halibut farming and research.
(Scale bar = 10 cm).



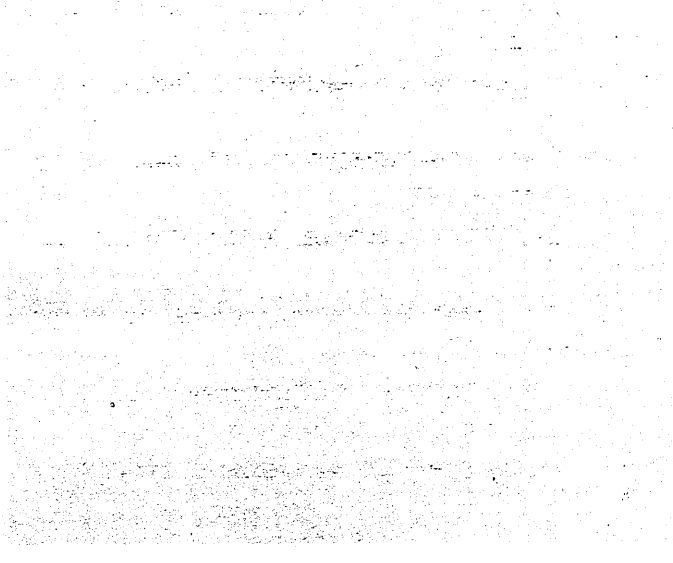
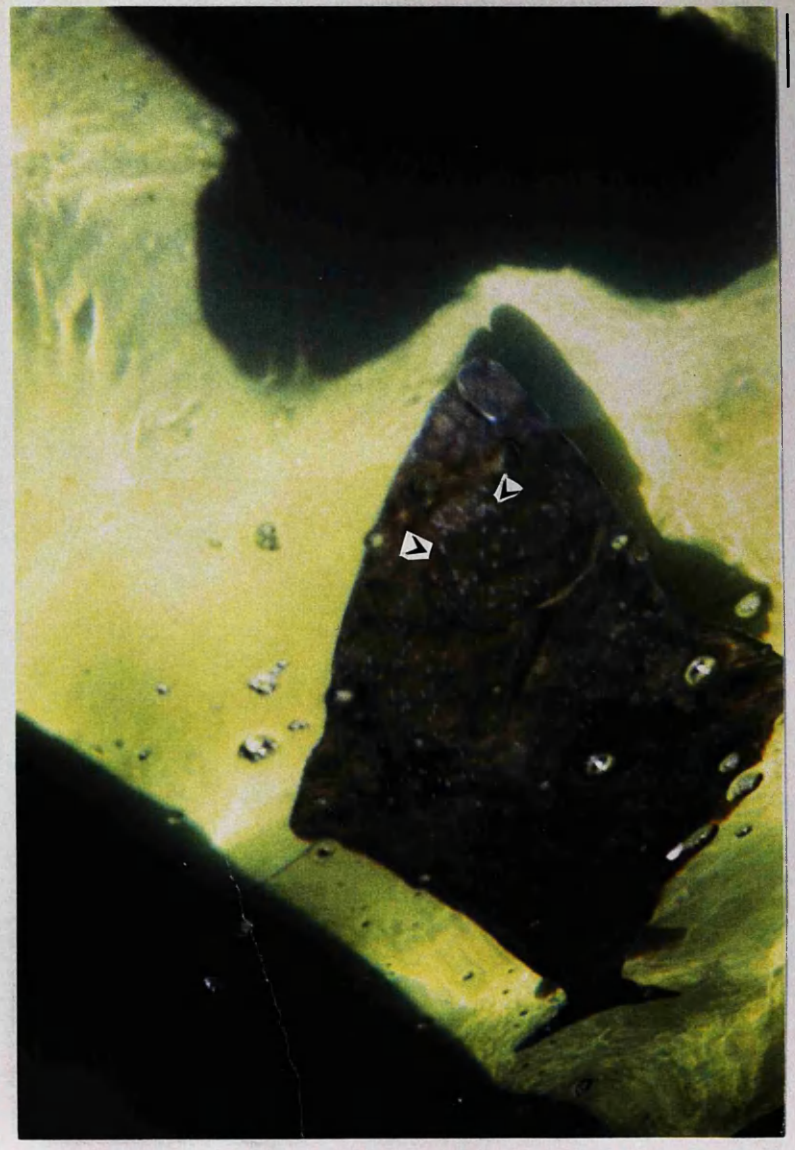
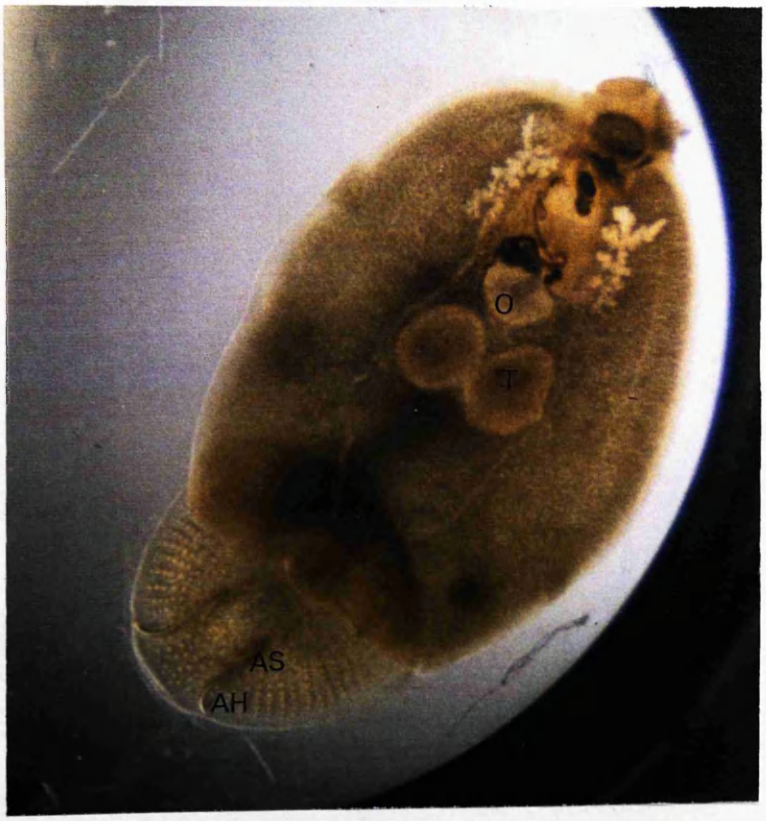


Figure 1.3 Heavily infected Atlantic halibut broodstock, *H. hippoglossus* with monogenean skin parasite, *Entobdella hippoglossi*.
(Scale bar = 10 cm)

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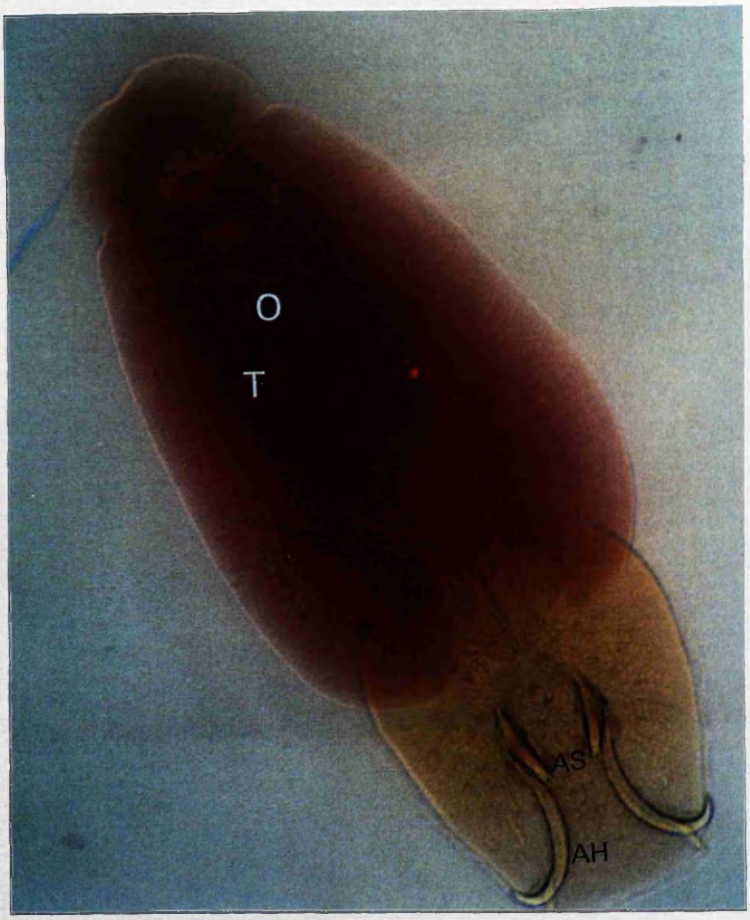
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Figure1.4 Live *Entobdella hippoglossi* from heavily infected Atlantic halibut broodstock, *H. hippoglossus*.
(**T**: testes, **O**: ovary, **AS**: accessory sclerites, **AH**: anterior hamuli).
(x 1 objective).

Figure 1.5 *Entobdella hippoglossi* stained with Mayer's paracarmine.
(**T**: testes, **O**: ovary, **AS**: accessory sclerites, **AH**: anterior hamuli).
(x 1 objective).

Chapter 2

GENERAL MATERIALS AND METHODS

2. GENERAL MATERIALS AND METHODS

2.1 Sampling sites and host fish

The Sea Fish Industry Authority at Ardtoe, Invernesshire and The Marine Environmental Research Laboratory (Stirling University) at Machrihanish, Argyll maintain halibut broodstock for culture and research purposes (Figure 2.1). Both sites thus allow access to these deep sea fish and hence make it possible to undertake a study of their parasites. For the population study of *Entobdella hippoglossi*, Ardtoe was sampled in March when the sea temperature was 5°C, while Machrihanish was sampled in May when the water temperature was 9.5°C.

In this part of the study, a total of 6 fish, 3 male and 3 female broodstock halibut, infected with the ectoparasitic monogenean, *E. hippoglossi*, were sampled. No length measurements were taken for the four Ardtoe fish (two females and two males), but the females weighed 20 and 10 kg respectively, whilst the males were 5 kg each. The two halibut from Machrihanish were weighed (one female and one male), the female was 20 kg and the male was 5 kg in weight. All fish were in spawning condition and records showed that there had been no treatments for parasites within the previous 6 months.

2.2 Parasite collection

Parasites on the dorsal surface of the halibut were collected from living and anaesthetized fish using blunt, round - ended forceps to avoid wounding the host. Prior to collecting the parasites, the host surface was mapped into four principal zones (front

dorsal, middle dorsal, rear dorsal and the ventral surface) as shown in Figure 2.2. It was not possible to map the collection of the parasites on the ventral surface because of the method of collection (see below).

The water level in the halibut holding tank was lowered to an approximate depth of 2 ft. A wooden ramp covered in plastic was placed in the tank. Once the fish had settled, individual halibut were gently manoeuvered onto the ramp, which was then gently lifted so that the fish was supported in a level position, just below the water surface. In this position, parasites were easily harvested from the dorsal surface as long as the fish remained still. Parasites were systematically collected from each zone and placed into marked plastic pots containing fresh aerated seawater at 35ppt.

On the ventral surface, parasites could not be collected from individual zones because of the potential damage that may be inflicted by inverting the fish, especially to the eyes, if the fish were to flap. Following collection and the mapping of parasites on the dorsal surface, the halibut was placed into an anaesthetic bath containing phenoxyethanol at a concentration of 50 ppm. The halibut was lightly anaesthetized, during which process most *E. hippoglossi* detached from the ventral surface of the body. Parasites were then filtered from the anaesthetic bath using a sieve. The halibut was then re-examined for any remaining parasites adhering to the dorsal or ventral surfaces. Parasites were then transported, on ice, to the Institute of Aquaculture, University of Stirling for processing.

Parasites were passed briefly through Berland's fluid (19 parts glacial acetic acid : 1 part formalin) to allow relaxation of the tissue before specimens were stored in 10 % neutral buffered formalin or in 80 % ethanol.

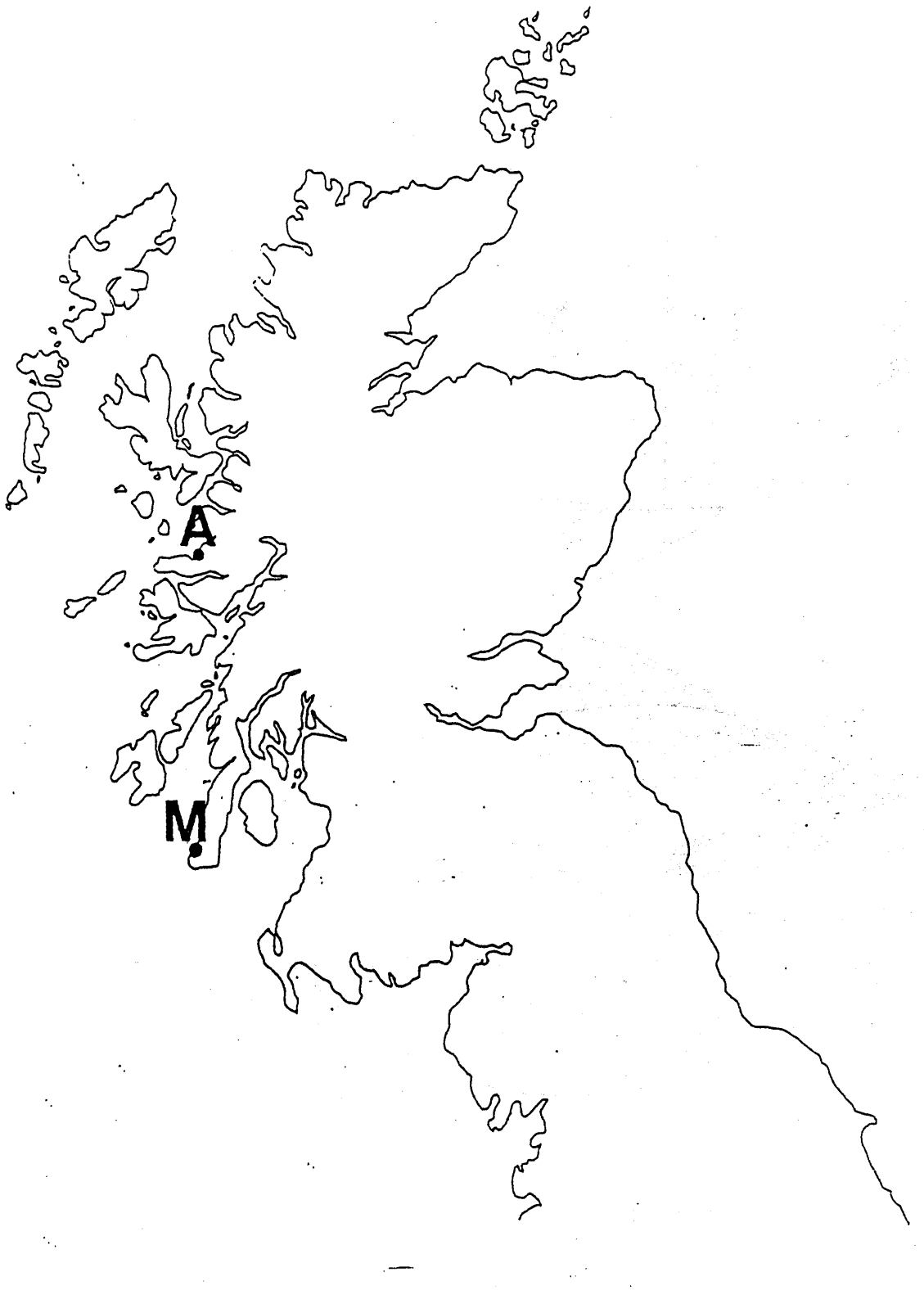

Figure 2.1 The location of sampling sites of *E. hippoglossi* in Scotland (A: Ardtoe, M: Machrihanish)

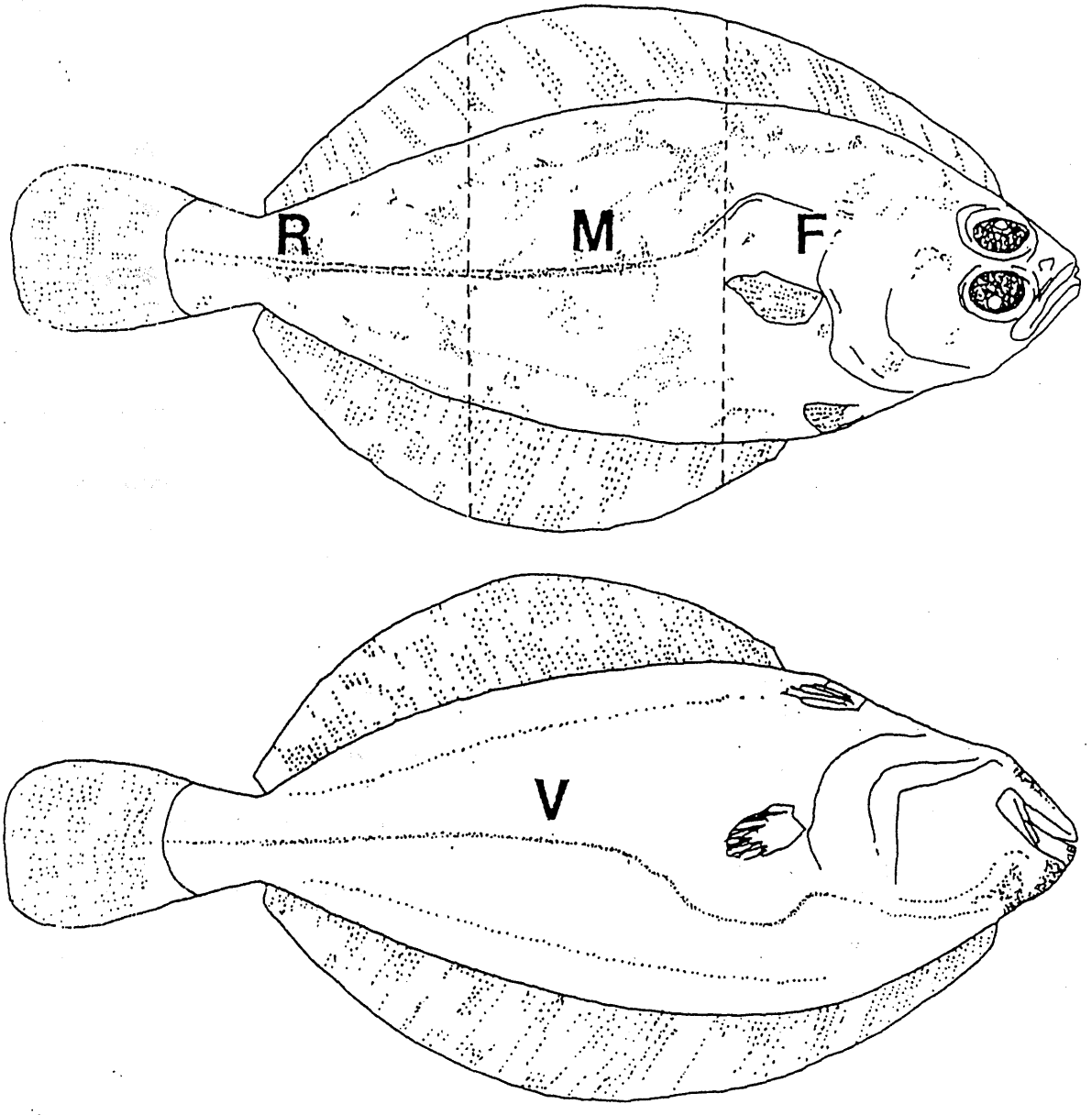

Figure 2.2 The map shows the four principal zones of the fish surface
(**F**: Front dorsal, **M**: Middle dorsal, **R**: Rear dorsal and **V**: Ventral)

2.3 Scanning electronic microscopy (SEM)

2.3.1 Eggs

The eggs of *E. hippoglossi* were collected from their container where they were held at 12°C. Specimens were fixed in 1 % glutaraldehyde at 4°C for 3 hours. They were then transferred into 3% glutaraldehyde at 4°C for 7 days. The egg material was then washed for 1 hour in 0.2 M cacodylate 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.

2.3.2 Oncomiracidia

After hatching from the eggs, the oncomiracidia were collected from their hatching chamber. Then, larvae were fixed in 1 % glutaraldehyde at 4°C for 3 hours. Subsequent processing was followed as described in paragraph 2.3.1.

Due to their small size, a dissection microscope was used to prevent loss of the specimens when transferring oncomiracidia from one solution to the next.

2.4 Parasite staining

The adults and newly hatched larvae were collected from the hatching chamber and pipetted very carefully into a watch glass. The parasites were then passed briefly through Berland's fluid to allow relaxation of the tissues before specimens were stored in 80 % ethanol. Fixed parasites were placed into undiluted Mayer's paracarmine solution for 15 minutes. After staining, the specimens were placed into 50 % acid alcohol for stain differentiation until the parasites turned a very faint pink colour. After differentiation, the specimens were transferred to 80 % alcohol and left for 15 minutes. From the 80 % alcohol, specimens were transferred to 100 % alcohol for 30 minutes for dehydration. After dehydration, the parasites were cleared with beechwood creosote overnight, then mounted in Canada balsam and allowed to dry. Completely dried glass slides were placed under the microscope to examine the morphology and the gonad development.

2.5 Histology

Blocks of skin from infection sites (front ventral) were cut from the parasitised juvenile halibut. For histological purposes samples were fixed in 10 % neutral buffered formalin. Tissues to be processed were placed in suitably sized cassettes, labelled and

autoprocessed in a histokinette (HISTOKINETTE 2000). They were blocked in molten wax using a Reichert - Jung wax embedder and then cooled rapidly on a freezing plate. The blocks were trimmed so that the desired tissues were brought to the surface of the blocks. Then the surface of the block was decalcified in RDC (rapid decalcifier from Histopath) for about two hours. The blocks were washed and cooled on a cold plate before being sectioned. A Leitz-Wetzlar microtome was used to cut 5 μm sections using Reichert-Jung disposable microtome blades. The sections were floated on water maintained at 40°C in a water bath and collected on prewashed wet glass slides. The slides were then marked with a diamond pen, dried on a hot plate and the wax melted in a 60°C oven overnight. For routine observation the sections were stained with haematoxylin and eosin (H + E).

For comparing the mucous cell number, size and thickness of the epidermal layer of halibut skin, the skin samples were also cut from uninfected fish. The skin samples were cut from 6 sites on each fish (dorsal front, dorsal middle, dorsal rear, ventral front, ventral middle and ventral rear). Samples were processed for histology as described in paragraph 2.4.

Specimens were stained for 5 minutes in 1 % Alcian blue with pH 2.5 and then in 1 % Periodic Acid- Schiff (PAS) for 5 minutes to visualize the mucous cells of the skin. Stained sections were processed following the methods given for the haematoxylin and eosin staining method.

2.6 Photography and drawing

Light micrographs were taken from fresh and fixed preparations of eggs, oncomiracidia and adult parasites. Light and phase contrast micrographs were taken with an Olympus BH-2 research microscope fitted with an automatic photomicrographic camera. Drawings were made using a drawing tube on a Leitz Wetzlar SH Lux compound microscope. Some free hand drawings were also made.

Chapter 3

POPULATION STRUCTURE AND DISTRIBUTION

3. POPULATION STRUCTURE AND DISTRIBUTION

3.1 INTRODUCTION

3.1.1 Environmental factors affecting the parasite population

Seasonal changes in nature are very clearly reflected in organic life. It is therefore natural to expect that parasites should not remain indifferent to the annual cycle of climatic changes (Dogiel, 1964). The impact of environmental factors on monogeneans has been widely studied in gyrodactylids (Scott, 1982; Scott & Anderson, 1984; Gelnar, 1987). The manner in which water temperature affects population dynamics of monogenean parasites has been investigated by a number of researchers (Scott & Nokes, 1984; Kamiso & Olson, 1986; Gelnar, 1987). Briefly, their results concluded that firstly, water temperatures directly stimulates parasite development and reproduction and secondly, by changing the immunological resistance of the host. Temperature is one of the major environmental factors which influences the population dynamics of *Dactylogyrus* species. High water temperature (20 – 26°C) was optimal for *Dactylogyrus vastator* Nybelin, 1924 and accelerates its development (Kollmann, 1970, 1972). However, Reda (1988) reported that high summer temperature (above 18°C) inhibited reproduction and caused a decrease in the number of *D. vastator*, whilst a temperature of about 4°C stimulated a new period of intensive reproduction. The viviparous Gyrodactylidae have a rapid development and short life span. When *Gyrodactylus alexanderi* Mizelle and Kritsky, 1967 was maintained at 15°C, it gave birth to two generations,

the first after an average of 1.6 days and the second after 6.9 days. Maximum longevity at 15°C was 28 days (Lester and Adams, 1974).

Bauer (1959) suggested that abiotic factors influence the occurrence of fish parasites on their hosts. He included the amount of light entering into the water, the depth, pH, oxygen content, salinity and water temperature. Prost (1959) added that water currents, suspended particles in the water and chloride also affect the monogenean populations.

Biotic factors influencing monogenean populations were discussed by Bauer (1959) and Engelbrecht (1963). They concluded that the density of the fish populations, reproductive behaviour, growth rates and migratory patterns could all modify parasite populations.

Skinner (1982) suggested that pollutants (ammonia, trace metals and pesticides) acted as stressors and caused physiological changes which altered host resistance to parasites. He revealed that three species of fish, yellow fin mojarra, *Gerres cinereus* Walbaum, 1792, gray snapper, *Lutjanus griseus* L., 1758 and timucu, *Strongylura timucu* Walbaum, 1792 from a polluted area which was degraded by discharges of agricultural, industrial and urban wastes, showed higher intensities of gill parasites than those from less polluted areas. When Atlantic cod, *Gadus morhua* L., 1758, was exposed experimentally to water-soluble oil fractions (WSOF), the prevalence and intensity of *Gyrodactylus* sp. were significantly greater in the oil treated groups than in controls 16-20 weeks after exposure (Khan and Kiceniuk, 1988).

3.1.2 Host size and sex

Parasites may a) be independent of the age of their host, b) decrease in abundance with the age of their host or c) increase in abundance with the age or length of their host. Winch (1983) found that *Atrispinum labracis* Beneden & Hesse, 1863 on the gills of the bass, *Dicentrarchus labrax* L., 1758 did not begin to infect its host until the host was at least 3 years of age. Fischer & Kelso (1990) found that the prevalence and mean intensity of the digenean trematode *Posthodiplostomum minimum* MacCallum, on the bluegill, *Lepomis macrochirus* Rafinesque and the largemouth bass, *Micropterus salmoides* Lacepede increased with increasing host length.The third group (c) is predominant in the natural condition (Fuentes and Nasir, 1990; Rodriguez and George-Nascimento, 1996). Some indications exist that intensity of infection also tends to decrease with host age or size in some cases (Ogawa, 1988).

Fuentes and Nasir (1990) reported that the infection rate of the monogenean gill parasite *Ligophorus mugilinus* Hargis, 1955 on silver mullet, *Mugil curema* Val, 1936 was not related to host sex.

3.1.3 Spatial distribution

Some parasites have a great specificity for certain sites on or in the host (Hanek and Fernando, 1978a, b). According to Llewellyn (1957), most monogeneans appear to have specific positions on the gills of their host. For example, *Gastrocotyle trachuri* Beneden and Hesse, 1863 was found mid-way along the primary lamellae, whilst *Pseudaxine trachuri* Parona and Perugia, 1890 was attached nearer to the distal tips

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3.1.3 Spatial distribution

Some parasites have a great specificity for certain sites on or in the host (Hanek and Fernando, 1978a, b). According to Llewellyn (1957), most monogeneans appear to have specific positions on the gills of their host. For example, *Gastrocotyle trachuri* Beneden and Hesse, 1863 was found mid-way along the primary lamellae, whilst *Pseudaxine trachuri* Parona and Perugia, 1890 was attached nearer to the distal tips

of the lamellae on the horse mackerel, *Trachurus trachurus* Castelnau. Llewellyn (1956) suggested that the site selection of polyopisthocotyleans was determined by the effect of the gill ventilating current. Arme and Halton (1972) reported that *Diclidophora merlangi* Kuhn in Nordmann, 1832 preferred the first gill arch of its host *Merlangius merlangus* L. Their suggestion was that the speed of the respiratory current may have an influence on the settlement of the infective larval stage or of immature worms entering the gill chamber via the respiratory current.

Site specificity is often presumed to result from active site selection by the parasite (Rohde, 1981). Specific microhabitats increase the chances of successful mating in monogeneans (Ktari, 1971). Most species of *Gyrodactylus* are known to be host specific and they also usually infect specific organs or tissues of fish (Cone and Odense, 1984; Ogawa, 1986). Harris (1988) described the site specificity of *Gyrodactylus turnbulli* Harris, 1986 on *Poecilia reticulata* Peters (guppy) and observed that this worm most frequently infected the caudal peduncle (42%) and caudal fin (40%). Cone and Cusack (1988) studied the behaviour of *Gyrodactylus colemanensis* Mizelle and Kritsky, 1967 on *Salvelinus fontinalis* Mitchill. The parasite attached anywhere on the body surface but the preferred site was the margin of the fins, particularly the caudal, pectoral and pelvic fins. Jensen and Johnsen (1991) found that the spatial distribution of parasites on the fish varied with infection intensities. As intensity increased, the distribution pattern of *Gyrodactylus salaris* Malmberg, 1957 on Atlantic salmon, *Salmo salar* L. was changed. At low intensity (<100), the dorsal fin was the principal site of attachment but when the intensity increased (>100), more parasites were located on the caudal fin, and when it exceeded 1000, the body surface was also heavily infected. Different species of *Gyrodactylus* attach to different sites on the same host. *G. masu* Ogawa, 1986 was

found on fins, body surface, gill filaments, and gill arches of salmonids (Ogawa, 1986). Cone and Odense (1984) observed that 4 out of 5 species of *Gyrodactylus* were primarily attached to the body surface and fins while in the fifth species, *G. salmonis* Yin and Sproston, 1948 more than 90 % of the worms were on the dorsal, ventral and pectoral fins. In a study of a mixed infection of two *Gyrodactylus* species, *G. colemanensis* and *G. salmonis*, on salmonids, *G. colemanensis* almost exclusively attached on the fin edges (>95%) while *G. salmonis* attached on the surface of the head, the body and the surfaces of the fins (Cone and Cusack, 1988).

3.1.4 Objectives

The objective of the present study was to determine the occurrence of *E. hippoglossi* on the Atlantic halibut, *H. hippoglossus* in its culture conditions and to provide information on the population structure and distribution of the parasite, particularly in relation to geographical location, host sex and spatial distribution over the host surface.

3.2 MATERIALS AND METHODS

3.2.1 Parasite collection sites

*Entobdella hippoglo*ssi were removed from the external surface of a total of 6 heavily infected Atlantic halibut held in 8 m diameter tanks at The Sea Fish Industry Authority at Ardtoe and in 4 m tanks at The Marine Environmental Research

Laboratory (Stirling University) at Machrihanish. Both sites had running water systems with aeration and input seawater directly from the sea at 34-35 ppt salinity. Of the 6 fish, 2 females and 2 males were from Ardtoe and 1 female and 1 male were from Machrihanish. Ardtoe was sampled in March when the sea temperature was 5°C, while Machrihanish was sampled in May when the water temperature was 9.5°C.

3.2.2 Collection of parasites

Parasites were collected as described in Chapter 2.

3.2.3 Morphometrics

3.2.3.1 Length and width

Individual parasites from the four principal body zones were measured, the length and the width of each parasite were measured using a stereo microscope (x 1- 4) with an eyepiece graticule. The total length was taken from the most anterior portion of the adhesive pad to the most posterior edge of the opisthaptor. The width was taken across the centre of the testes, which represented the widest part of the body. Figure 3.1 shows the dimensions of the length and width measurements taken from parasites within the present study.

3.2.3.2 Size distribution

All measured parasites were divided into 8 size classes based on the parasite body length; less than 3 mm, 3- 5 mm, 5- 7 mm, 7- 9 mm, 9- 11 mm, 11- 13 mm, 13- 15 mm and over 15 mm. The number of parasites at each site (Ardtoe and Machrihanish), for host sex (male and female), surface (dorsal and ventral) and the 4 different zones (front dorsal, middle dorsal, rear dorsal and ventral) were counted (refer Figure 2.2 in Chapter 2).

3.2.3.3 Somatic indices

The presence of testes and ovaries was ascertained. The length and width of the testes from a subsample (100 parasites) of parasites were measured. These data were analysed for each host surface (dorsal and ventral) and for the four principal zones (front dorsal, middle dorsal, rear dorsal and ventral total) of each fish.

3.2.3.4 Hamuli length

The hamulus length for each parasite within the subsample of parasites was measured. Both hamuli were measured on a subsample of 30 parasites and the length measurements were found to be the same for both hamuli. Subsequently, therefore, only one hamulus was measured. It was selected for its clarity. This is shown in Figure 3.1.

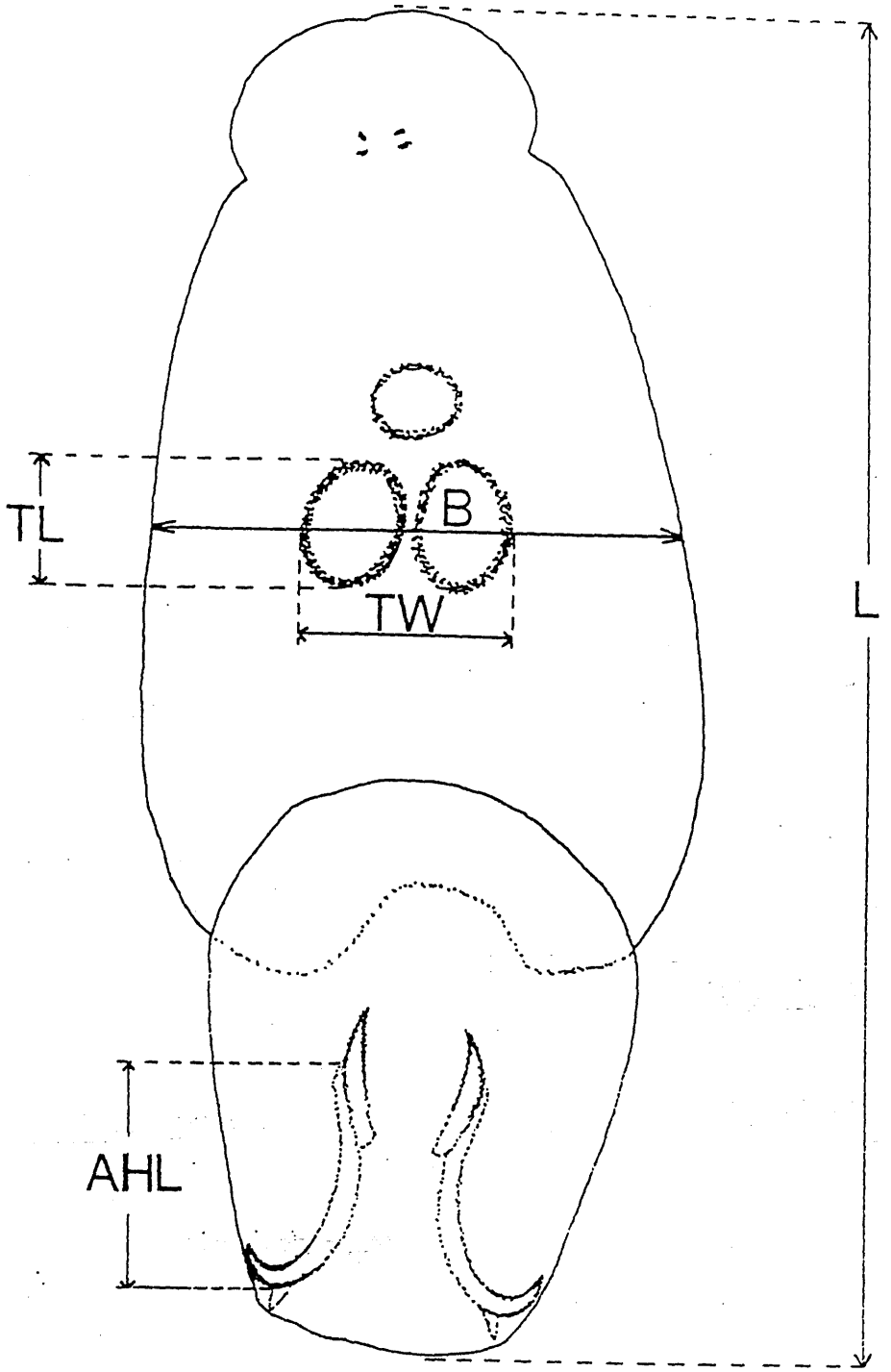


Figure 3.1 Diagramatic representation of *Entobdella hippoglossi* showing the dimensions measured. (**L**: Total length, **B**: Total width, **TL**:Testes length, **TW**: Testes width, **AHL**: Anterior hamulus length).

3.2.3.5 Statistical analysis

Re – sampling test was used to test the differences between parasite burdens from different sexes (male and female) and different sides (dorsal and ventral). The non – parametric Tukey test was used for comparing parasite burden from four different sites (front dorsal, middle dorsal, rear dorsal and ventral).

The non – parametric Dunn's test was used for comparing the mean length and width of parasites from different sites (Ardtoe and Machrihanish), different sexes (male and female), different sides (dorsal and ventral) and four different sites (front dorsal, middle dorsal, rear dorsal and ventral).

Testes length and hamuli length were compared using the non- parametric Dunn's test.

All findings were considered significant at values of $P<0.05$.

3.3 RESULTS

3.3.1 Body length and width of *E. hippoglossi*

3.3.1.1 A comparison between sites (Ardtoe vs Machrihanish) (Table 3.1)

Although the total number of parasites for the two sites, Ardtoe and Machrihanish, were similar, 1228 (n = 4) and 1282 (n = 2) respectively, the mean intensity from Machrihanish was 641 ± 233.3 while that from Ardtoe was 307 ± 276.7. The parasite burden on Machrihanish fish was twice than that of Ardtoe fish.

The mean length and width of parasites from the Ardtoe halibut were greater than those collected from halibut maintained at Machrihanish ($P<0.05$).

The relationship between the body length and width is shown in Figure 3.2. It was demonstrated that the parasite length and width were highly positively correlated (r^2 = 0.955 in Machrihanish and r^2 = 0.926 in Ardtoe) in both sites.

3.3.1.2 A comparison of the parasite profile with the sex of the host (Table 3.2)

For the purpose of the test the parasites from all the females and from all the males from both Ardtoe and Machrihanish were pooled. The total number of parasites on the female host (n = 3), 1817, was more than that on the male halibut (n = 3), 693. The mean intensity of the parasite from male hosts was 231 ± 226.6 while that of the parasites from female hosts was 605 ± 244.7 ($P < 0.05$).

The mean length and width of parasites on female hosts were significantlylonger and wider than those on the male fish ($P<0.05$).

Table 3.1 The mean length, width and number of *E. hippoglossi* from two sites

Location	Ardtoe		Machrihanish	
	Length	Width	Length	Width
Total number	1228	1228	1282	1282
Mean (mm)	10.63	4.97	6.04	2.62
SD	3.27	1.78	2.44	1.18
Median (mm)	11.4	5.2	5.6	2.3
Minimum	0.97	0.29	1.46	0.49
Maximum	16.99	9.71	16.90	7.67

Figure 3.3 shows the relationship of parasite length and width for parasites collected from female and male hosts. The widths were found to be positively correlated with the lengths (r^2 =0.964 on male halibut and r^2 = 0.958 on female halibut) in both male and female hosts.

Table 3.2 The mean length and width of *E. hippoglossi* from both host sexes

Location	Male		Female	
	Length	Width	Length	Width
Total number	693	693	1817	1817
Mean (mm)	7.87	3.65	8.45	3.82
SD	3.77	1.89	3.64	1.92
Median (mm)	7.5	3.4	7.8	3.3
Minimum	1.55	0.58	0.97	0.29
Maximum	16.89	9.03	16.99	9.71

3.3.1.3 A comparison of the parasite profile on different surfaces of the host (dorsal vs ventral) (Table 3.3)

Again data from males and females from Ardtoe and Machrihanish were combined. The total number of parasites collected from the dorsal surface was 1256 (n = 3) and the ventral surface was 1254 (n = 3). The mean intensity on the dorsal surface was 209 ± 199.8 while that on the ventral surface was 209 ± 159.9. The parasite burdens from dorsal surfaces and ventral surface, therefore, were almost the same.

However, the mean length and width of parasites on the ventral surface were larger than those on the dorsal surface ($P< 0.05$). The parasites body width was found to be strongly positively correlated with the parasite length ($r^2 = 0.958$ on the dorsal surface and $r^2 = 0.948$ on the ventral surface) for both surfaces (Figure. 3.4).

Table 3.3 The mean length, width and number of *E. hippoglossi* from both sides of halibut

Location	Dorsal		Ventral	
	Length	Width	Length	Width
Total number	1256	1256	1254	1254
Mean (mm)	6.90	3.07	9.67	4.48
SD	3.10	1.58	3.70	1.95
Median (mm)	6.3	2.6	10.3	4.7
Minimum	1.46	0.49	0.97	0.29
Maximum	16.51	8.25	16.99	9.71

3.3.1.4 A comparison of the parasites collected from the four principal skin zones (front dorsal, middle dorsal, rear dorsal and the ventral side of the halibut) (Tables 3.4 and 3.5)

On the ventral surface, parasites could not be collected from individual zones because of the potential damage that may be inflicted by inverting the fish, especially to the eyes, if the fish were to flap (refer Chapter 2.2). Only the dorsal surface could be mapped as turning over the halibut could only be carried out

following anaesthetising and, as a result of this, many of the parasites on the ventral surface detached. These were, therefore, treated as a single group.

The mean intensity of the parasite from the ventral surface was higher than that from the front dorsal region, middle dorsal region and rear dorsal region. Though more parasites were found within the front zones on the dorsal surface, this observation was not significantly different from other regions on the same surface (dorsal surface). However, there was a significant difference statistically between parasite numbers from the ventral surface and the rear dorsal ($P<0.05$)(Table 3.4).

The mean length and width of the parasites collected from the four principal zones are summarised in Table 3.5. Parasites occupying the ventral surface of their host were significantly longer and wider than those found on the other zones of the fish ($P<0.05$). Further, the parasites in the front zone were longer in length and wider in width than parasites from the middle zone which in turn were longer and wider than parasites from the rear zone ($P<0.05$). The relationship between parasite length and width for the four regions is shown in Figure. 3.5. The widths were positively correlated ($r^2 = 0.948$ in the ventral zone, $r^2 = 0.957$ in the front dorsal zone, $r^2 = 0.923$ in middle dorsal zone and $r^2 = 0.914$ in the rear dorsal zone) with the lengths in the four principal zones.

Table 3.4 The number of *E. hippoglossi* collected from the principal zones of their halibut host

Location	Front dorsal (n = 6)	Middle dorsal (n = 6)	Rear dorsal (n = 6)	Ventral total (n = 6)
Total number	704	334	218	1254
Mean intensity	117.3 ± 77.8	55.7 ± 93.1	36.3 ± 50.9	209 ± 159.9
Minimum	12	0	0	17
Maximum	231	236	112	493

Table 3.5 The mean length and width of *E. hippoglossi* collected from the principal zones of their halibut host

Location	Front dorsal		Middle dorsal		Rear dorsal		Ventral total	
	Length	Width	Length	Width	Length	Width	Length	Width
Mean (mm)	8.09	3.68	5.73	2.41	4.86	2.11	9.67	4.48
SD	3.33	1.73	1.87	0.91	1.83	0.89	3.70	1.95
Median (mm)	7.8	3.4	5.5	2.2	4.7	1.9	10.3	4.7
Minimum	1.46	0.49	2.14	0.78	1.55	0.58	0.97	0.29
Maximum	16.51	8.25	13.11	6.41	10.68	5.63	16.99	9.71

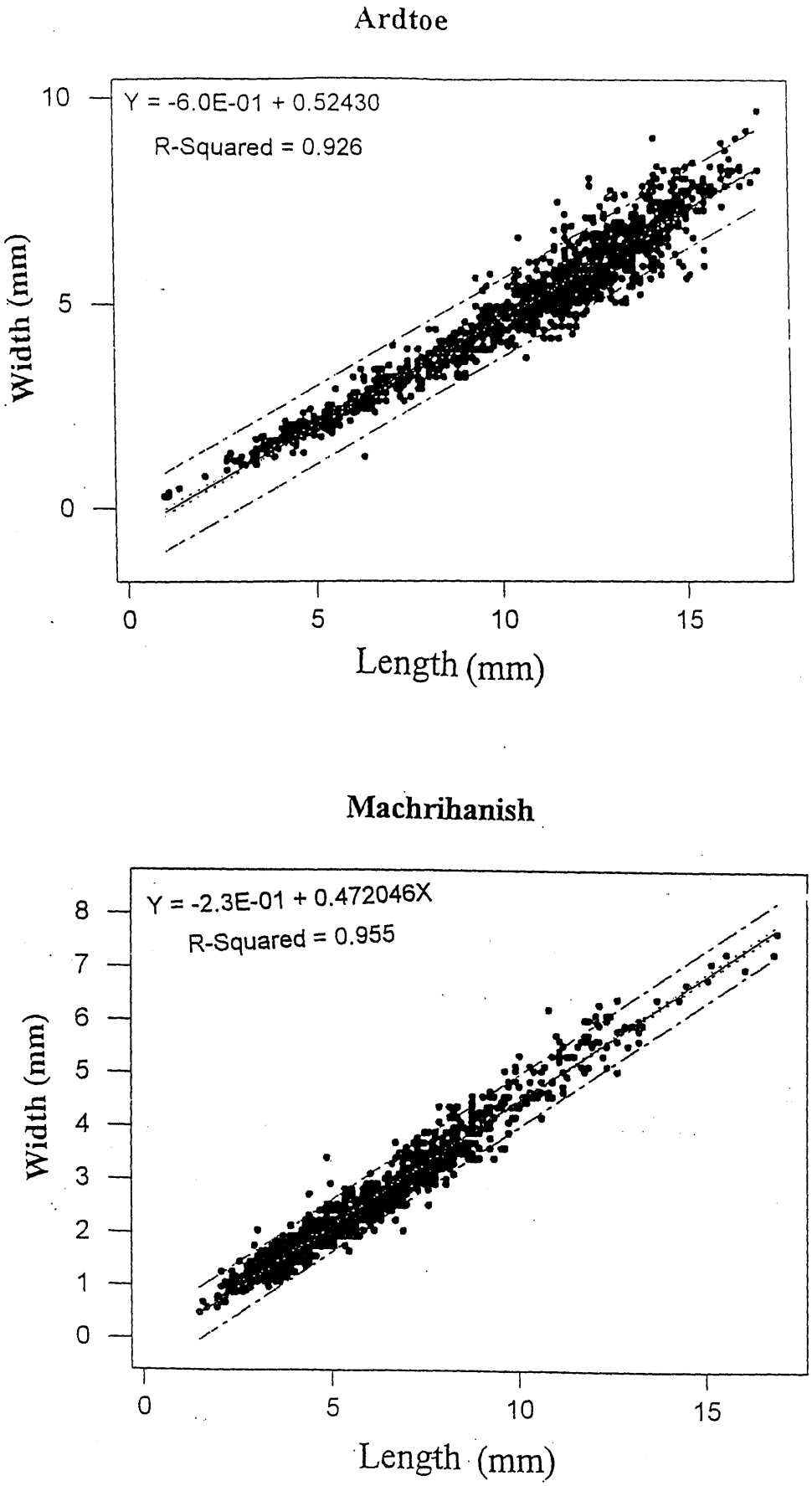


Figure 3.2. Relationship between total length and total width of *E. hippoglossi* on *H. hippoglossus* at the different sample sites Ardtoe and Machrihanish.

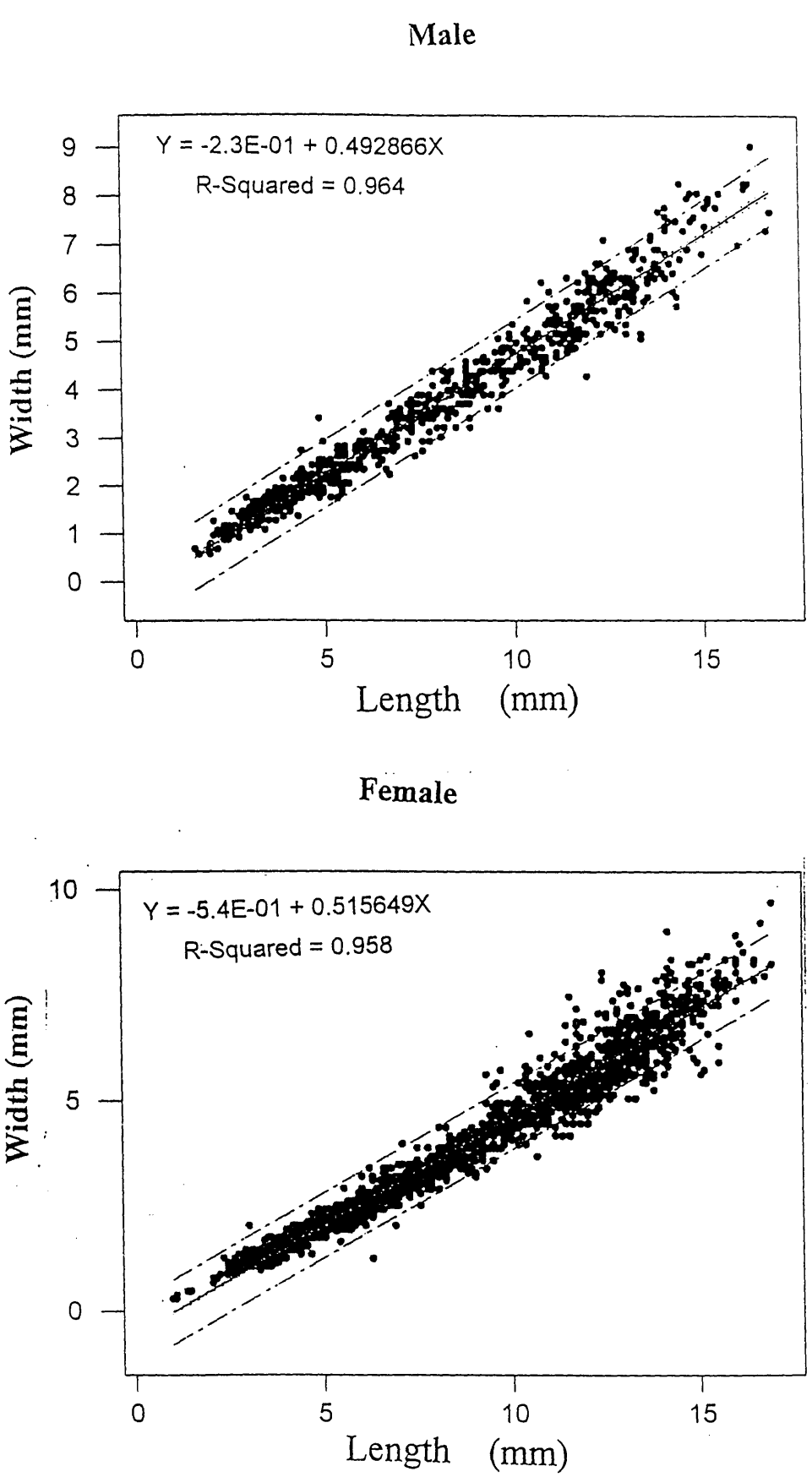


Figure 3.3. Relationship between total length and total width of *E. hippoglossi* on the different sexes of the host *H. hippoglossus*.

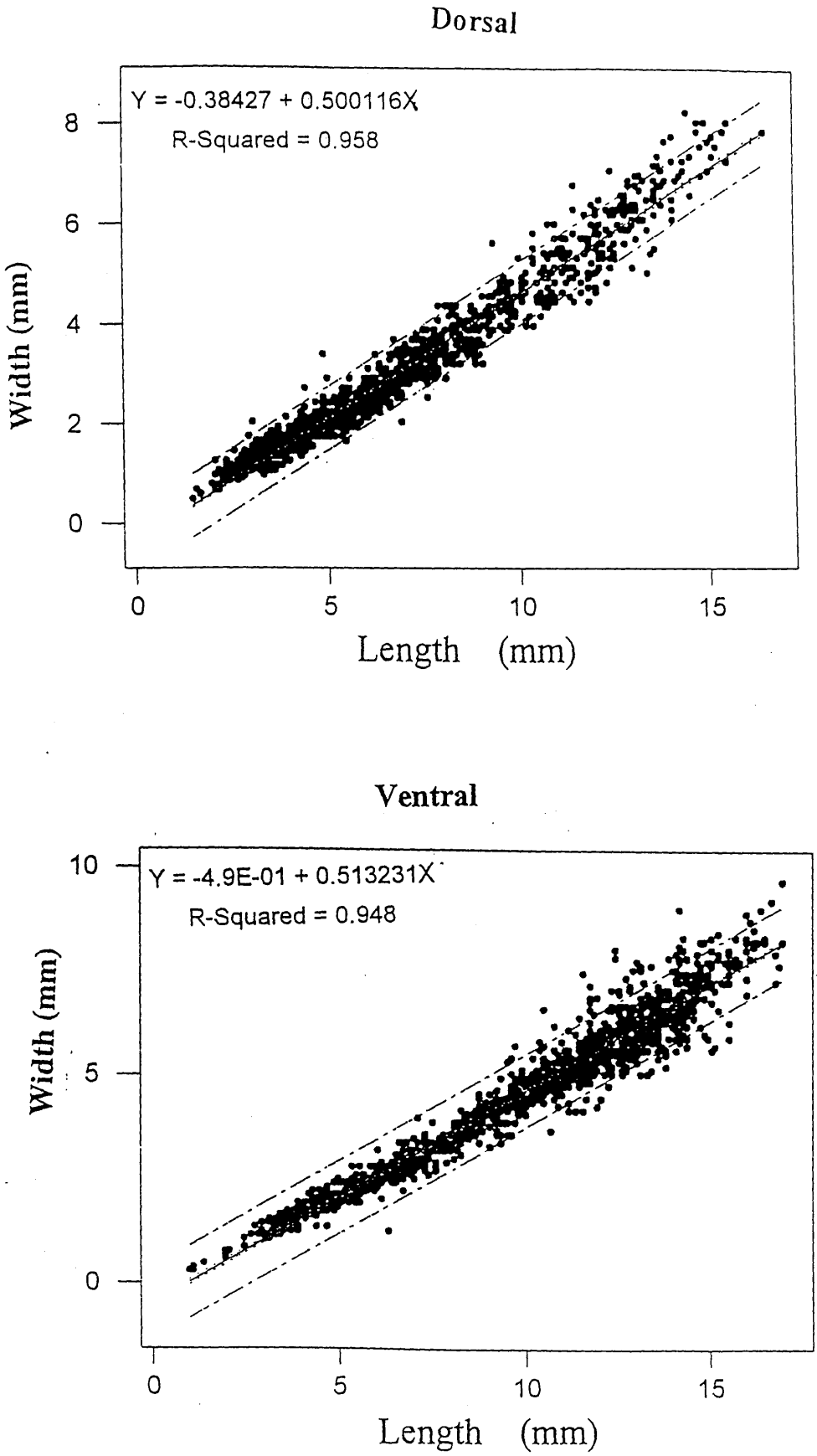


Figure 3.4. Relationship between total length and total width of *E. hippoglossi* on the different surfaces, dorsal and ventral of the host *H. hippoglossus*.

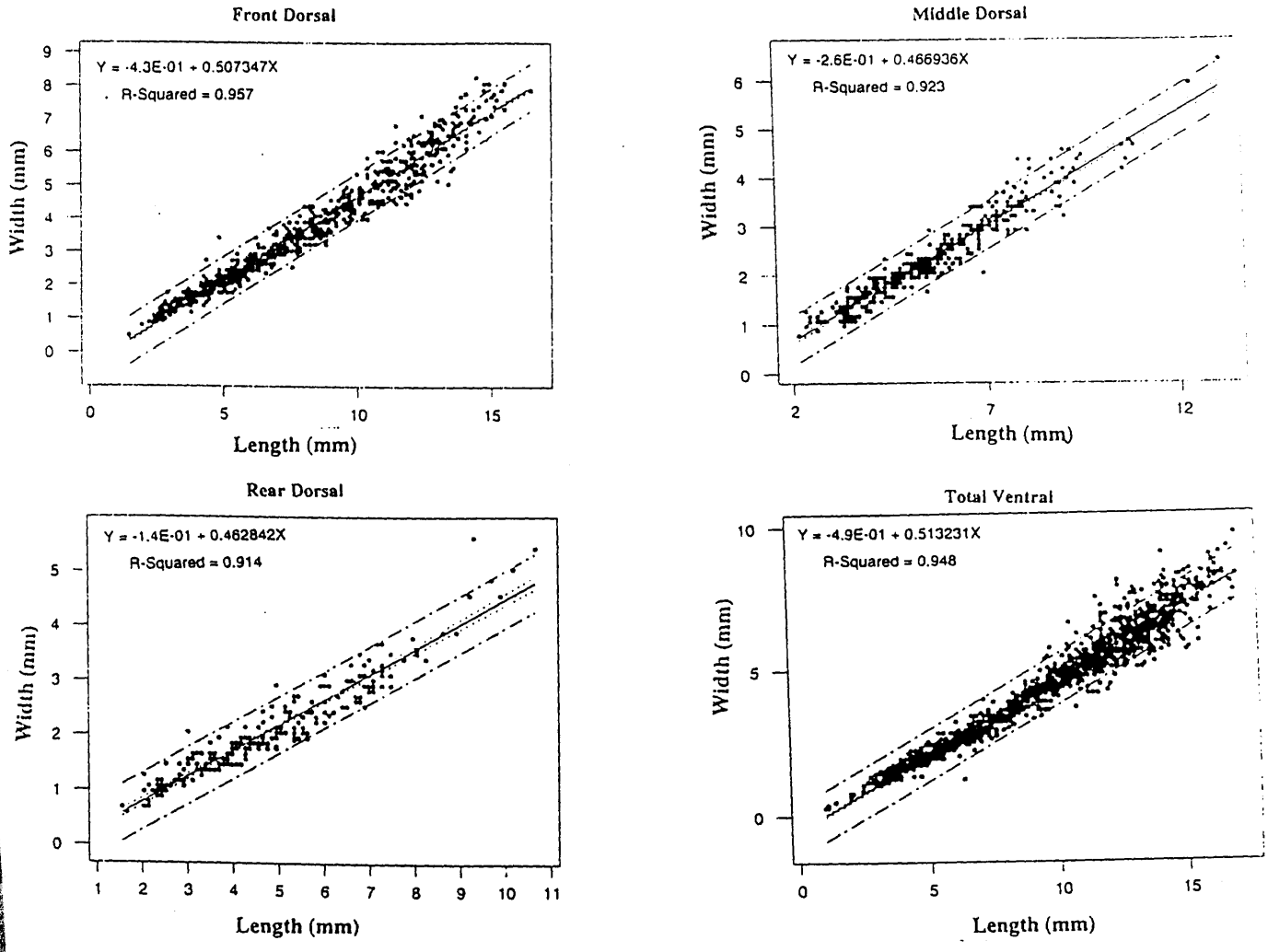


Figure 3.5. Relationship between total length and total width of *E. hippoglossi* on the 4 different principal zones of the host *H. hippoglossus*

3.3.2 Size distribution of *E. hippoglossi* on Atlantic halibut

3.3.2.1 A comparison between sites (Ardtoe vs Machrihanish) (Fig. 3.6)

The dominant size class of parasite in the Machrihanish population of *E. hippoglossi* was the 3-5 mm class which represented 31.9.% of the total parasite population whilst the percentage of the same size class of parasites collected from the Ardtoe population was 6.7 %. In comparison, the longest size class of parasite in the Ardtoe population was the 11 - 13 mm (26.1 %). The same size class in the Machrihanish population was 3.6 %. In Ardtoe, the smallest size class, less than 3 mm was 0.98 % whilst the same size class in Machrihanish was 6.63%. The size class over 13 mm was 27.93 % and 1.17 % from Ardtoe and Machrihanish respectively.

3.3.2.2 A comparison between sexes (Fig. 3.7)

The size distributions of *E. hippoglossi* on both male and female halibut were similar, however, the majority of the parasites on the female host were 5 - 7mm (22.95 %) whereas on the male 23.95 % of the total parasite belonged to the 3 – 5 mm size class.

The smallest size class, less than 3 mm, was 6.93 % on the male host and on the female host 2.7 %. The biggest size class, over 15 mm, was similar on both sexes (2.02 % and 2.81 %, male and female, respectively)

3.3.2.3 Dorsal and ventral differences in the population structure (Fig. 3.8)

The 5 - 7 mm class represents the dominant size class in the parasites collected from the dorsal surface (27.7 %) whilst 14.3 % of the same size class was collected from the ventral surface. On the ventral surface, 20.9 % of all parasites measured between 11 -13 mm, whereas 8.4 % of the dorsal population fell into this size class. On the dorsal surface, the smallest size class, less than 3 mm, comprised 5.97 % whilst the same size class on the ventral surface was 1.75 %. The over 13 mm size class was 5.02 % and 23.53 % from dorsal surface and ventral surface, respectively.

3.3.2.4 The population structure of *E. hippoglossi* in the four principal zones (front dorsal, middle dorsal, rear dorsal and total ventral) (Fig. 3.9)

The parasites on the front dorsal zone were distributed quite widely between 3-13 mm in size. The majority of parasites in the front dorsal and the middle dorsal regions were 5 - 7 mm (22.3 % of the front dorsal parasites and 38.0 % of the middle dorsal parasites). In the rear dorsal region, parasites measuring 3 - 5 mm (41.7 %) were the dominant size class but on the ventral zone, the dominant size class was the 11- 13 mm (20.9 %). In the front dorsal zone, parasites were quite equally distributed between the size classes 3 - 5 mm, 5 - 7 mm, 7 - 9 mm, 9- 11 mm and 11- 13 mm (87.8 %) in the front dorsal zone. In the middle dorsal zone, most parasites belonged to the size classes 5 - 7 mm and 7 - 9 mm (90.7 %). The parasites in the rear dorsal zone were principally in the 3- 5 mm and 5- 7 mm (87.2 %) size classes. In the ventral zone, 54.2 % of all parasites measured between 11 mm and 15 mm and 4.4 % measured over 15 mm. The smallest size class, less than

3 mm, was 16.1 % in the rear dorsal whilst the same size in other zones (front, middle and ventral) was 3.8 %, 3.9 % and 1.6 % respectively. The size class over 13 mm represented 25.5 % in the ventral zone and 8.8 % in the front zone. There were no parasites measuring over 13 mm in the middle dorsal zone. Furthermore, there was no parasite over 11 mm in the rear dorsal zone.

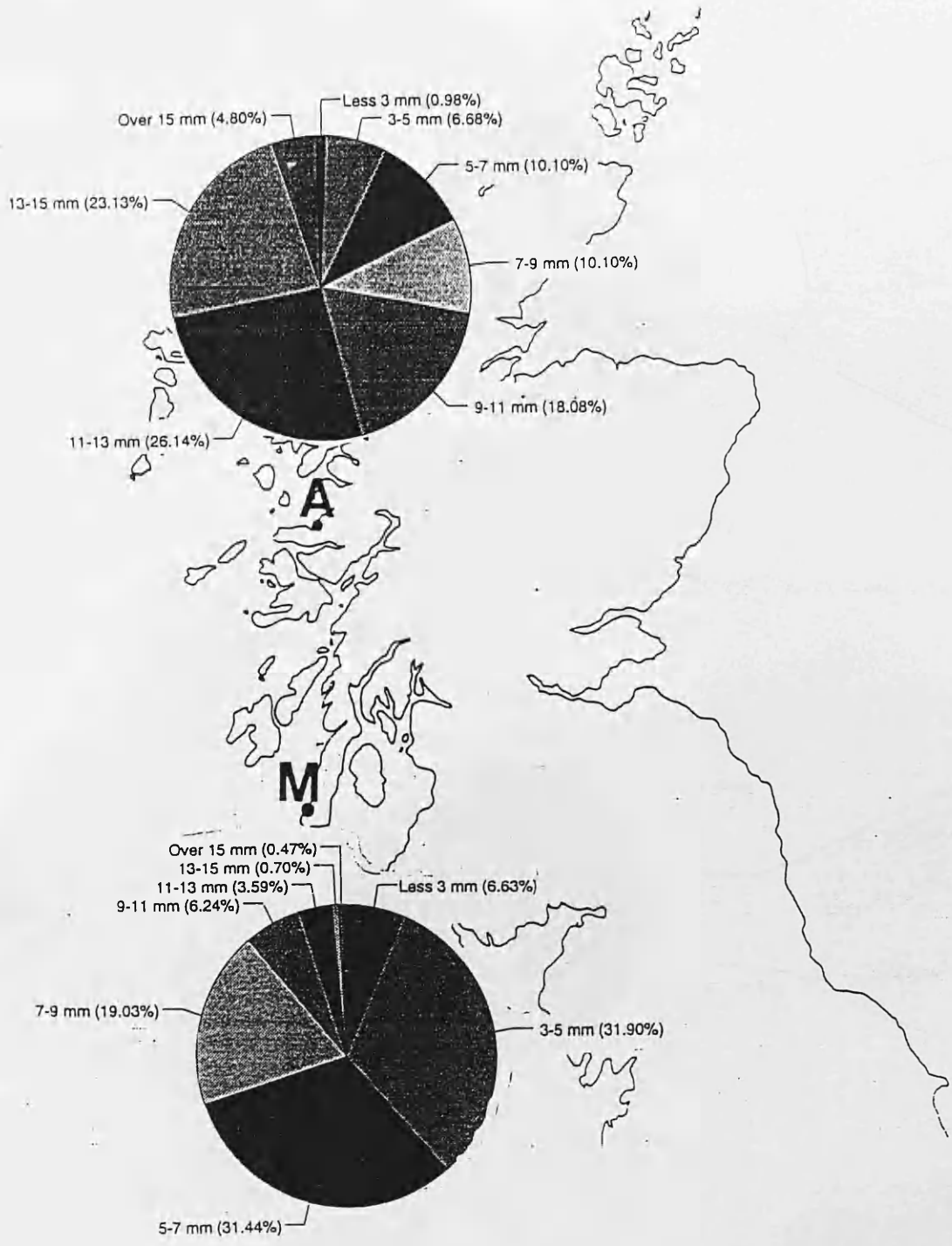
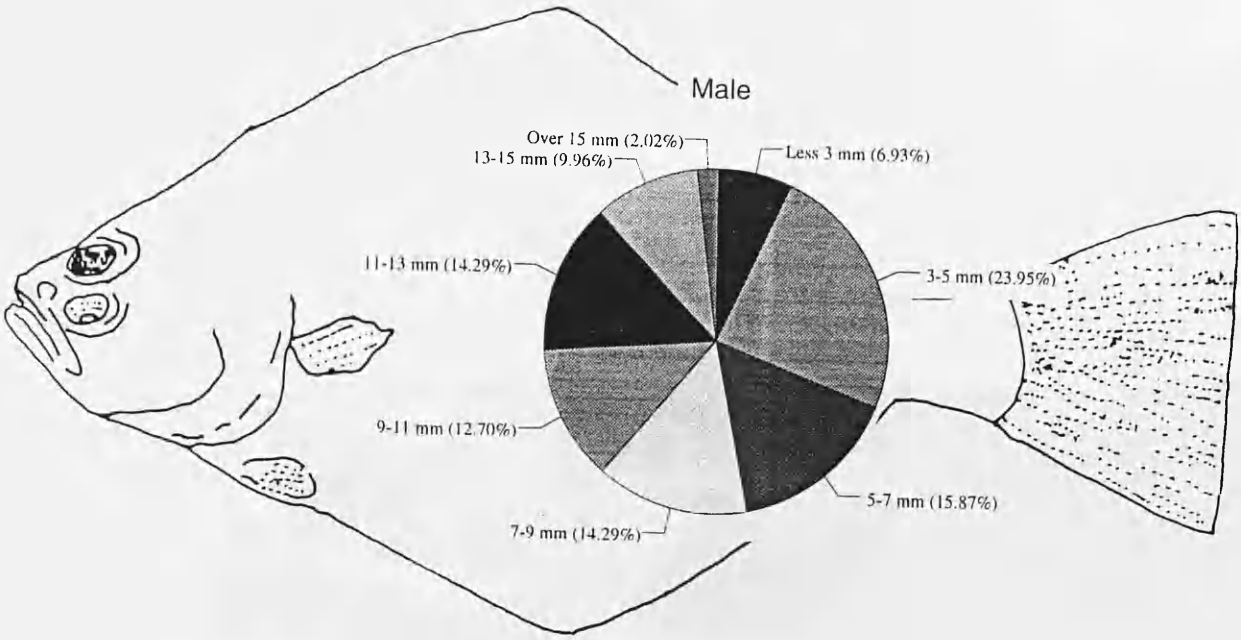


Figure 3.6. The population structure of *E. hippoglossi* on the different sites.
(A: Ardtoe, M: Machrihanish)



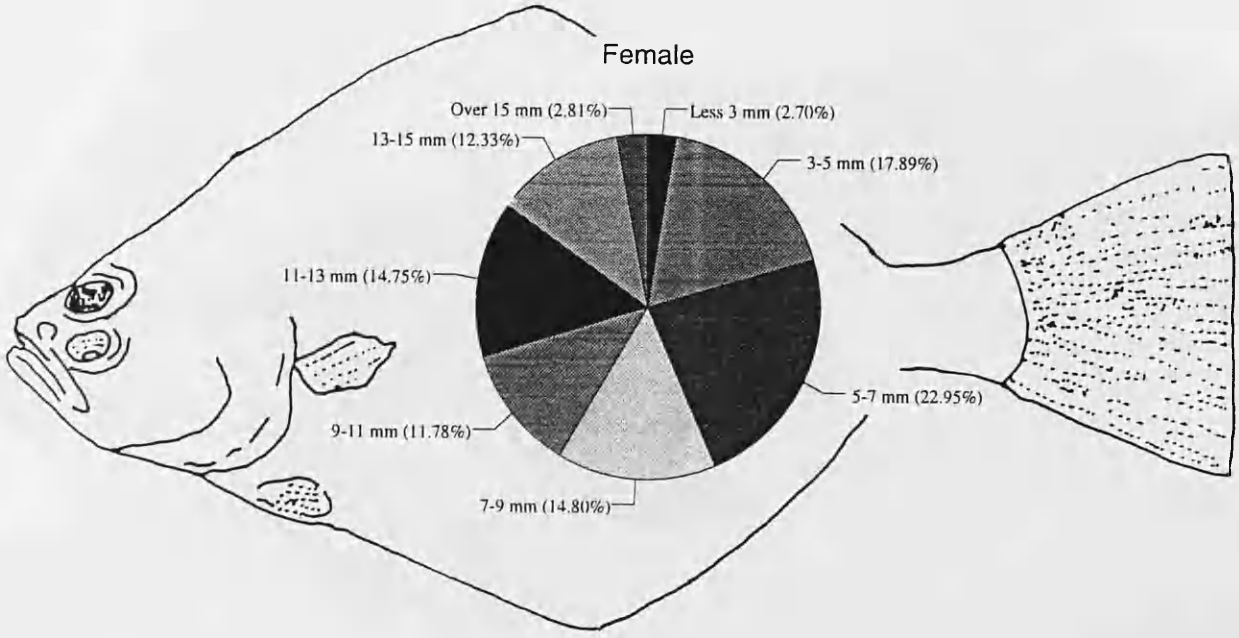


Figure 3.7. The population structure of *E. hippoglossi* on the different sexes of halibut.

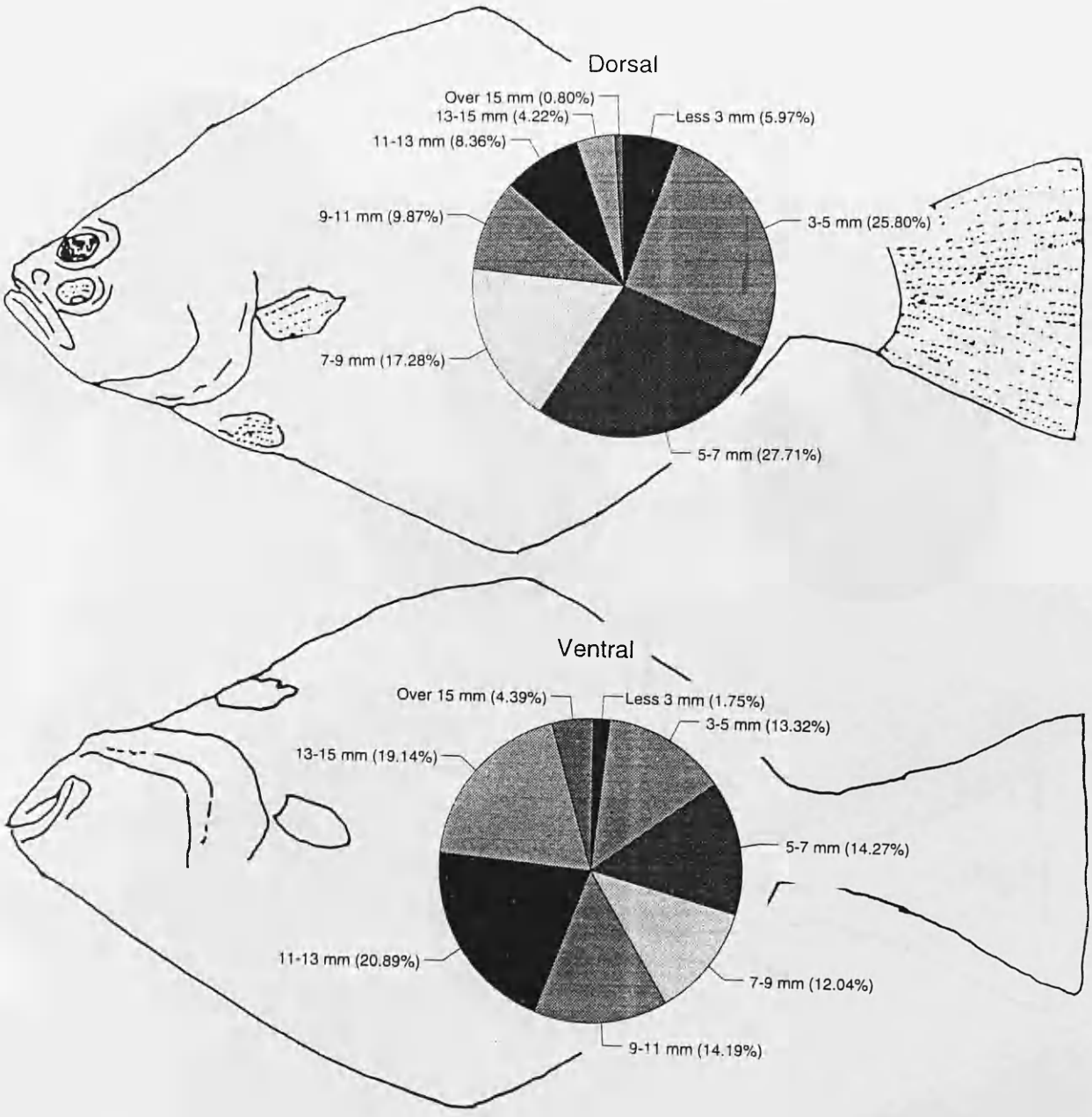


Figure 3.8. The population structure of *E. hippoglossi* on different sides of the halibut.

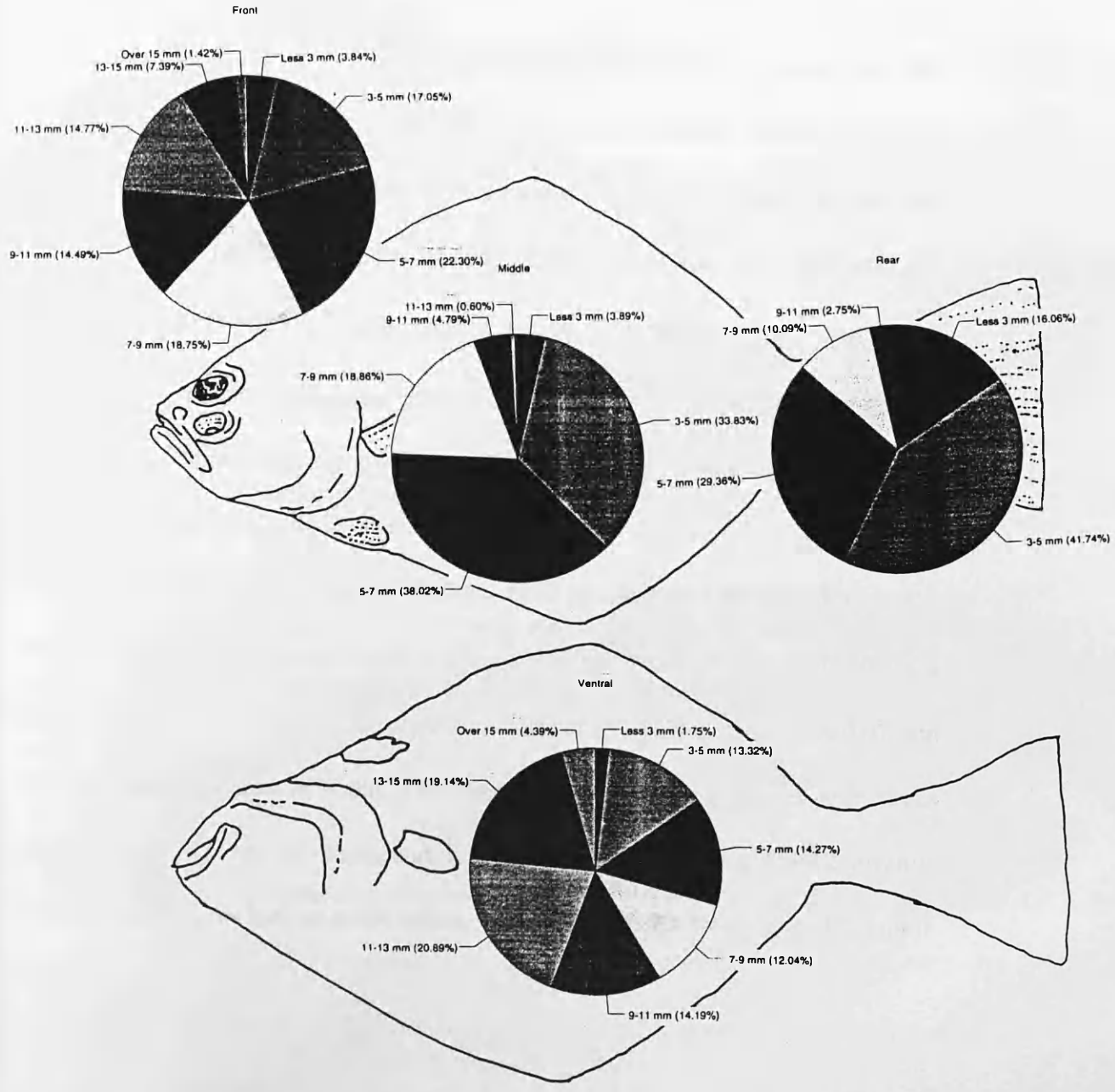


Figure 3.9. The population structure of *E. hippoglossi* on the 4 principal zones of halibut.

3.3.3 Somatic indices

3.3.3.1 A comparison between sites

The gonadal development of *E. hippoglossi* is asynchronous (protandrous). The testes appear before the ovary develops. Of the 1282 parasites collected from the Machrihanish halibut, 30 parasites were found to have undeveloped gonads. The mean length and width of parasites lacking sexual organs was 2.6 ± 0.56 mm and 1.0 ± 0.27 mm respectively. The smallest length of parasite having testes but no ovary was 1.5 mm and the biggest parasite measured 3.6 mm in length. Amongst the 30 juvenile parasites, only one parasite measuring 1.9 mm in length and 0.58 mm in width had neither testes nor ovary.

In the Ardtoe population, 12 out of the total 1228 parasites had developed no sexual organs. The mean length and width of those parasites was 2.5 ± 1.12 mm and 1.0 ± 0.52 mm respectively. The smallest parasite without an ovary or testes was 0.97 mm in length and 0.29 mm in width. The biggest parasite lacking ovaries with testes however, was 4.2 mm in length and 1.75 mm in width. Among the 12 juvenile parasites, 3 parasites had no testes either. All these parasites were under 1.5 mm in length.

3.3.3.2 A comparison of somatic indices of parasites collected from different sexes of host

Fourteen out of a total 693 parasites collected from male halibut were found to have either no ovary or testes or testes only. The mean length and width of these parasites were 2.5 ± 0.62 mm and 1.0 ± 0.32 mm. The smallest length of parasite was 1.6 mm

and the largest parasite with an undeveloped ovary was 3.6 mm. Among the 14 parasites, only one parasite measuring 1.9 mm in length and 0.58 mm in width had neither testes nor ovary. On the female hosts, 28 out of a total of 1817 parasites had no developed sexual organs. The mean length and width of these parasites was 2.6 ± 0.82 mm and 1.0 ± 0.37 mm. The smallest parasite without an ovary or testes was 0.97 mm in length and 0.29 mm in width. The biggest parasite measured 4.2 mm in length and 1.7 mm in width. Among these 28 parasites, 3 also had no testes. All these parasites were under 1.5 mm in length.

3.3.3.3 A comparison of somatic indices of parasites collected from the dorsal and ventral surfaces of host fish

Twenty-two parasites out of a total of 1256 from the dorsal surface of the host were found to have no developed ovary. All parasites from the dorsal surface of fish had testes. The mean length and width of parasites without an ovary was 2.6 ± 0.52 mm and 1.0 ± 0.22 mm. The smallest length of parasite was 1.5 mm and the largest parasite without an ovary was 3.6 mm. On the ventral surface, 20 parasites out of a total of 1254 parasites had no developed sexual organs. The mean length and width of these parasites was 2.5 ± 0.96 mm and 1.0 ± 0.47 mm. The smallest parasite without an ovary or testes was 0.97 mm in length and 0.29 mm in width. The largest parasite was 4.2 mm in length and 1.7 mm in width. Amongst these 20 parasites, 3 parasites had no testes either. All these parasites were under 1.5 mm in length.

The length and breath of the testes of parasites from the dorsal surface were 0.95 ± 0.27 and 1.53 ± 0.43. Those of parasites from the ventral surface were 1.0 ± 0.32

and 1.69 ± 0.54. Although there is no statistical difference, testes length and width of the parasites from the ventral surface were larger than those of the parasites from the dorsal surface.

3.3.3.4 Four principal zones (front dorsal, middle dorsal, rear dorsal and total ventral) (Table 3.6)

All the parasites from the dorsal surface had testes but 4 parasites from the ventral surface had no testes. Comparing the size of the sexual organs of the parasites from 4 principal zones, the parasites from the ventral surface had the biggest reproductive organs, although there was no significant difference when analysed statistically.

3.3.4 Hamuli

3.3.4.1 Dorsal and ventral

The average length of the anterior hamuli measured from parasites on the dorsal surface was 0.71 ± 0.18 mm, whilst those of parasites on the ventral surface averaging 0.83 ± 0.24 mm in length were significantly larger ($P < 0.05$). When hamuli length was correlated with the parasite length from both surfaces, dorsal and ventral, Figure. 3.10 shows that the length of the anterior hamuli are strongly positively correlated with the parasite length ($r = 0.778$, $P < 0.01$).

Table 3.6 The summary of somatic indices of parasites from four principal zones

Zones	Front	Middle	Rear	Ventral
Total parasites	704	334	218	1254
Testes but no ovary	12 (1.7 %)	6 (1.8 %)	4 (1.8 %)	20 (1.6 %)
No testes or ovary	0 (0 %)	0 (0 %)	0 (0 %)	4 (0.3 %)
Mean testes length	0.96 ± 0.3	0.81 ±0.4	0.85 ± 0.2	1.04 ± 0.3
Mean testes width	1.57 ± 0.4	1.34 ± 0.6	1.32 ± 0.3	1.69 ± 0.5

3.3.4.2 Four principal zones (Front dorsal, Middle dorsal, Rear dorsal and ventral total)

The mean hamuli length of parasites collected from the ventral zone was bigger than that of the parasites collected from the 3 other principal zones on the dorsal surface ($P < 0.05$). The mean hamuli lengths of parasites in the ventral, front dorsal, middle dorsal and rear dorsal zones were 0.83 ± 0.24 mm, 0.72 ± 0.18 mm, 0.64 ± 0.21 mm and 0.61 ± 0.10 mm, respectively (Figure.3.11).

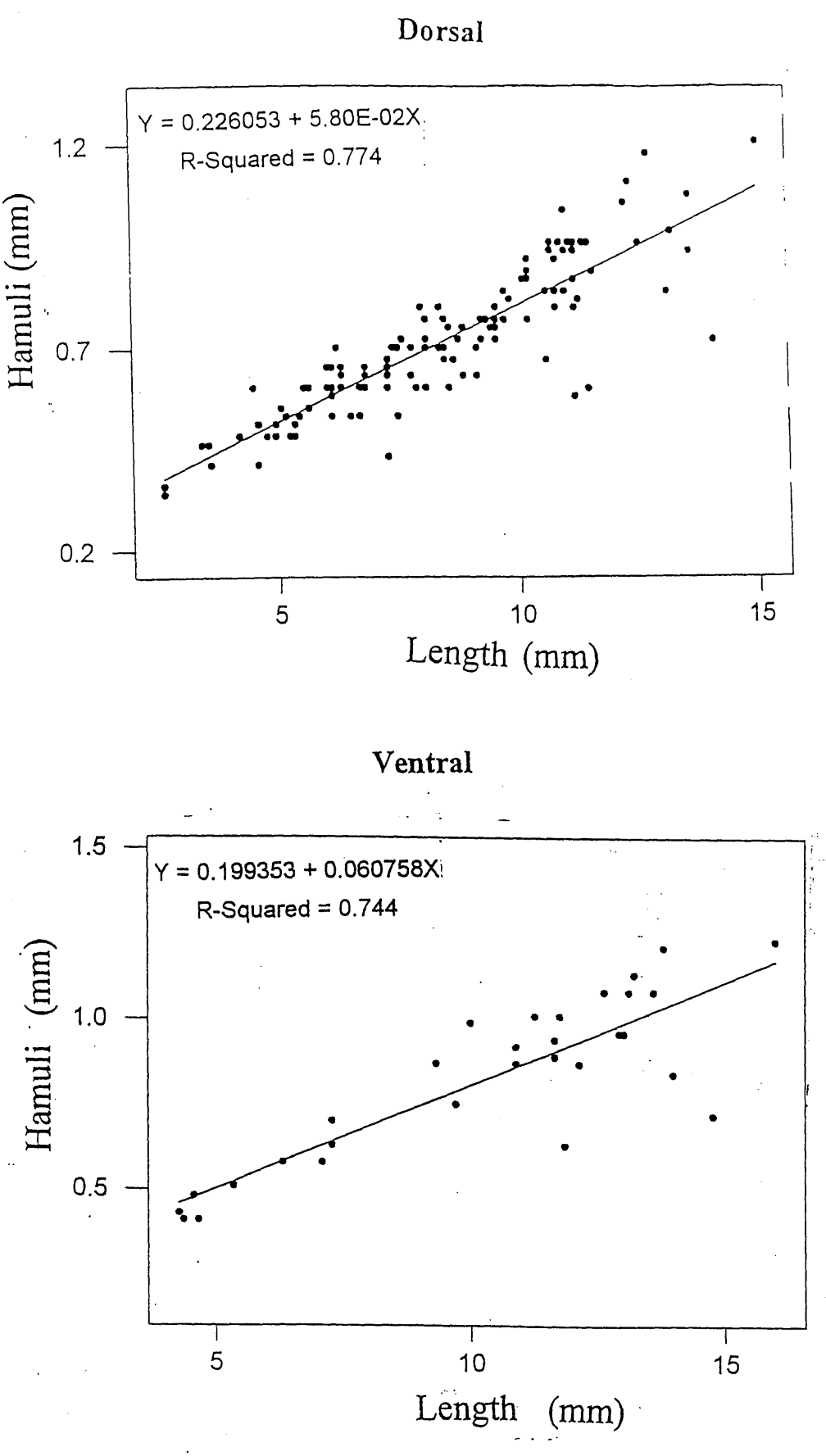


Figure 3.10. Relationship between total length and anterior hamuli length of the parasites on the dorsal and the ventral surfaces.

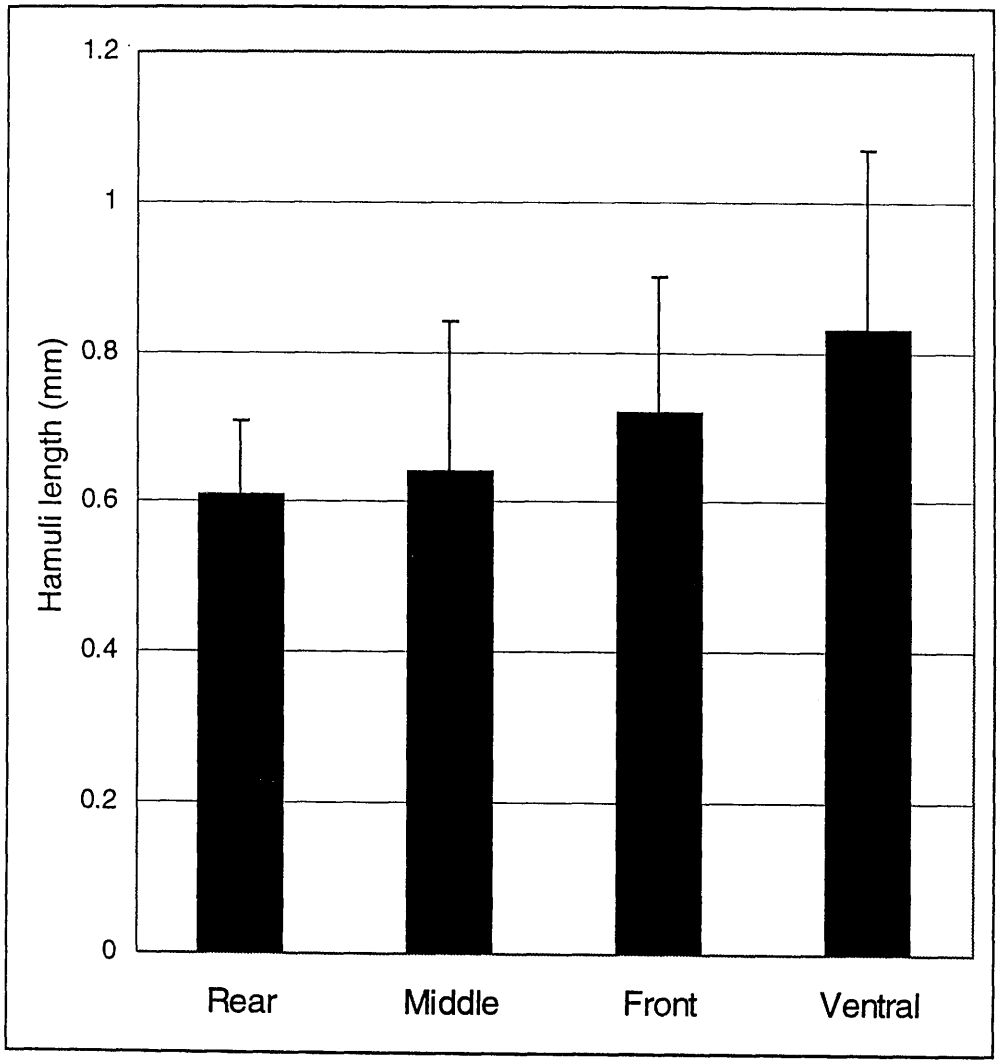


Figure 3.11. The mean anterior hamuli length of the parasites on 4 different principal zones (ventral, front dorsal, middle dorsal, rear dorsal).

3.4 DISCUSSION

3.4.1 Environmental influences

The results from the present study indicated that the two geographically different areas did not have a similar parasite population structure. The mean intensity of *E. hippoglossi* from Machrihanish fish was higher than that of parasites from Ardtoe. The mean length however, of parasites from Ardtoe fish was bigger than that from Machrihanish. When the parasites were taken from their host, the water temperature in Machrihanish was 9.5°C in May while at Ardtoe it was 5°C in March.

The findings from the present study show very strong evidence that the parasite population in Machrihanish was younger than that in Ardtoe. The dominant parasite size class range was 5 - 9 mm, 82.4 %, from Machrihanish whilst the same range class comprised 26.9 % of the Ardtoe population. In the Ardtoe population, 67.3 % of the parasites belonged to the largest parasite range of 9-15 mm. In comparison, only 29.6 % of the Machrihanish population was in this size class. In addition, 30 out of 1282 (2.3%) parasites from the Machrihanish fish had not developed either ovary or testes, while in Ardtoe fish, 12 out of 1228 (1.0 %) were in this category. These data suggest that the parasite population at Machrihanish were apparently younger whilst the population at Ardtoe was of predominantly older worms. Each species of monogenean has an optimum temperature for the maximum efficiency of reproduction (Chubb, 1977). The optimum temperature for reproduction and propagation for *Dactylogylus vastator* lies between 24-28°C according to Paperna

(1964). Pilcher, Whitfield and Riley (1989) suggested that a *Dichlidophora merlangi* Kuhn in Nordmann, 1832 population increased at an optimum of 7-11°C, with progressively declining reproductive success below 7°C and above 11°C. The population of the gill parasite, *Bivagina tai* Yamaguti, 1938 on cultured red sea bream, *Pagrus major* increased when the water temperature was about 20°C (Ogawa, 1988). He suggested that increasing water temperature must be an essential factor to facilitate the growth of the parasite population due to a shorter incubation time of the egg and to a shorter maturation time on the host. There has not been any study on the optimal temperature for *E. hippoglossi* reproduction. However monogenean ectoparasites must be affected by the host environment. The optimal temperature for 3-5 kg Atlantic halibut was suggested to be around 10°C by Bjørnsson and Tryggvadottir (1996). Therefore, it may be that the optimum temperature for reproduction of *E. hippoglossi* might be around 10°C. That may explain why the parasite burden at Machrihanish was higher than at Ardtoe.

Jansen and Bakke (1991) found that the average life –span of *G. salaris* was up to 5 weeks and was negatively correlated to temperature, but the potential for population growth was positively correlated to temperature. The water temperature strongly affected both the birth and mortality processes of *G. salaris* and these determine the parasite population growth. Unfortunately, in the present study, there was no observation of the life span or generation time for *E. hippoglossi*. However, regarding the findings of the above authors, it may be assumed that the generation time of the parasites in Machrihanish was shorter than that in Ardtoe. Therefore, the *E. hippoglossi* population structure at Machrihanish showed smaller parasites were dominant whilst a bigger parasite class was dominant in Ardtoe.

There may be a further reason why Ardtoe's parasites were bigger than Machrihanish's. If two groups of parasites or animals have the same amount of space and food, the group with the smaller number would get a better chance than the bigger population group to compete for food and space. However, in the present study, the initial population history was not known, but the number of small parasites indicated that the parasite population in Machrihanish was actively growing or, at least, the generation time of parasites may have been shorter than the parasites in Ardtoe.

3.4.2 Host sex and size influence on the parasite population

One of the most important factors influencing the composition of the parasite fauna is age or size and sex of host (Dogiel, 1964). Schram and Haug (1988) observed that 3-4 female caligid *Lepeophtheirus hippoglossi* infected the male hosts whilst 5-6 were found on females. *E. hippoglossi* was also found more on females than on males. This might be due to the more active spawning behaviour of males, making it difficult for settling of oncomiracidia of *E. hippoglossi* and larvae of *L. hippoglossi.* Schram and Haug found parasites only from adult halibut and none from juveniles, which might suggest that the parasites did not infect until the adult population congregated for spawning. In the present study, the mean intensity on female hosts was 606, whilst that on male hosts was 231, similar to what Schram and Haug (1988) found on wild halibut. However, in this study the smallest size class, less than 3 mm, was 6.9 % and 2.7 % of the population on the male and female hosts, respectively. It seems that the difference in the population structure on the different

sexes is not only due to the host spawning behaviour as Schram and Haug suggested, since immature young worms were clearly able to settle on the male hosts. The size of male and female halibut was different in the present study. Usually, the weight of the female was 2- 4 times greater than that of the male. This might be one of reasons why male halibut had a lower parasite population than the female. It seems that the bigger surface of female halibut was providing more food and a larger surface for attachment of the parasites. However, it is unclear why the smallest size class of parasites on the male host was more than that on the female host, but the following hypotheses are suggested. First, the parasite growth rate between male and female hosts might be different. Second, more oncomiracidia attach to the male host then, as Schram and Haug (1988) suggested, parasites might fall off due to aggressive behaviour of male halibut.

3.4.3 Parasite distribution on the host

In the present study, the numbers of parasites from the dorsal and ventral surfaces were almost the same. On the dorsal surface where their precise location could be studied, however, the parasites were not distributed randomly. The parasites were mainly found in the front region on the dorsal surface of the halibut. The spatial distribution of the oncomiracidia may hold the key to the infection dynamics. Kearn (1963a) also observed a larger number of *E. soleae* on the lower surface while smaller parasites were found on the upper surface of heavily infected sole, *Solea solea*. He found that the free-swimming oncomiracidia invaded the anterior part of the upper surface of the fish, which is the only part exposed when the fish is buried

in the sand on the bottom of the sea. After a short period of development, the parasites on the upper surface migrated to the lower surface of the host.

Strong evidence that *E. hippoglossi* also migrated over the host surface was found in the present study. In contrast to *E. soleae* from the sole, oncomiracidia of *E. hippoglossi* seem to invade the posterior dorsal part of the halibut. Then they migrate from the posterior to anterior part of the dorsal surface and finally they orientate towards the ventral surface of the host.

Mostly, site specificity studies have been concerned with gill parasites. Hanek and Fernando (1978a, b) found that there was a significant preference for the anterior medial section of the hemibranchs by the monogeneans of pumpkinseed sunfish *Lepomis gibbosus* L., 1758 and rock bass *Ambloplites rupestris* Rafinesque, 1817. With regard to Monogenea on marine fish, Llewellyn and Macdonald (1980) found that there was a distinct preference for the 2nd and 3rd gill arches by diclidophoran gill parasites of trisopteran (gadoid) fishes. Most parasites attached themselves to the middle third of the gill arches and to proximal regions of the primary lamellae. Rohde (1978) showed that the most frequent location of parasites on the gills of marine fish caught off the Great Barrier Reef of Australia, was gill arch I with moderate levels in II and III and least frequently on gill arch IV. Spatial distribution could be due to centrifugal migration of the parasites on the gill arches (Lambert and Maillard, 1975). How the oncomiracidia of parasites reach the gills of the host fish is not clear. The larvae may be carried passively into the mouth of the fish with the respiratory current and then be swept onto the gills (Bovet, 1967; Paling, 1969). Alternatively, the larvae may possibly attach anywhere on the surface of the host fish and subsequently migrate over the surface to the opercular cavity and gills (Kearn, 1968).

In the present study, looking at the population structure on the 4 principal zones, 3-7 mm size classes were the majority groups on the 3 dorsal zones, whilst 11-13 mm was the dominant size class on the ventral surface. However, the parasites which had undeveloped sexual organs were almost equally distributed on the 4 principal zones (1.4 –1.8 %). It suggested that the oncomiracidia can invade every part of the host body, in cultured conditions at least, then they migrate to their final destination, the ventral surface of the host.

Kearn (1988) found that most adult and large immature specimens of *E. soleae* from the common sole, *Solea solea*, were settled on the lower surfaces of their host. He observed that the parasites migrated forward from the tail on the top surface then moved to the lower surface of the host. Kearn (1984) suggested that the advantages of migration from the upper surface to the lower surface were increasing opportunity for exchanging spermatophores, having a better position to attach eggs to sand grains and avoiding predators. It is assumed that *E. hippoglossi* migrated for the same reasons as *E. soleae* in the present study. However more precise studies are needed to confirm this in *E. hippoglossi.*

Chapter 4

EGGS

4. EGGS

4.1 INTRODUCTION

4.1.1 Egg laying behaviour

Monogenean parasites lay eggs which generally fall free of the host, drop in the water column and develop at the bottom until hatching. However, some eggs from Monogenea attach to the host surface. Egg bundles of *Dionchus remorae* MacCallum, 1916 attach to gill filaments of the remora, *Echeneis naucrates* L. by a loop of eggshell material (Whittington, 1990). The eggs of *Nitzschia sturionis* Abildgaard, 1794 were firmly cemented by the appendages to the mucous membrane of the buccal cavity of the sturgeon (Bychowsky, 1957). Roubal (1994) confirmed that the gill parasite, *Lamellodiscus acanthopagri* L. attached eggs to the gill lamellae of *Acanthopagrus australis* Guenther using a thorn-like egg filament.

4.1.2 Egg production

Many studies have determined the egg output in various monogeneans. Some of them examined egg production by parasites detached from the host (Llewellyn, 1957; Anderson, 1981; Finlayson, 1982) while others were based on the egg production of parasites still attached to the host (Tinsley and Owen, 1975; Jackson, 1982). Generally, most monogeneans lay fewer than 100 eggs/ parasite/ 24 hours and many of them produce fewer than 25 eggs. In contrast, Combes (1972) found that *Polystoma integerrimum* Froelich, 1791 laid from 1000 to 2500 eggs in one day,

whilst Tinsley (1983) found that *Pseudodiplorchis americanus* Rodgers and Kuntz, 1940 and *Neodiplorchis scaphiopodis* Rodgers, 1941, which infect the urinary bladder of amphibians probably produces no more than 250 eggs annually. When the egg output of dactylogyrid monogeneans were compared, different results were obtained depending on whether the parasites were attached or detached from the host (Paperna, 1963a). Prost (1963) found that the detached *Dactylogyrus anchoratus* (Duj., 1845) Wagener, 1857 produced more eggs than when attached to the host.

Many factors influence the egg laying rates of parasites. Kearn (1985) has shown that the egg production of *E. soleae* increased as the adult parasite increased in size. Egg production, however, may slow down or decrease as the parasites near the end of their life.

Temperature, oxygen and salinity significantly affect the egg production of parasites (Prost, 1963; Combes, 1972; Houlihan and Macdonald, 1979; Anderson, 1981; Winch, 1983). The effects of host starvation on the egg production in the blood feeding *Protopolystoma xenopodis* Price, 1943 was studied by Jackson (1982). Egg output was reduced by 50 % when parasites were transferred from well fed hosts to hosts which had been starved for 6 months. Other factors may affect egg laying rate. Parasite burden was positively related to egg laying rate in some monogenean infections (Tinsley and Owen, 1975; Jackson and Tinsley, 1988). Conversely, *Polystoma integerrimum* decreased their egg laying rate as the number of parasites increased (Combes, 1972).

Macdonald and Jones (1978) found that *Diplozoon homoion gracile* Bychowsky and Nagibina, 1959 laid more eggs at night and suggested that this might lead to the

accumulation of eggs in sheltered, night time resting areas sought out by the host, *Barbus meridionalis* Risso,1826.

4.1.3 Egg shape

Monogeneans produce eggs of different shapes and sizes and there is a remarkable diversity among the genera. 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 (Baer and Euzet, 1961).
Many monogenean eggs have filamentous apical extensions of eggshell material. Bychowsky (1957) proposed the terms "filament" and "little foot" to distinguish between extensions of the distal (= leading) and proximal (= trailing) apices of a fusiform egg during its passage from the ootype into the uterus. Later the same extensions were called "appendages" because of the difficulty of applying these terms to tetrahedral eggs (Kearn, 1963b). The eggs of parasites may be joined together by their appendages. Some monogeneans such as *E. soleae* (Kearn, 1963a) *and Acanthocotyle lobianchi* Monticelli, 1890 (Kearn, 1967a) have sticky material on the appendages. The eggs of *Hexabothrium squalonchocotyle* were attached together by the fusion of the opercular and abopercular appendage (Kearn, 1986), while eggs of *Diclidophora luscae* Beneden and Hesse, 1864 and *Dactylocotyle denticulata* Olsson, 1876 are attached together by the entanglement of their slender and coiled appendages (Kearn, 1986).

4.1.4 Objectives

It is suggested that the reproductive biology of *E. hippoglossi* might be the same as that of *E. soleae.* However, knowledge of the spawning behaviour and egg production of *E. hippoglossi* is far from complete. The aim of the present study was to investigate the biology of reproduction of *E. hippoglossi* on the Atlantic halibut from aquaculture systems and to compare it to that of *E. soleae* which is closely related to *E. hippoglossi.*

4.2 MATERIALS AND METHODS

4.2.1 Egg spawning observations

Entobdella hippoglossi were maintained at 12°C. The water was changed daily using sterilised sea water (33 ppt) which was allowed to adjust to 12°C before replacing the parasites.

Parasites were monitored every hour under an Olympus (CH2) binocular microscope to observe their spawning behaviour. Parasites were observed briefly and then they were returned to the incubator. Important spawning activities were photographed using an Olympus BH-2 research microscope fitted with an automatic photomicrographic camera.

4.2.2 Egg laying studies

Twenty parasites were randomly harvested from each of the dorsal and the ventral surfaces of 2 infected male and 2 female Atlantic halibut, *H. hippoglossus*. The length of the males was 70 cm and 65 cm and the length of females was 104 cm and 102 cm. Parasites infecting the dorsal surface of the host were kept separate from those individuals parasitising the ventral surface. Individual parasites were kept and maintained in 33 ppt seawater at 12°C in plastic petri dishes measuring 5 cm in diameter by 1 cm in depth. After 24 hours, eggs which had been released were counted using an Olympus binocular microscope (x 4).

4.2.3 SEM study of eggs

Eggs of *E. hippoglossi* were collected from egg laying chambers and eggs of *E. soleae* were received from Dr. G. C. Kearn, University of East Anglia, England. They were processed following the procedures described in Chapter 2.

4.2.4 Statistics

The non-parametric Kruskal - Wallis test was used for comparing the egg laying rate of the parasites from different sexes of halibut and different sides of host (dorsal and ventral). The same test was used for analysing the measurements of the parasite length from different host sexes and different sides. All data were considered significant at values of $P<0.05$.

4.3 RESULTS

4.3.1 Egg spawning observations using the light microscope

Parasites were found to strongly attach themselves to the bottom of a plastic container after removing them from their hosts and moved actively over the surface of their container. The eggs were discharged from the uterus by very powerful contractions of the anterior part of the parasite.

Figure 4.1 shows the eggs (E) being expelled from the genital pore (GP) of the parasite (P). The tetrahedral shaped egg emerged first, followed by the egg filament. The manner in which eggs were released from the body of the parasite was that the filaments were entwined, to the extent that eggs were laid in a chain-like fashion. The eggs became entangled thus: as the first egg was laid it emerged from the body of the parasite, but the egg filament was still within the parasite. The parasite produced second and subsequent eggs before the filament of the first egg was fully released from the body of the parasite. This manner of egg laying meant that the egg filaments entwined internally so that they were linked in a chain-like fashion externally (Figure 4.2). As the eggs were laid in a string or chain rather than as a discrete bundle, eggs were occasionally found attaching to the haptor part of the mother parasite (Figure 4.3) where they then aggregated together to form an egg mass (Figure 4.4). Producing eggs in a chain was the usual manner in which this parasite species distributed their eggs.

However, a second mechanism was occasionally observed. Unusually, the parasite laid eggs with an 'egg ball'. It was believed that this represented a real alternative strategy of egg laying and not an artificial *in vitro* observation. Here, the parasite laid her eggs and then all the eggs appeared to be anchored together attached to a

ball of unknown material but possibly secreted by the genital complex (Figure 4.5). The precise manner in which these eggs were laid was unknown since, although the parasite was closely observed, this mechanism was never seen. The number of eggs attached to the 'egg ball' varied, some egg balls bearing as many as a hundred eggs (Figure 4.6) with others bearing only a few eggs (Figure 4.7). These two types of egg masses developed normally and then successfully hatched as seen in later observations.

A third mechanism was found where some parasites laid single eggs. These eggs, although normal in their shape, were found not to develop any further. *i.e.* were sterile (Figure 4.8). The sterile eggs decomposed in the seawater so that when observed later, only the appendage was found in the egg container.

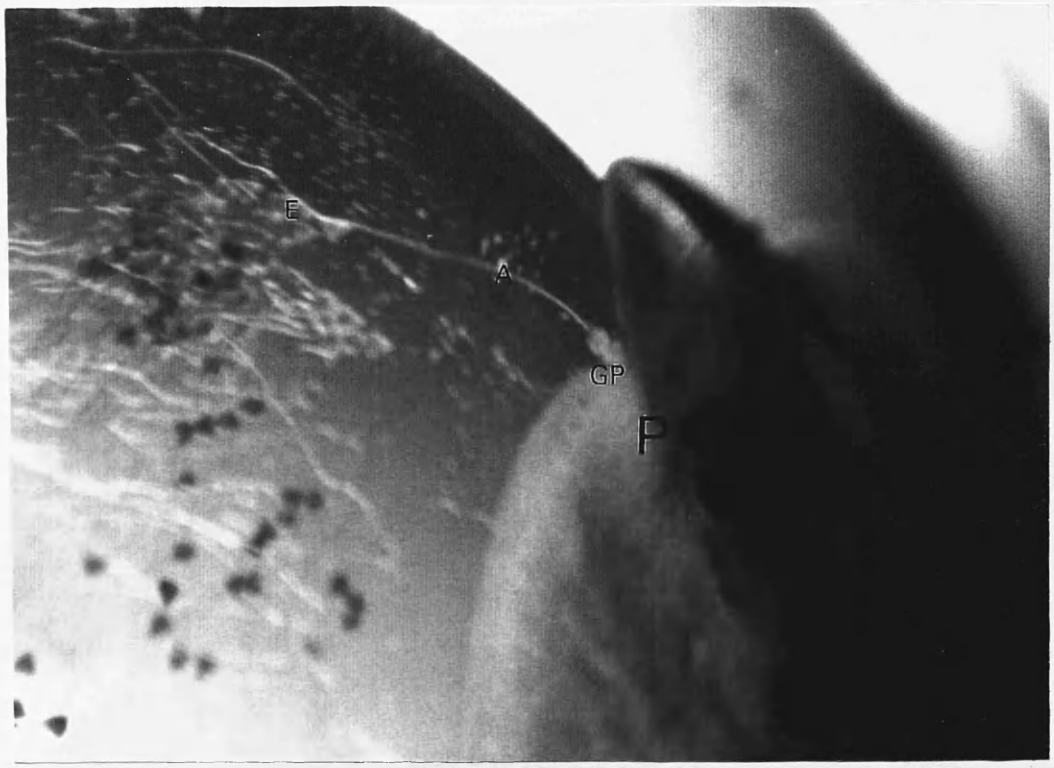


Figure 4.1 Light microscopy observation of *E. hippoglossi in vivo* discharging an egg through the genital pore. Note the act of egg-laying appeared to involve muscular effort involving contraction of the general body musculature as well as of the muscles associated with the reproductive tract.
(**E:** eggs, **GP:** genital pore, **P:** parasite). (x 1 objective).

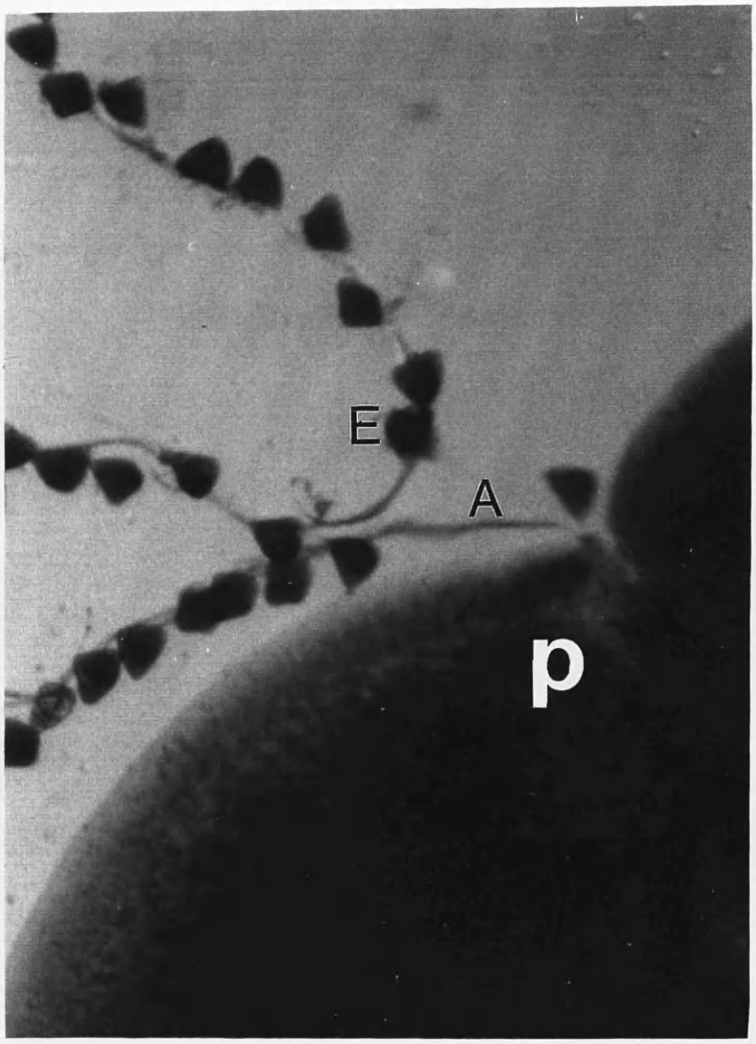


Figure 4.2 Light microscopy observation of *E. hippoglossi* laying eggs in a chain-like fashion. Note the tetrahedral shaped egg emerged first, followed by the egg filament.
(**P:** parasite, **E:** egg, **A:** appendage). (x 4 objective).

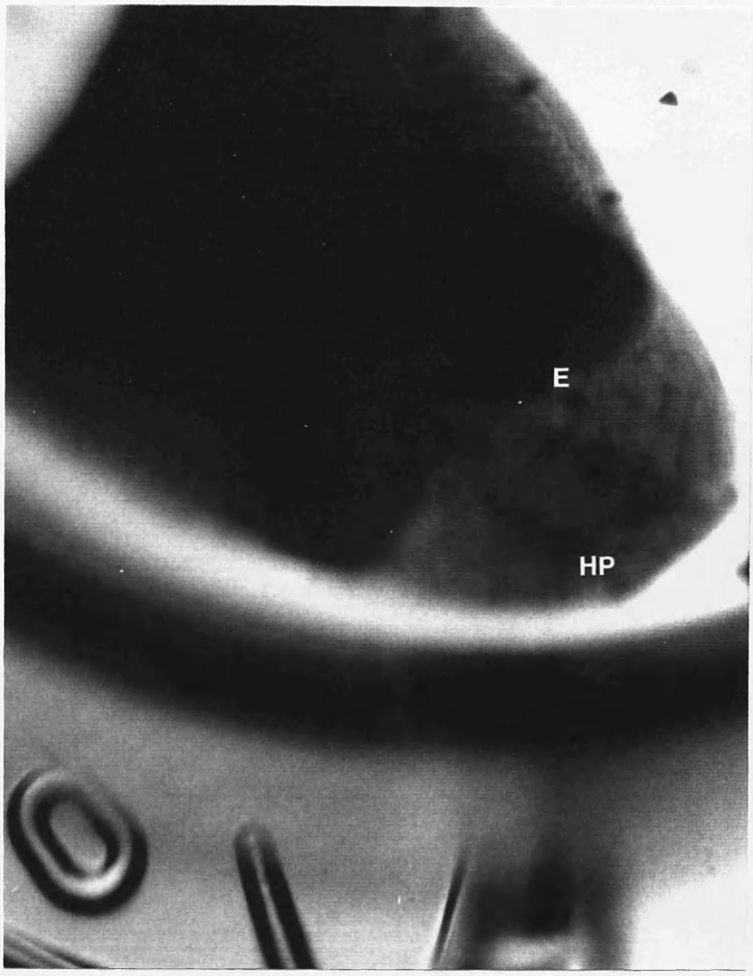
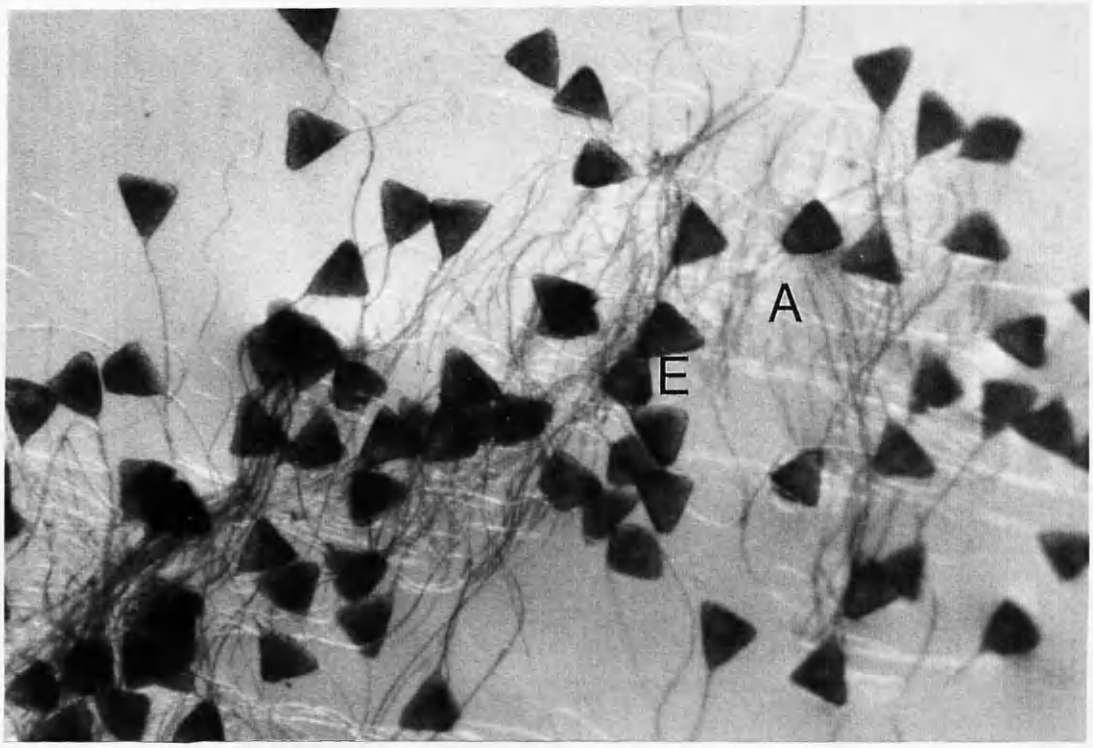


Figure 4.3 Light microscopy observation showing the eggs of *E. hippoglossi* are found attached to the haptor of the mother parasite. Note they then aggregated to form an egg mass outside the parasite.
(P: parasite, **E:** egg, **HP:** haptor). (x 1 objective).


A
E

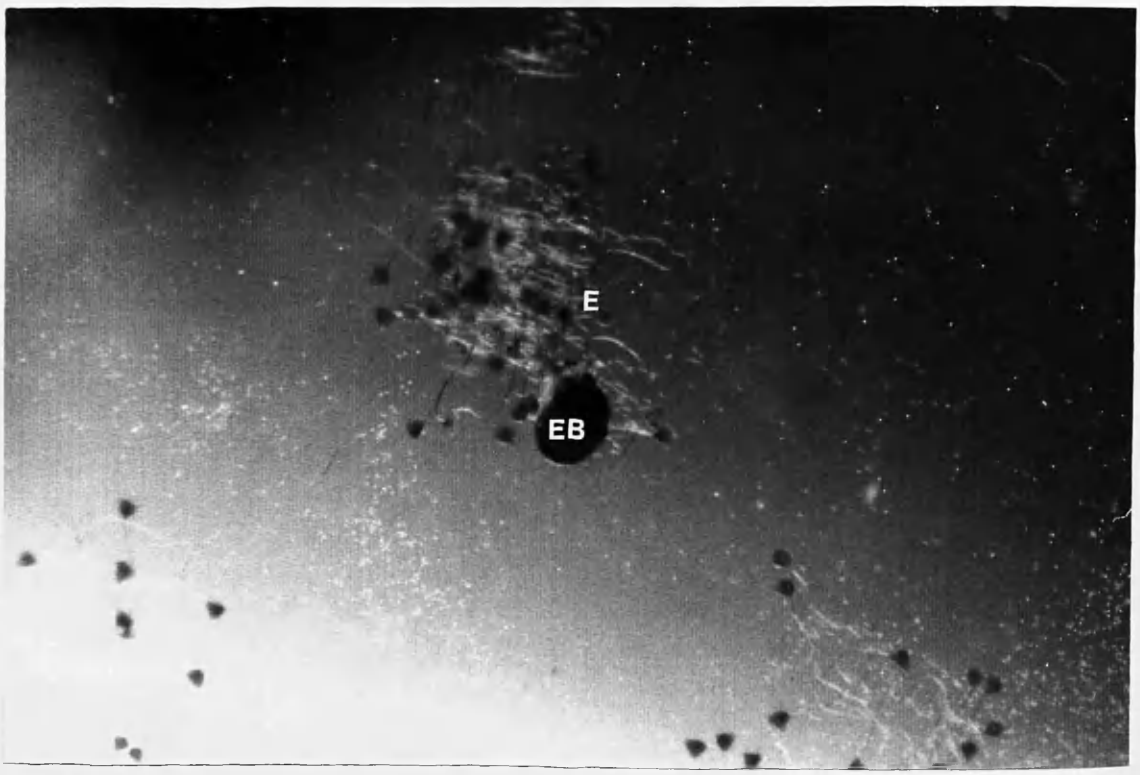
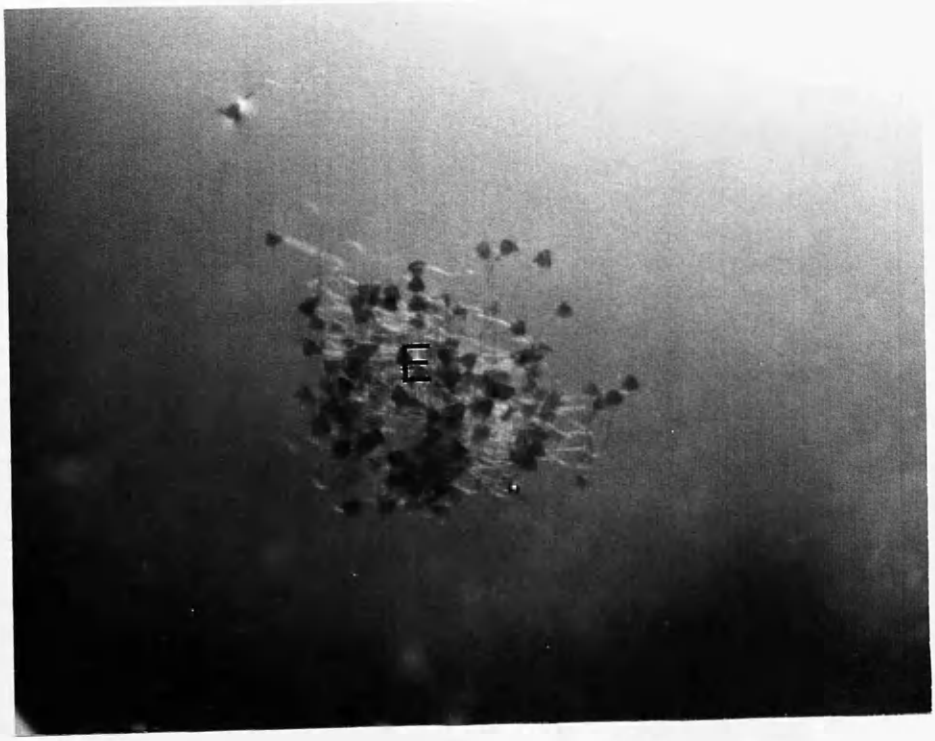

E
EB

Figure 4.5 Light microscopy observation of *E. hippoglossi* egg mass showing one type of pattern of egg production where eggs are laid in a chain-like style then entwined by their filaments. (**E:** egg, **A:** appendage). (x 4 objective).

Figure 4.5 Light microscopy observation showing one of the egg laying patterns of *E. hippoglossi.* Note all of the eggs appeared to be anchored together attached to a ball of unknown material. (**E:** egg, **EB:** egg ball). (x 1 objective).


E

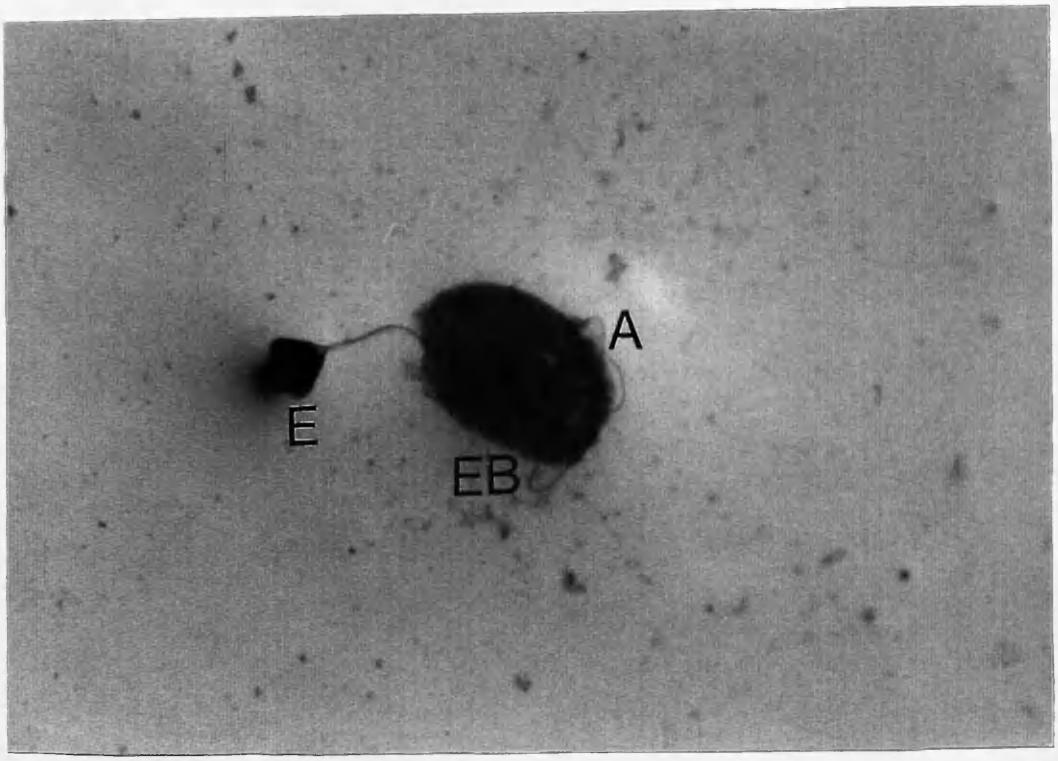

A
E
EB

Figure 4.6 Light microscopy observation showing eggs of *E. hippoglossi* combined together with an egg ball. Note one egg ball has as many as a hundred eggs and eggs are anchored with egg ball.
(**E:** egg). (x 1 objective).

Figure 4.7 Light microscope observation showing a single egg attached to a large egg ball. Note the egg attached to the egg ball with entwined appendage.
(**E:** egg, **EB:** egg ball, **AP:** appendage). (x 4 objective).

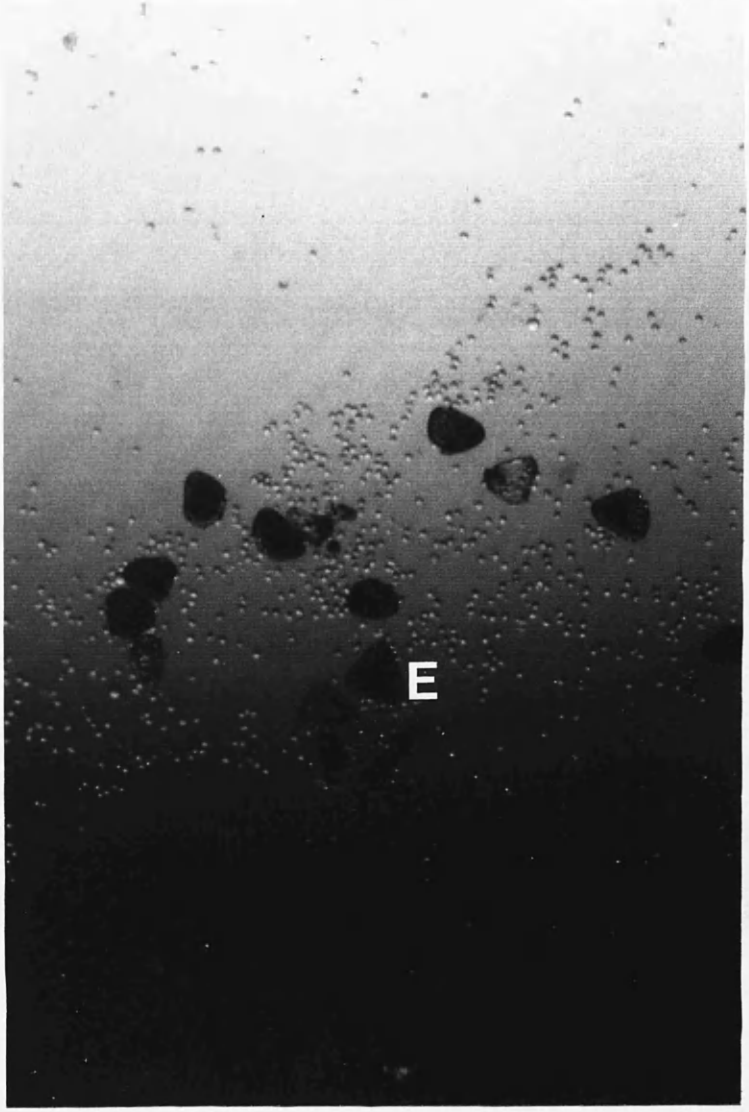


Figure 4.8 Light microscopy observation showing eggs laid singly. Note the singly laid eggs are thought to be sterile and rapidly decompose.
(**E:** egg). (x 4 objective).

4.3.2 Egg laying rate

4.3.2.1 A comparison between parasites collected from male and female hosts

There was no significant difference between the mean number of eggs (total number of eggs/ total number of parasites) produced by parasites from the 2 male hosts (26.9 ± 10.4 eggs, n = 40) and eggs produced by parasites from the 2 female hosts (35.2 ± 9.9 eggs, n = 40) ($P>0.05$). It was found that the same number of worms from both male and female fish (20 out of 40, 50 % each) were found to be laying eggs. Although there was no significant difference statistically, the average number of eggs produced by the spawning parasites only (total number of eggs/ the number of spawning parasites) was higher for those collected from the female hosts (70.3 ± 16.6 eggs, n=20) than produced by spawning parasites only from the male hosts (53.8 ± 19.2 eggs, n=20) (Figure 4.9).

The mean length of the parasites from the males was 8.3 ± 0.4 mm, n = 40 and from the females was 9.3 ± 0.4 mm, n = 40. When compared, they were not significantly different ($P>0.05$). Figure 4.10 illustrates the length frequency distribution of parasites from male and female hosts. The pattern of distribution was the same on both hosts.

4.3.2.2 Egg laying rate; dorsal and ventral surfaces compared

The mean number of eggs produced (total number of the eggs/ total number of the parasites) by parasites from the ventral surface (53.2 ± 13.2 eggs, n = 40) was significantly greater than those produced by parasites from the dorsal surface (8.9 ± 2.8 eggs, n = 40)($P < 0.05$). Also, it was found that a greater proportion of worms on the ventral surface were found to be spawning eggs (26 out of 40, 65 %) than on the dorsal surface (14 out of 40, 35 %). The number of eggs produced by spawning parasites only was significantly greater ($P < 0.05$) on the ventral surface (81.8 ± 18.0 eggs, n=26) than on the dorsal surface (25.4 ± 6.1, n=14) (Figure 4.11).

Figure 4.12 shows that larger parasites laid significantly more eggs than smaller parasites on both dorsal and ventral surfaces of their halibut host ($P<0.05$). A regression line was calculated for the relationship between parasite length and egg productivity for both dorsal and ventral surfaces (see Figure 4.12). This showed that there was no significant difference between the slopes of the lines and the R-squared values between the two sides (dorsal and ventral). The length of parasites on the dorsal surface accounts for 27.3 % of the variability in egg productivity, whilst the parasite length accounts for 33.7 % of the variability in egg productivity from parasites on the ventral surface.

The parasites on the ventral surface were significantly larger (9.4 ± 0.34 mm, n=40) than those parasites on the dorsal surface (8.2 ± 0.4 mm, n=40) ($P<0.05$). This observation was supported by a larger scale study in chapter 3, where the average length of parasites on the dorsal surface was found to be 6.9 ± 3.1 mm (n = 1256), whilst parasites on the ventral surface measured 9.7 ± 4.5 mm (n = 1254).

Figure 4.13 illustrates the length frequency distribution of parasites comparing dorsal and ventral surfaces of the fish. Whereas the pattern of distribution was the same on both surfaces, there was a displacement towards the smaller size worms on the dorsal surface.

The different size distribution of parasites on the ventral and dorsal surfaces of halibut therefore explained the observed higher number of eggs produced on the ventral surface of the fish compared to the dorsal surface. For parasites on both surfaces however, there were major differences in the egg productivity of similar sized parasites. For example, a parasite collected from the ventral surface measuring 10 mm in length produced approximately 80 eggs in a 24 hour period whilst a similar sized parasite from the dorsal surface laid c.35 eggs over the same time period. This suggests that there are factors other than worm size which enhance egg production on the ventral surface of the fish.

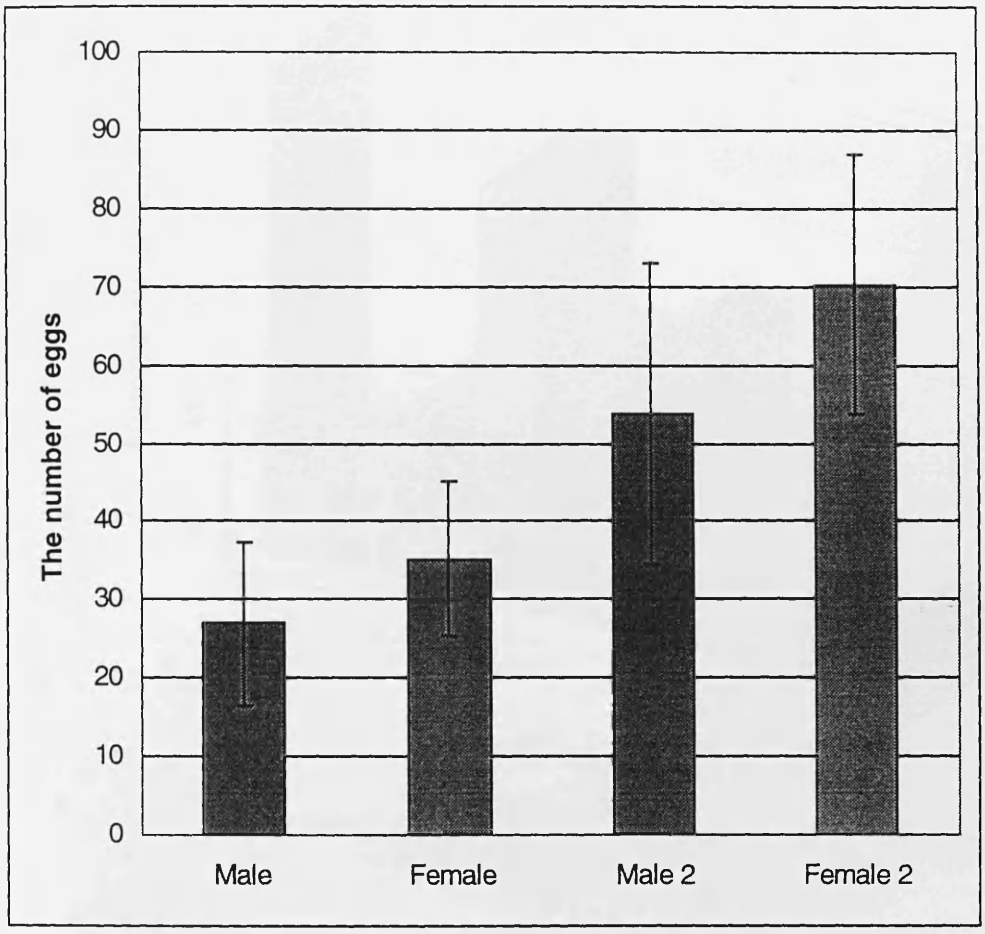


Figure 4.9 The mean number of eggs produced by parasites collected from male and female hosts.

Male: The total number of eggs was divided by the total number of parasites from the male host (n=40)

Female: The total number of eggs was divided by the total number of parasites from the female host (n=40).

Male2: The total number of eggs was divided by the spawning parasites only from the male host (n=20).

Female2: The total number of eggs was divided by the spawning parasites only from the female host (n=20).

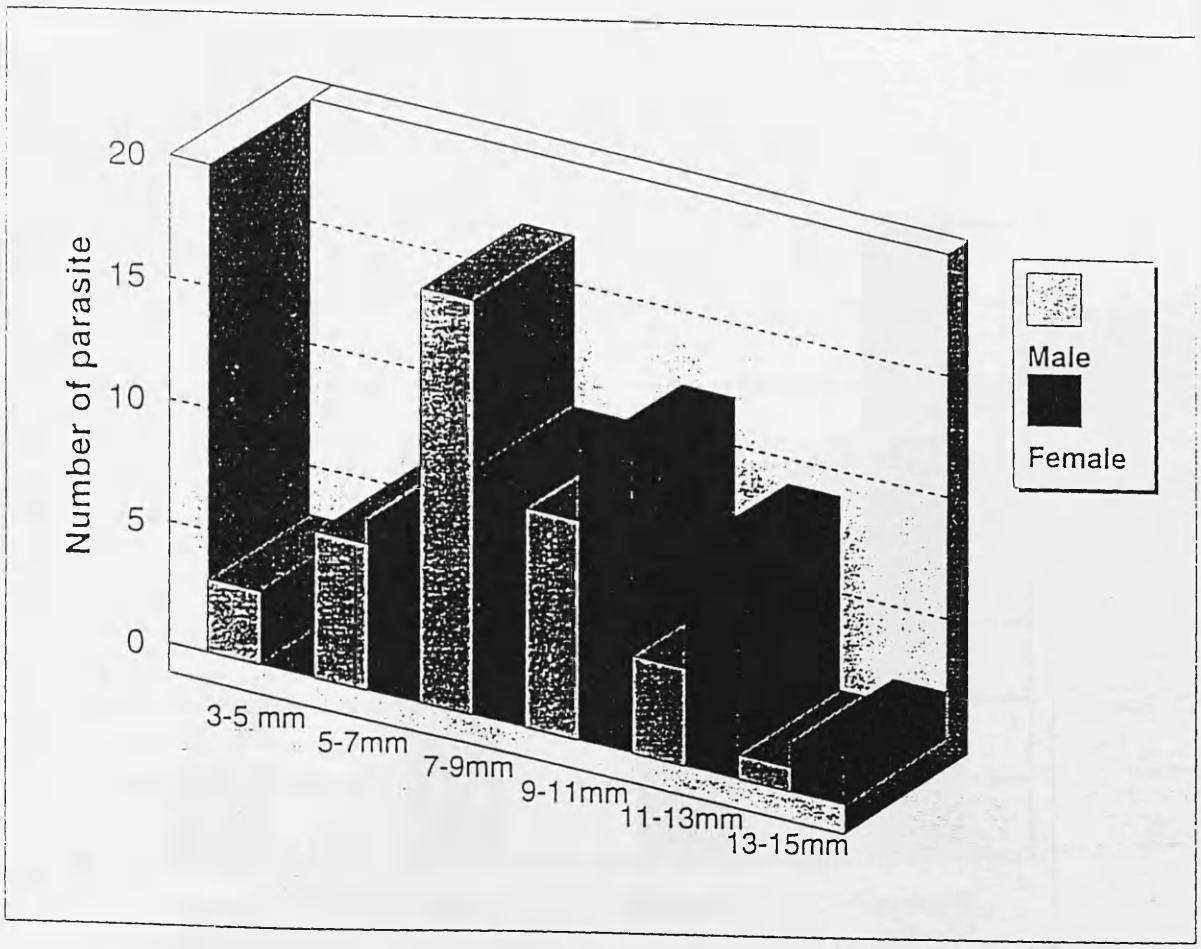


Figure 4.10 The length frequency distribution of parasites collected from male and female hosts and used to calculate the egg laying rate.

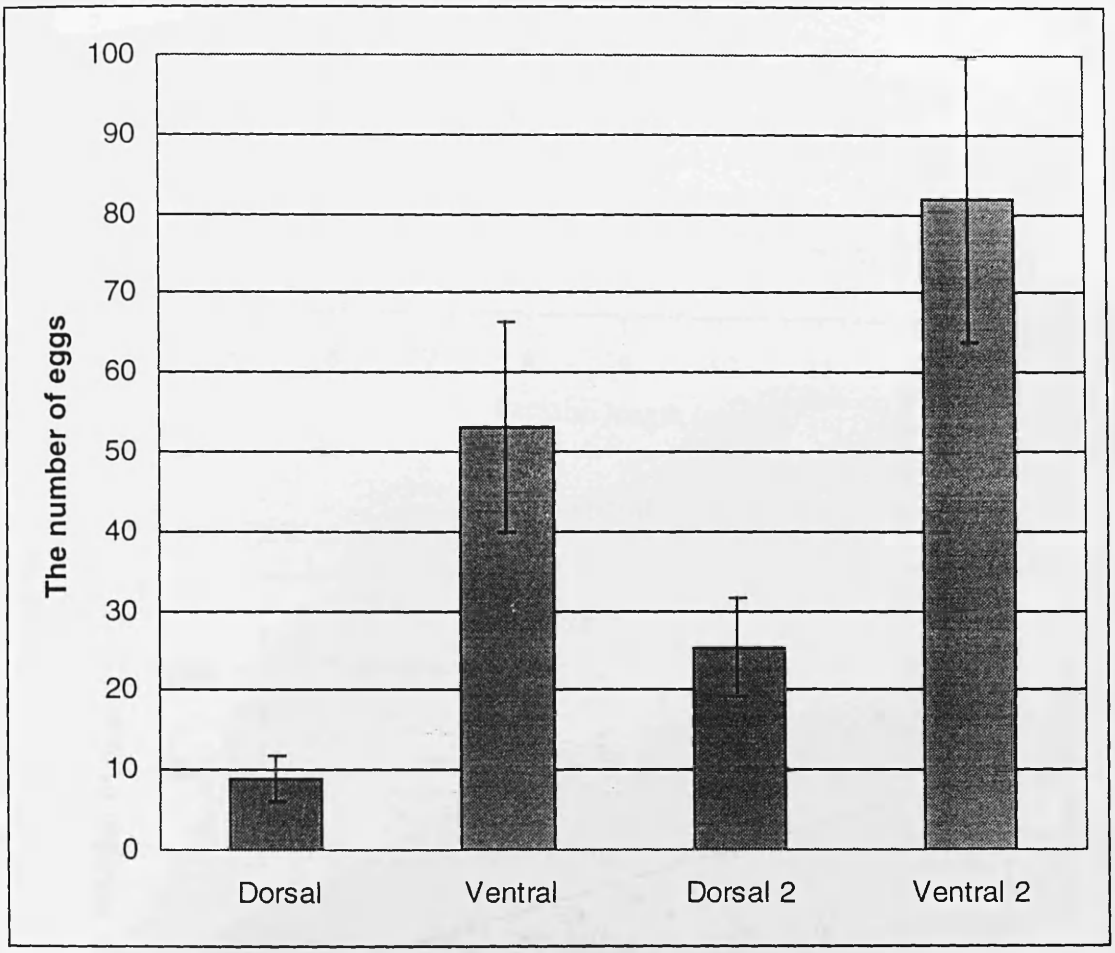


Figure 4.11 The mean number of eggs produced by parasites on the dorsal and ventral surfaces of the fish.

Dorsal: The total number of eggs divided by the total number of parasites from the dorsal surface of the hosts (n = 40).

Ventral: The total number of eggs divided by the total number of parasites from the female hosts (n = 40).

Dorsal 2: The total number of eggs divided by spawning parasites only from the dorsal surface of the hosts (n = 14).

Ventral 2: The total number of eggs divided by spawning parasites only from the ventral surface of the hosts (n = 26).

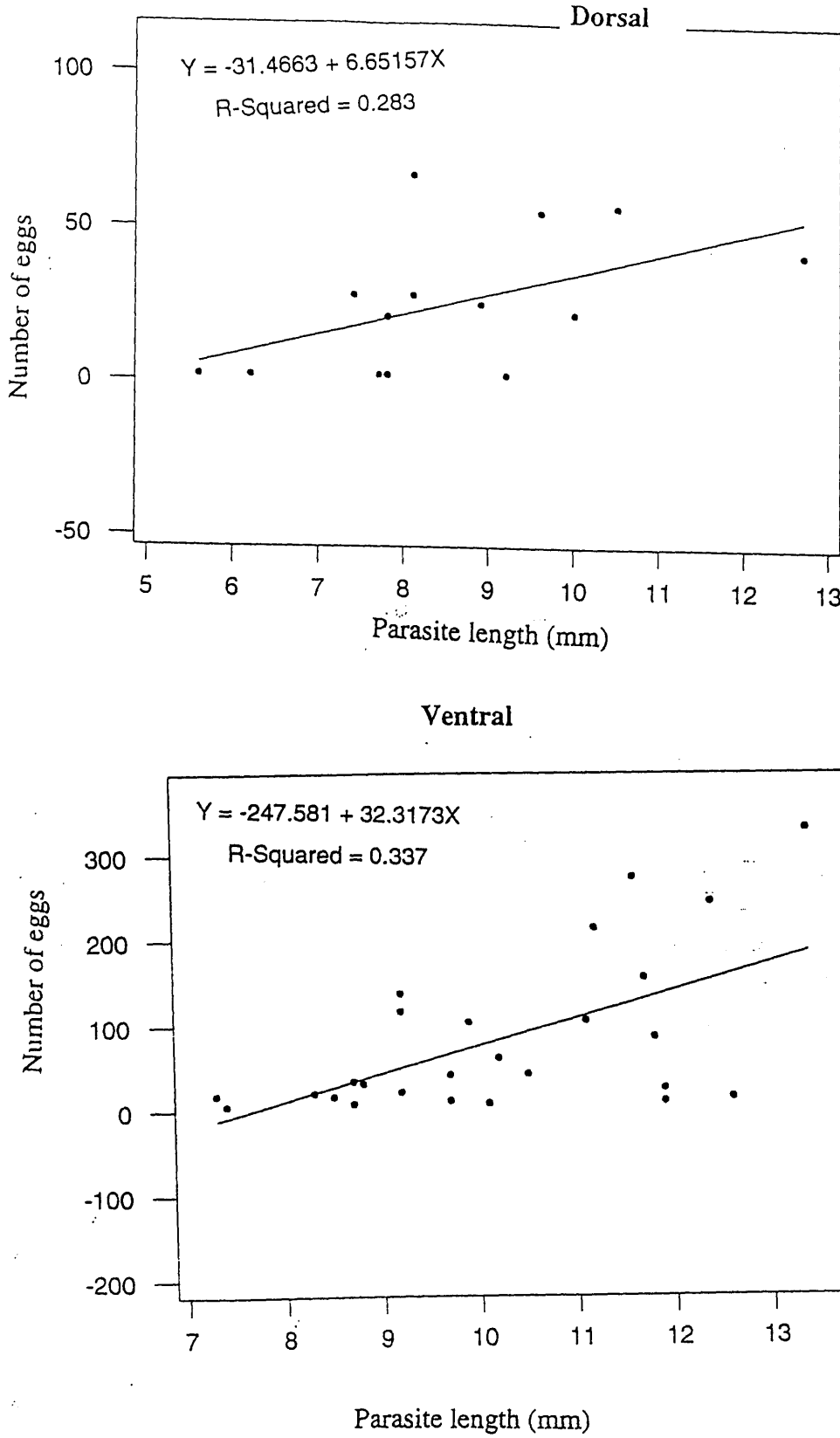


Figure 4.12 The relationship between parasite length and egg laying rate on the dorsal and ventral surface of the hosts dorsal surface (n = 14) and ventral surface (n = 26)).

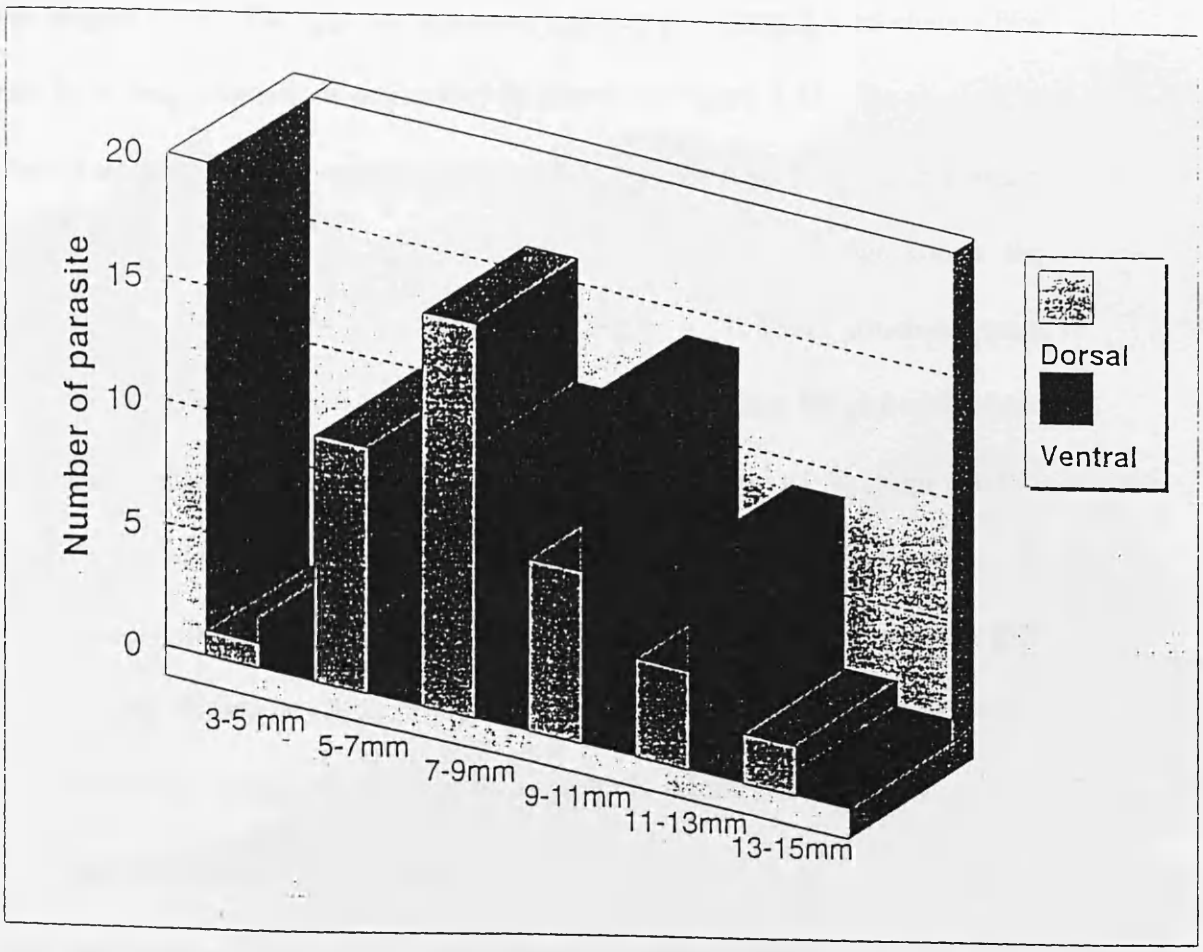


Figure 4.13 The length frequency distribution of parasites collected from the dorsal and ventral surfaces of the fish and used for the egg laying rate studies.

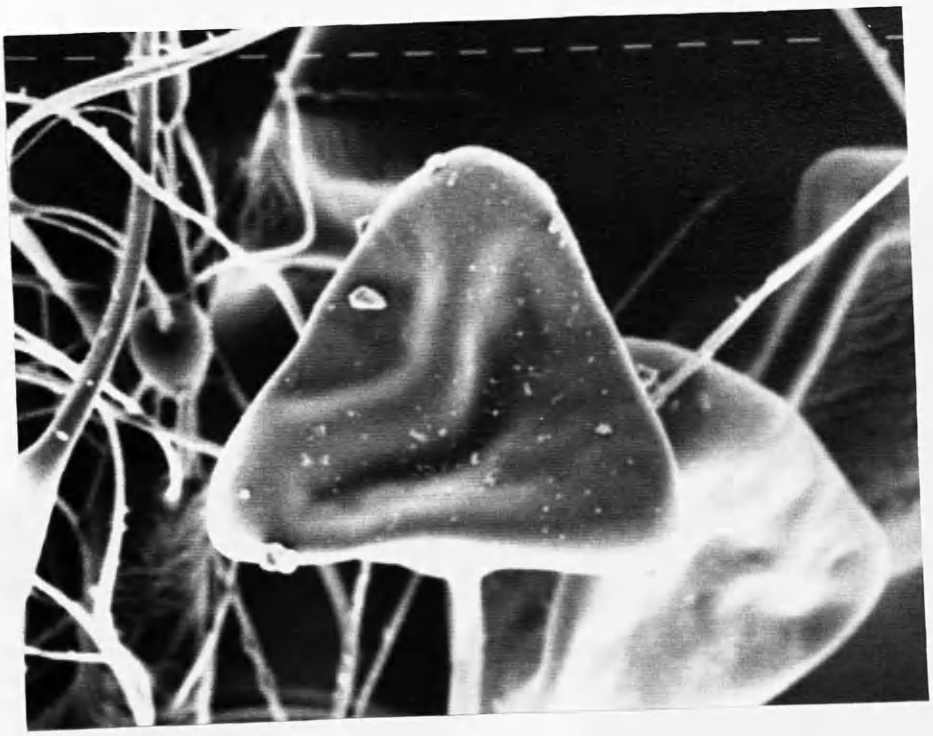
4.3.3 SEM study of eggs

4.3.3.1 Egg structure of *E. hippoglossi*

The newly produced eggs of *E. hippoglossi* are yellowish in colour and tetrahedral in shape. The edges of each side of the tetrahedron measure 175.3 ± 9.2 μm (n = 40) in size (Figure 4.14). The eggs are entwined together in a complicated chain - like fashion by a long filament or appendage as shown in Figure 4.15. The appendage attached to one corner of the egg (proximal apex) and entwined at its free end within the egg bundle (Figure 4.16). Closer examination of the appendage shows the presence of buoy – like structures as shown in Figure 4.17. These structures were not a regular size and were not spaced regularly. Individual eggs have an operculum formed at an apex opposing that bearing the appendage (distal apex) (Figure 4.18). Figure 4.19 shows the operculum on one apex of the egg and that the egg surface is pitted. The function of the operculum is clearly evident, operating as a hatching gate for the emerging oncomiracidium (Figure 4. 20). The empty egg with the operculum removed following hatching by the oncomiracidium is shown in Figure 4.21.

4.3.3.2 Egg structure of *E. soleae*

The size and shape of *E. soleae* egg was similar to that of the *E. hippoglossi* egg. However, the eggs of *E. soleae* were not entwined together, instead each individual egg was firmly attached to the surface of the egg container using sticky droplets. Figure 4.22 shows a single egg of *E. soleae* attached to the bottom of the container with sticky droplets. Closer examination of the sticky droplets showed that they were quite regularly arranged at intervals of about 30 - 40 μm and were 30 μm in size. A single egg had around 10 sticky droplets on its appendage (Figures 4.23 and 4.24).



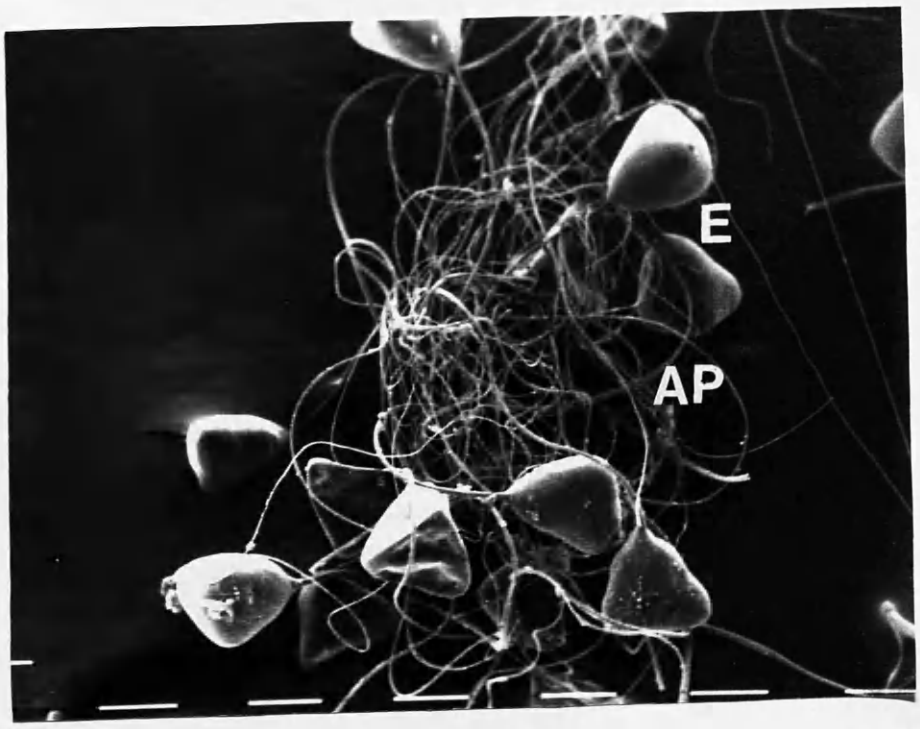
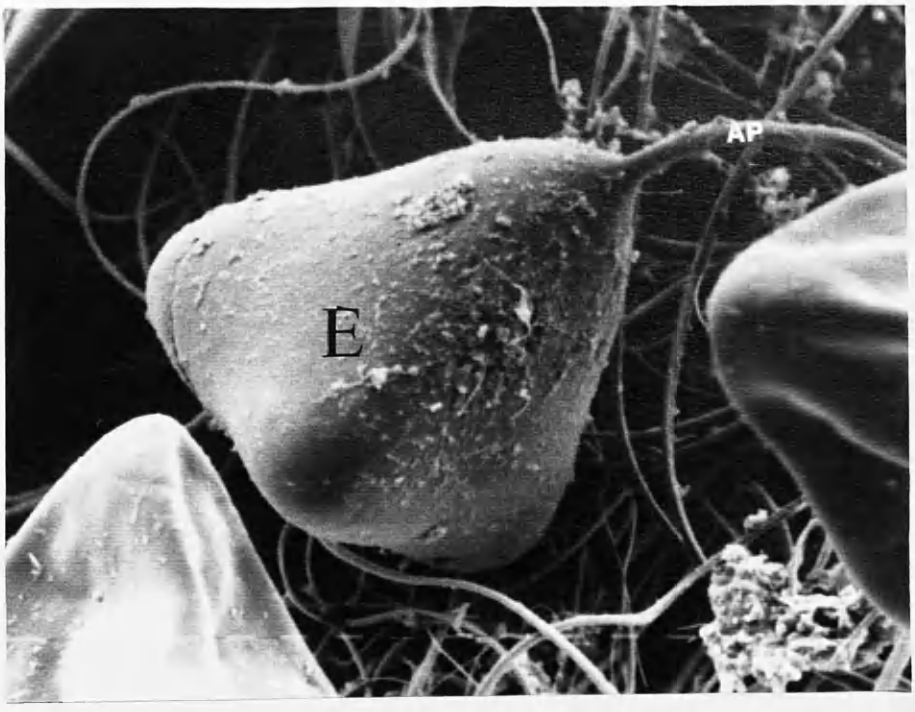

E
AP

Figure 4.14 Scanning electron micrograph showing tetrahedral shaped eggs of *E. Hippoglossi.* (Scale bar = 10 μm)

Figure 4.15. Scanning electron micrograph showing the eggs entwined together by their long appendages. Note eggs entwined by the long appendage which attaches to one corner of the egg (proximal apex). (**E**: egg, **AP**: appendage). (Scale bar = 100 μm).


AP
E

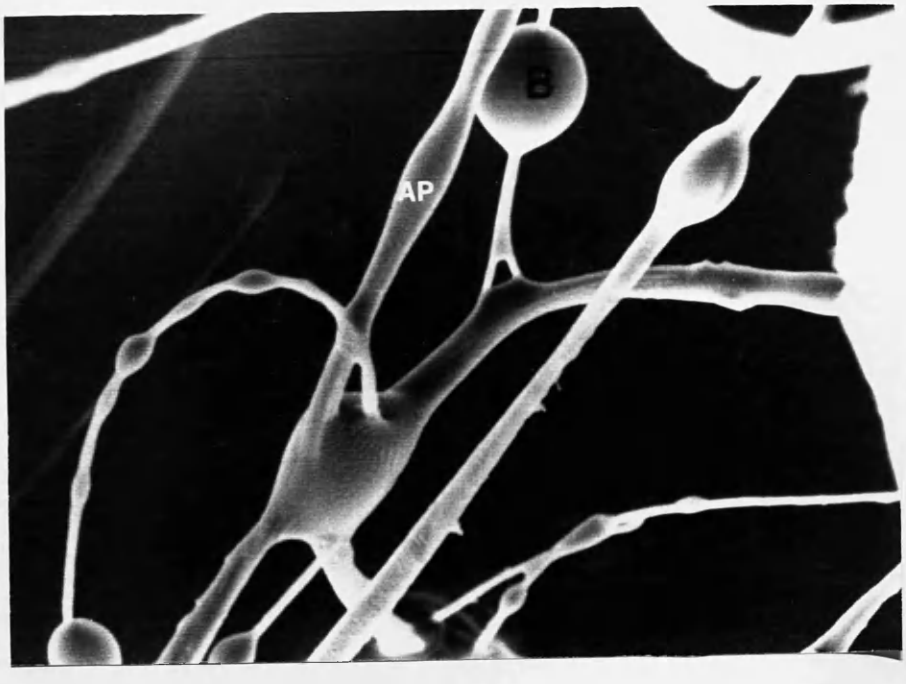
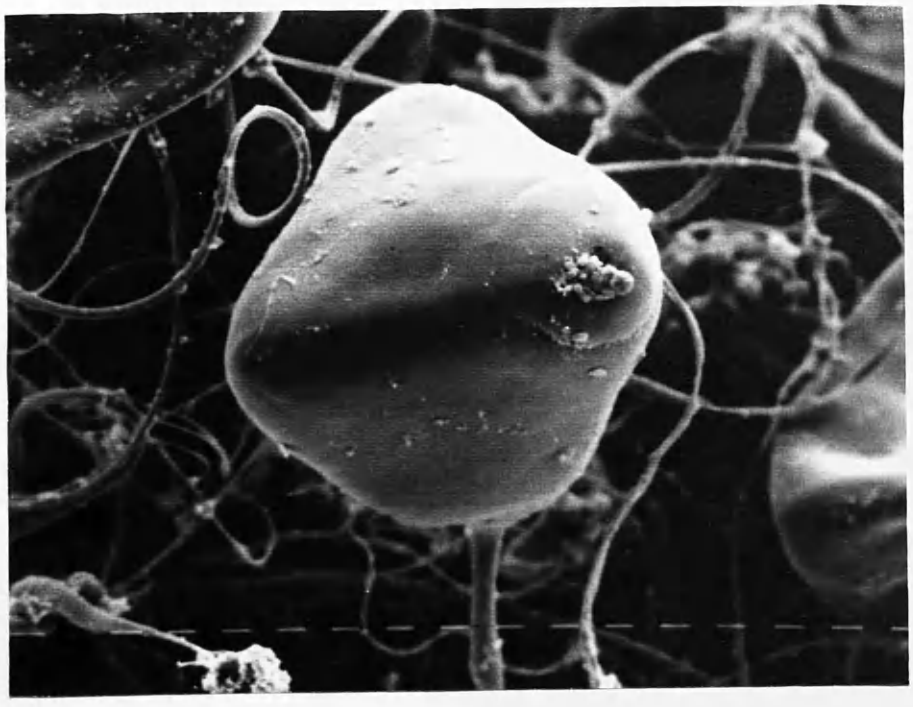

B
AP

Figure 4.16. Scanning electron micrograph showing a single egg. Note the appendage attached to one apex of the egg.
(**E**: egg, **AP**: appendage). (Scale bar = 10 μm)

Figure 4.17 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.
(**AP**: appendage, **B**: buoy like structure). (Scale bar = 10 μm)



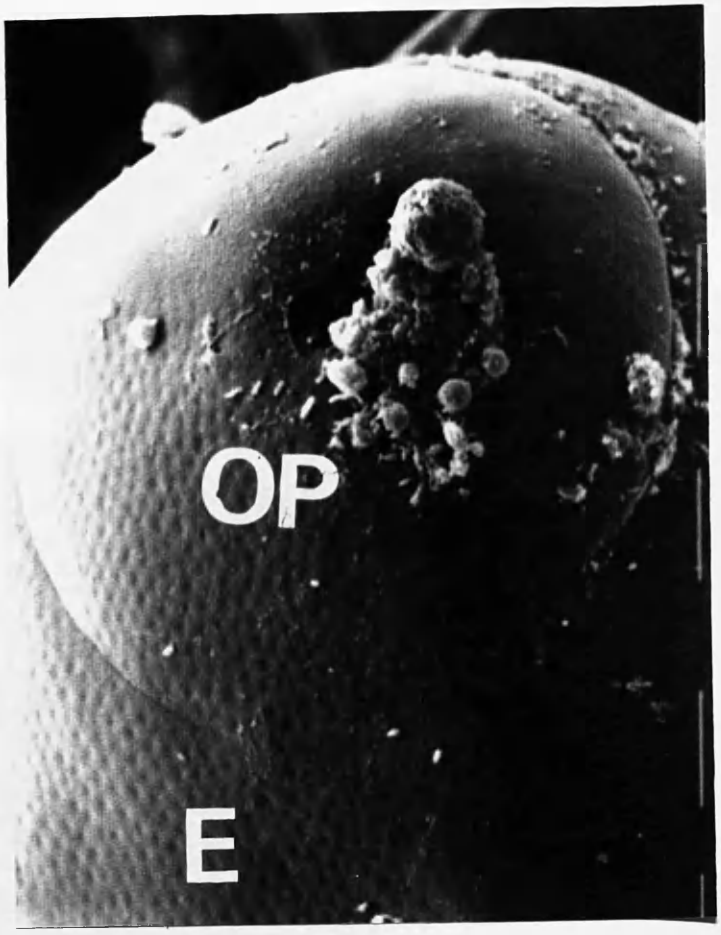
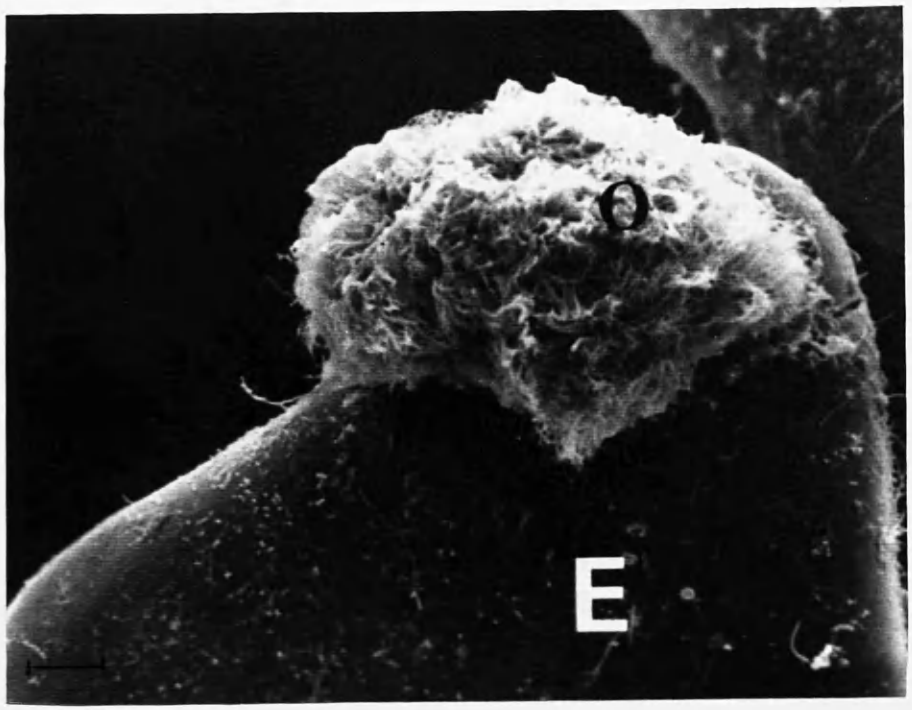

OP
E

Figure 4.18 Scanning electron micrograph showing an egg has an operculum forming an apex opposing that of the one bearing the appendage (distal apex). (Scale bar = 10 μm)

Figure 4.19 Closer examination of scanning electron micrograph showing the operculum and the pitted surface of the egg.
(**E**: egg, **OP**: operculum). (Scale bar = 10 μm).


O
E

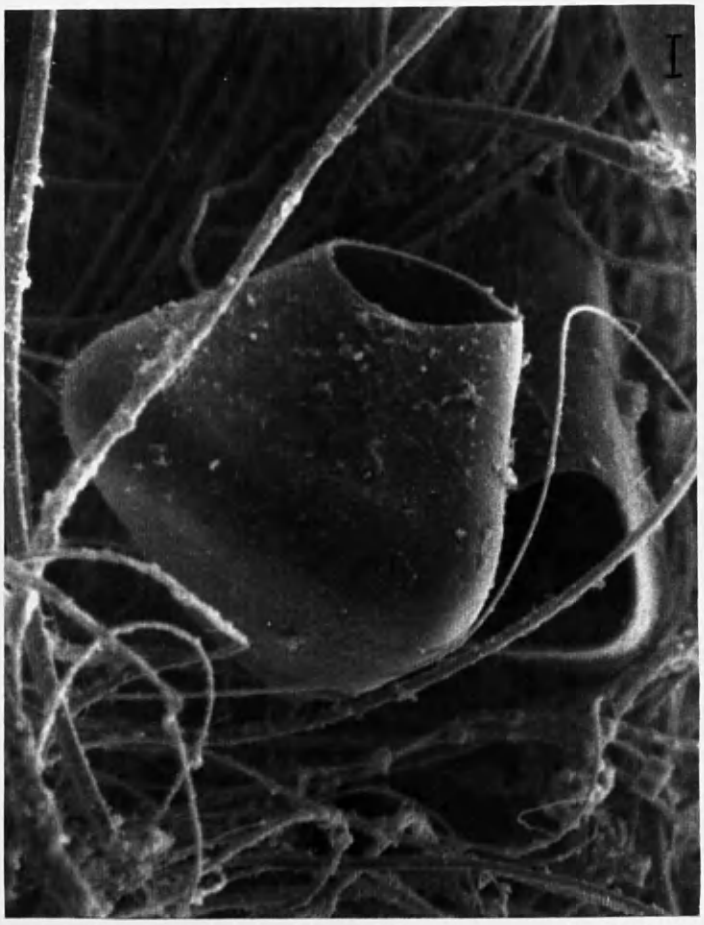
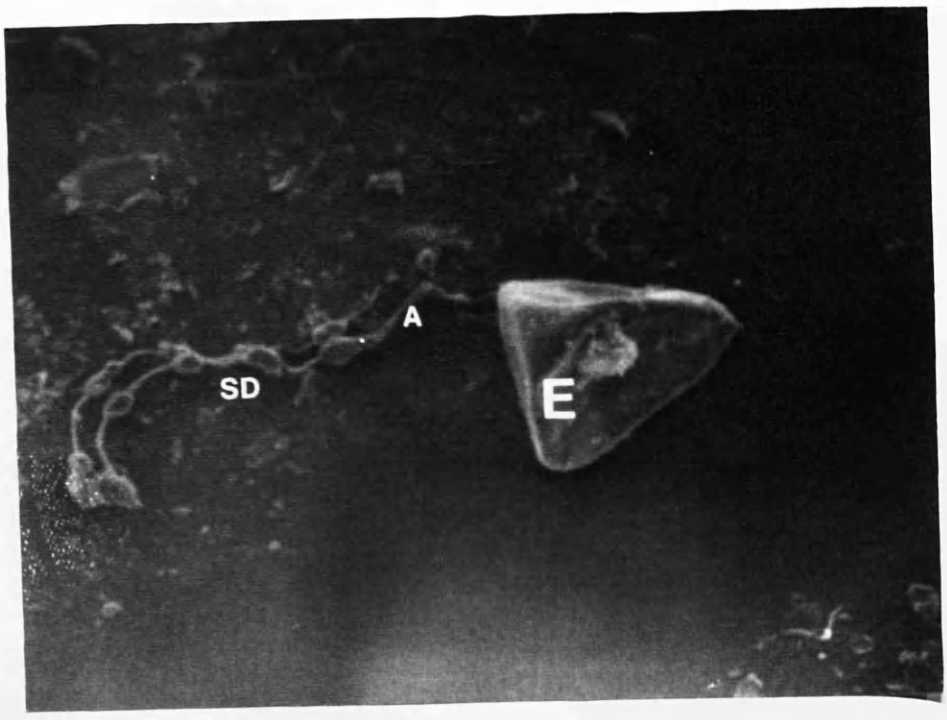


Figure 4.20 Scanning electron micrograph showing the function of the operculum. Note the oncomiracidium emerging through the operculum as a hatching gate.
(**E**: egg, **O**: oncomiracidium). (Scale bar = 10 μm)

Figure 4.21 Scanning electron micrograph showing the empty eggs after hatching. (Scale bar = 10 μm).


A
SD
E

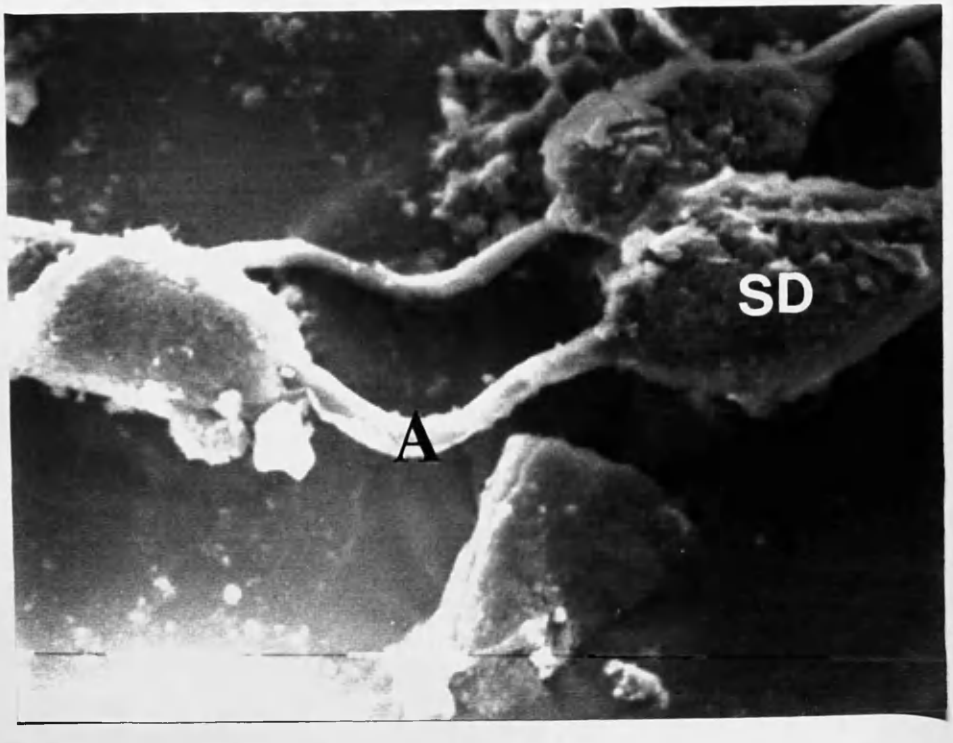

SD
A

Figure 4.22 Scanning electron micrograph showing a single egg of *E. soleae* with appendage and sticky droplets.
(**E**: egg, **SD**: sticky droplet, **A**: appendage). (Scale bar = 100 μm)

Figure 4.23 The closer examination of scanning electron micrograph showing sticky droplets of egg of *E. soleae*. Note the sticky droplets sized about 30 μm arranged at 30 - 40 μm intervals along the appendage.
(**SD**: sticky droplet, **A**: appendage).
(Scale bar = 10 μm)

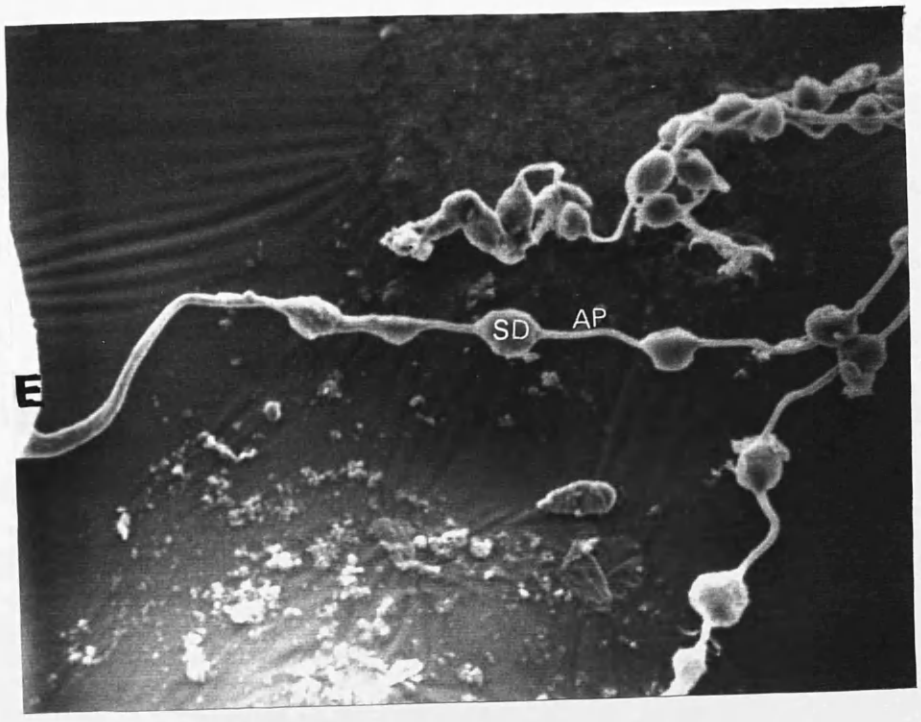


Figure 4.24 Scanning electron micrograph showing a single egg of *E. soleae* has around 10 sticky droplets on its appendage. Note the sticky droplets are quite regularly arranged at intervals of about 50 µm and are30 µm in size. (**E**: egg, **SD**: sticky droplet, **AP**: appendage).
(Scale bar = 10 µm).

4.4 DISCUSSION

4.4.1 Egg spawning behaviour

After removal from the host, the parasites firmly attached themselves to the bottom of a plastic petri dish and continued to produce eggs. *E. hippoglossi* produced tetrahedral eggs in common with other members of the Capsalidae. The act of egg-laying in *E. hippoglossi* appeared to involve muscular effort involving contraction of the general body musculature as well as of the muscles associated with the reproductive tract. Kearn (1985) made the same observation of egg laying behaviour in *E. soleae*. Also, a similar energetic spawning has been described in other polyopisthocotyleans by Remley (1942) in *Microcotyle spinicirrus* MacCallum, 1918 and in *Diclidophora denticulata* by Frankland (1955).

Kearn (1985) 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

In the present study, there were 3 patterns of egg laying by *E.hippoglossi*. The first was a chain-like pattern where the eggs were attached together by their appendages and were then expelled. This kind of egg laying pattern was the most commonly found in the present study. After they were expelled from the uterus, they were entwined outside of the parasite body. Baer and Euzet (1961) suggested that eggs with long appendages that become entangled to form bunches would float in the sea but, in the present study, it was found that the eggs of *E. hippoglossi* appeared to sink in seawater. 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* Guberlet, 1933 and egg chains were observed by Dillon and Hargis (1968) in *Erpocotyle callorhynchi* Manter, 1955. Similar egg chains have been reported from *Microcotyle caudata* Goto, 1894 and from *Rhinecotyle crepitacula* Euzet and Trilles, 1960 (Euzet and Wahl, 1970). Bovet (1959) observed that the coiled appendage of *Diplozoon paradoxum* uncoiled during laying and became entangled with the appendages of other eggs so that most of the eggs were held off the bottom. He suggested that this 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.*

The second pattern was the 'egg ball' style where the parasites laid eggs attached to a large ball. It seemed that this style also has an advantage. When eggs combine together and sink to the bottom of the sea, they can resist water currents, providing a substrate for eggs. It was presumed that this was one of the normal egg laying patterns of *E. hippoglossi,* not only occurring *in vitro.* There are several possible ways in which these eggs could be produced:

1) Eggs are combined and retained inside the uterus which are then released as an egg ball.
2) Parasites lay eggs which become attached on the mother's body and form an egg ball externally.
3) Eggs are laid in the usual fashion but the filaments remain inside the parasite entwined within the parasite. The egg mass is then laid with the egg ball which may be one large sticky droplet that binds all the filaments together.

The third pattern of laying eggs was producing them singly. The eggs were found not to develop and rapidly broke up. There was no clear reason why the singly produced eggs did not develop. It may be that they were sterile before being expelled

4.4.2 Egg laying rate

In the present study, there was no significant difference in egg laying rates between parasites from male and female hosts. However, significantly lower numbers of eggs were produced by parasites on the dorsal surface compared to the higher number of eggs from the ventral surface. The size at which *E. hippoglossi* started laying eggs was calculated to be around 5 - 6 mm (see Figure 4.12). The smallest parasite found laying eggs was around 5.5 mm, with most parasites laying eggs at 9 mm in length.

The time taken to assemble a single egg has been obtained by direct observation of the egg assembly process in a few monogeneans. The time taken by *Enterogyrus globidiscus* to lay individual eggs was 20-30 minutes according to Nilakarawasam (1993). According to Shaharom – Harrison (1983), the time by *Cichlidogyrus sclerosus* Paperna and Thurston, 1969 was approximately 45 minutes. Jahn and Kuhn (1932) found an average time of 5 minutes (range 2-10 minutes) for the production of a single egg by *Epibdella melleni* MacCallum, 1927 and Kearn (1985) calculated that *E. soleae* 5 mm in length produced 30 eggs per day at 12°C. It seems likely that the time taken by each species to lay eggs varies greatly depending on its biology and the surrounding environmental conditions.

In the present study, the egg laying rate of the parasites was clearly dependent on the origin of the parasites. The egg laying rates of parasites from males and females

were similar, 2.2 eggs and 2.9 eggs per hour, respectively. However comparing the rate of egg laying by parasites on the dorsal and ventral surfaces, there was a significant difference, 3.4 eggs per an hour and 1.1 eggs per an hour, respectively. This could be explained by the mean length of parasites on the ventral surface which were longer than that on the dorsal surface. Thus, the bigger parasites laid more eggs than the smaller ones. However, there were big differences in the egg productivity of even similar sized parasites on the dorsal and ventral surfaces, suggesting that there were factors which enhanced egg production on the ventral surface of the fish other than worm size.

Many researchers found that several factors affect the egg laying rate of parasites. For example, the optimal temperature for egg production varies between parasite species. Egg production of *D. vasta*tor and *D. lamellatus* Achmerow, 1952 was highest at 28°C (Paperna, 1963b; Molnár, 1971). On the other hand, *D. anchoratus* (Duj., 1845) Wagener, 1857 appeared to produce more eggs at 23°C (Prost, 1963). Egg production of *Polystoma integerrimum* from the bladder of grass frog *Rana temporaria* L. was higher at 4°C or at 8°C than at higher temperatures in the range 12-20°C (Combes, 1972).

A positive relationship between numbers of eggs produced and the parasite intensity was recorded for *Protopolystoma xenopodis*, *Polystoma integerrimum* and *Cichlidogyrus sclerosus* (Combes, 1972; Tinsley and Owen, 1975; Shaharom – Harrison, 1983; Jackson and Tinsley, 1988). Population density might affect parasite egg production in two ways; first, intraspecific competition for limited food resources might cause a negative effect on egg production (Combes, 1972; Jackson and Tinsley, 1988), and second, some parasites might react to unfavourable

conditions and high population density by increasing fecundity to maintain parasite numbers (Tinsley and Owen, 1975; Shaharom-Harrison, 1983). One of these could be one of the reasons why the egg production of parasites from the ventral surface was higher than that of parasites from the dorsal surface. However, further investigations are necessary to compare differences between the dorsal and ventral surfaces. For example, the different mucous content of the two surfaces or the maturity of the parasite from different surfaces and not only the length of the parasites.

4.4.3 Egg structure

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*. Monogenean eggs with sclerotized protein shells are strongly resistant to external factors. Eggs of *Dactylogyrus lamellatus*, *D. extensus* Mueller and Van Cleave, 1932 and *D. vastator* can over - winter at the bottom of dried ponds (Musselius and Ptashuk, 1970; Paperna, 1963b; Prost, 1963). A tanned protein shell might be expected to resist enzymatic attack during the passage through the gut of a small predator, as suggested for *Diplozoon homoion gracile* by Macdonald and Jones (1978). There is no report demonstrating the resistance of *E. hippoglossi* except Svendsen and Haug (1991) who tried to treat the eggs of *E. hippoglossi* using freshwater, hot freshwater (50°C) and formalin (500 ppm). They reduced the hatching rate of eggs with these treatments but still the eggs were able to hatch. Their results suggested that the eggs of *E. hippoglossi* also have a strong resistance to outside factors.

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* have appendages bearing sticky droplets. *Acanthocotyle lobianchi* Monticelli, 1890 (Kearn, 1967a) and *Entobdella australis* Kearn, 1978 (Kearn, 1978) have appendages with sticky droplets, and *Calicotyle kröyeri* Diesing, 1850 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. 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. 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 sea which is very deep. 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. The eggs of the freshwater parasite *Discocotyle sagittata* Brinkmann, 1952 have no appendage or adhesive material. Paling (1965) suggested the eggs of *Discocotyle sagittata* would be carried downstream by water currents and deposited where the water is slow moving. Thomas (1964) observed that the heaviest infections of this parasite occur in stretches of river having a sedimentary bottom of mineral particles which would also favour the settling of eggs.

Chapter 5

ONCOMIRACIDIA

5. ONCOMIRACIDIA

5.1 INTRODUCTION

5.1.1 Egg development

The duration of egg development of monogeneans varies from species to species (Kearn, 1975; Ogawa, 1988; Kearn *et al.*, 1992a). Most monogeneans show a decrease in the development time as the temperature increases. Eggs of *Dactylogyrus lamellatus* Achmerow, 1952 hatched in 4 days at 18°C, 2.5 – 3 days at 23°C and 1.5 days at 26°C (Molnár, 1971) while the eggs of *Dictyocotyle coeliaca* Nybelin, 1941 had a long incubation period of between 112-147 days at 10°C (Kearn, 1970). Eggs of the polyopisthocotylean *Bivalgina tai* Yamaguti, 1938 hatched in 26 and 6-8 days at 10 and 30°C, respectively (Ogawa, 1988). Kearn (1963b) found that *E. soleae* hatched in 27 days at 12°C.

When the larva in the egg of *E. soleae* was fully developed, appropriate stimuli, such as light, host mucus and mechanical disturbance were needed to trigger larval hatching (Kearn, 1986). Light seems to be an important influence on egg hatching in many monogeneans. Some worms hatch during darkness or in reduced illumination (Kearn, 1982). For example, the eggs of *Heteraxine heterocerca* (Goto, 1894) Yamaguti, 1938 hatched mainly at dusk (Kearn *et al.*, 1992a). In contrast, some monogenean eggs hatched during the light as occurs in the capsalid *Benedenia seriolae* Yamaguti, 1934 (Kearn *et al*, 1992a) and in the frog parasite, *Polystoma integerrimum* Froelich, 1791 (Macdonald and Combes, 1978).

5.1.2 Morphology of oncomiracidium

Most monogeneans are oviparous and the newly hatched young is a larva which is called the oncomiracidium. A few monogeneans are viviparous and the newly born young resemble the parents (Llewellyn, 1957). According to Bychowsky (1957), there are two phases of activity of oncomiracidia. First, a free swimming phase in search of the host and second, a creeping or gliding phase on the surface of the host. In the first phase, movement is purely by means of epidermal cilia, but in the second phase, muscular movements take place using the haptor.

Extensive studies of the structure of the oncomiracidia of marine monogeneans have been carried out by Kingston, Dillon and Hargis (1969). They studied the morphology of ten species of Monogenea from sixteen species of fish from the Chesapeake Bay area. They showed that monopisthocotylean oncomiracidia usually have four pigmented eyespots, fourteen marginal hooks on the haptor and that the cilia are situated in three zones: the anterior, middle and posterior regions of the larvae.

Amongst capsalid parasites, Kearn *et al.* (1992a) described the oncomiracidium of *B. seriolae* from the skin of yellowtail *Seriola quinqueradiata* Temminck and Schlegel, and Whittington and Kearn (1993) examined the oncomiracidium of *B. lutjani* from the pelvic fins of *Lutjanus carponotatus* and *Capsala martinierei* Bosc, 1811 from the skin of the sunfish *Mola mola* L., 1758 (Kearn, 1963c). Recently, Whittington, Kearn and Beverly-Burton (1994) described the oncomiracidium of *Benedenia rohdei* n.sp. from the gills of *Lutjanus carponotatus*. They found that the oncomiracidium of *B. rohdei* was closely similar to the oncomiracidium of *B. seriolae* and *B. lutjani*. From these reports, it would appear that the morphology of

oncomiracidia of capsalid monogeneans is generally similar. There are four eyespots with lenses, three ciliary epidermal plates, a pharynx and a disc-shaped haptor having 14 marginal hooklets and three pairs of median sclerites, called accessory sclerites, anterior hamuli and posterior hamuli.

Within the genus *Entobdella*, Kearn (1963b) described the oncomiracidium of *E. soleae* and Kearn (1974b) compared the glandular and excretory systems of three entobdellid oncomiracidia, *E. hippoglossi*, *E. diadema* and *E. soleae*.

5.1.3 Ciliary epidermal plates

Oncomiracidia are the only dispersal stage in the life cycle of most monogenean parasites. They exhibit structural specializations of the ciliary epidermal plates adapted to the task of finding and invading the host. Therefore, a knowledge of ciliary plate is important to understand the behaviour and biology of these invasive stages. The ciliary epidermal plates of most oncomiracidia are distributed in three zones, an anterior, a middle and a posterior zone. There are two patterns of distribution of ciliary epidermal plates: the plates may be isolated from each other (Ancyrocephalidae, Calceostomatidae, Dactylogyridae) or contiguous (Capsalidae, Monocotylidae) (Lambert, 1981). Using the silver staining method, many authors have described the number and arrangement of miracidial epidermal plates of digenean parasites (Peters, 1966; Dimitrov, McCarthy and Kanev, 1991; Bell, Gibson and Sommerville, 1997). Wagner (1961) reported differences of epidermal plates distribution in the three species of schistosome cercariae. Bell *et al.*, (1997) found that three species of Strigeidae had different distributions of the sensilla

whilst the ciliary epidermal plates of all three species of miracidia were arranged in the same formula.

Some authors have described ciliary epidermal plates of the oncomiracidia of monogenean parasites: Owen (1970) *Discocotyle sagittata* (Bartels, 1834) Die, 1850 on the gills of *Salmo trutta* L; Tinsley and Owen (1975) *Protopolystoma xenopodis* of the African clawed toad; Lambert (1977) Polystomatidae monogeneans of marine fishes; Whittington (1987) *Rajoncocotyle emarginata* Olsson, 1876 on *Raja brachyura* Lafont, 1873 using SEM; *Polystomoides australiensis* on Australian freshwater turtle, *Chelodina pleurodira* by Pichelin (1995a) and *Parapolystoma johnstoni* Pichelin, 1995 from *Litoria nyakalensis* (Amphibia) by Pichelin (1995b).

5.1.4 Host finding behaviour

In order to maintain their life cycle, parasites must be able to consistently reproduce themselves, and their offspring must find and infect a host. Rogers (1962) clearly stated " evolution of infection is of prime importance in the evolution of parasitism."

Great interest has been shown in the behaviour of the miracidium and cercaria of larval digeneans. The behavioural 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 substance. Kearn (1980) also studied the response of *E. soleae* to light and gravity.

5.1.4 Objectives

Information on the oncomiracidia of *E. hippoglossi* is lacking. Therefore, this study set out to investigate the morphology of the oncomiracidium and to establish the host finding behaviour of the oncomiracidia based on laboratory experiments.

5.2 MATERIALS AND METHODS

5.2.1 Light microscopy and Scanning electron microscopy (SEM) studies

Two preparation methods were used for the study with the light microscope: i) some live oncomiracidia were placed onto a glass slide and then mounted in a drop of sea water, carefully covered with a coverslip, and observed; ii) other parasites were stained using Mayer's paracarmine and mounted in Canada balsam.

i) Mayer's paracamine staining method

The staining method is described in Chapter 2

ii) SEM fixation

Newly hatched oncomiracidia of *E. hippoglossi* were fixed into cacodylate buffered 1 % glutaraldehyde while parasites were still alive. They were then moved into 3 % glutaraldehyde after 1 hour. The material was then processed, mounted and observed as described for eggs in Chapter 2

5.2.2 Ciliary epidermal plates of *E. hippoglossi* and *E. soleae*

Newly hatched oncomiracidia were carefully pipetted from the hatching chamber and then placed into an embryo dish. The sea water was replaced with a hot solution (60-65°C) of 0.5 % silver nitrate and placed in the dark for 5 minutes. The specimens were then washed 5-10 times in distilled water before being placed under an ultraviolet lamp (325 nm) for 3 minutes and then washed 5-10 times in distilled water. The oncomiracidia were then transferred to an embryo dish containing 1 part glycerine and 9 parts 80% alcohol which was allowed to evaporate. They were then mounted in glycerine. The ciliary epidermal plates were mapped using 100x objective and bright field illumination, the illustrations being drawn with the aid of a drawing tube.

5.2.3 Swimming speed and geotactic response

5.2.3.1 Vertical swimming speed

A glass tube, 150 cm in length and 1 cm in diameter was sealed at one end with paraffin tape. Sea water at 12°C was used to fill the glass tube. Newly hatched (within 30 minutes) oncomiracidia were used. Individual 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).

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 behaviour of the oncomiracidia did not differ between light and dark. Therefore, this experiment was carried under ambient laboratory illuminated conditions. 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.

5.2.3.2 Responses to variable swimming distance and water pressure

From the previous experiment, a characteristic behavioural pattern shown by the oncomiracidia was noted. After arriving at the bottom of the glass tube, the parasite continued swimming, moving upwards and downwards within 10 cm of the bottom of the tube. Therefore, further testing was needed to check whether the oncomiracidium would keep moving up and down in the same pattern under different water pressures and different swimming distances. Seven different lengths of glass tube were used for testing the up and down swimming behaviour. The 1 cm diameter glass tubes were cut by a diamond glass cutter to 10cm, 30 cm, 50 cm, 70 cm, 130cm and 150 cm lengths. Sea water at 12°C was used to fill the glass tubes, then five newly hatched oncomiracidia were put at the top surface of each glass tube. The locations of the parasites were checked at 5minutes, 30 minutes, 2 hours, 6 hours and 12 hours. Every glass tube was kept in a constant temperature room set at 12°C.

5.2.4 Two- armed- chamber trials (responses to various stimuli)

The chemotactic behaviour of *E. hippoglossi* oncomiracidia was investigated using a two-armed - chamber based upon the design utilised by Bell (1995). The two-armed-chamber (Figure 5.1) was used to quantify the chemotactic behaviour of *E. hippoglossi* oncomiracidia in response to differing stimuli. The system was soaked in natural sea water for 10 days before being used 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 5.1. Every experiment was repeated 5 times. The results obtained from the 4 experiments were statistically analysed using Wilcoxon's two- related-samples test for non-parametric data. Findings were considered significant at values of $P < 0.05$.

Table 5.1 The stimuli for investigating the chemotactic responses of *E. hippoglossi* oncomiracidia

Test arm	Central reservoir	Control arm
Light		**Dark**
Halibut mucus		**Dark**
Mucus		**Light**

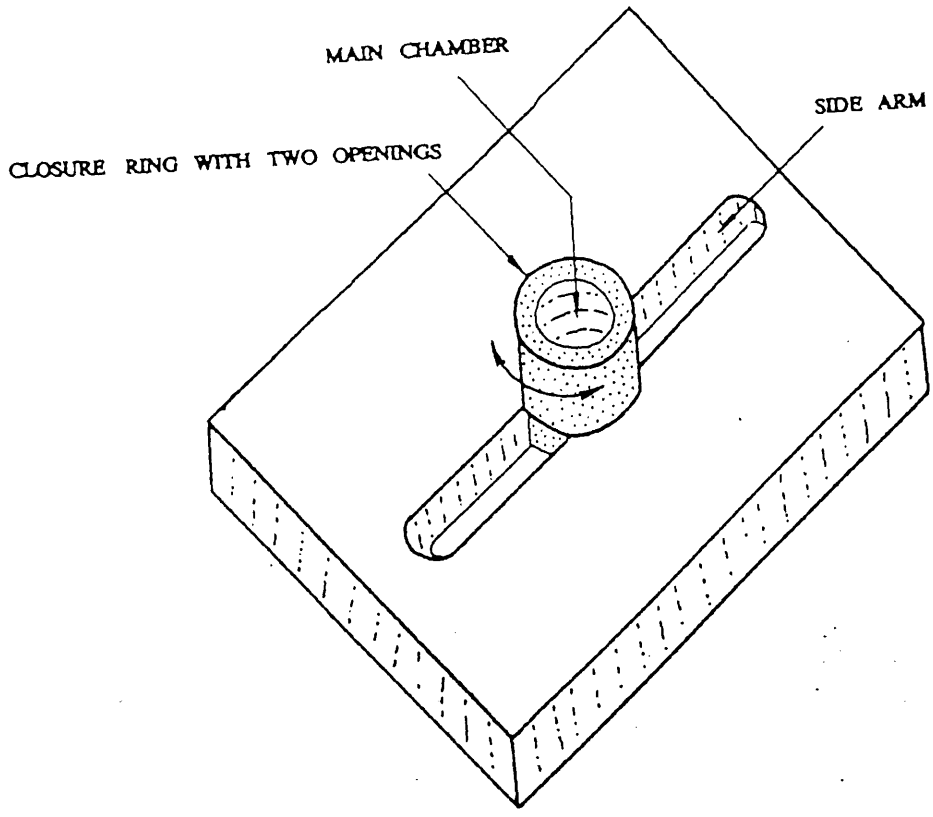


Figure 5.1. Two - arm - chamber for qualification of the oncomiracidial host - finding behaviour (after Bell, 1995). Chamber shown at approximately actual size.

5.3 RESULTS

5.3.1 Light microscopy and SEM findings

Larvae began to hatch after 27 - 30 days incubation at 12°C when exposed to an illumination pattern of 12L : 12D. The eye spots of the oncomiracidium were clearly seen around 7 days before hatching (Figure 5.2). The eggs became transparent, then the larva inside the egg could be seen clearly. The larva in the egg began to move 2 or 3 days prior to hatching and the movement became stronger and the ciliary movement became faster and more active.

The anterior region of the oncomiracidium emerged first through the operculum of the egg, whilst the posterior region of the body remained within the egg or apparently trapped by the operculum of the egg for a few minutes. Figure 5.3 illustrates the method by which the oncomiracidium movement is restricted by the partially closed operculum. During the period that the larva remained trapped in the egg by the operculum, the cilia in the posterior part were moving very actively. An SEM picture of a "trapped" oncomiracidium is shown in Figure 5.4. The larva showing the 3 ciliated zones is attached to the egg by its posterior haptor. A closer view is shown in Figure 5.5. The close up examination shows the ciliated posterior part is captured by the gap between the egg and operculum. Whether the emerging oncomiracidium was trapped or whether it simply paused there for a purpose is not clear. Once they were completely released from the egg, the free oncomiracidia swam rapidly and actively.

The swimming larvae, completely emerged from the egg, measured 200 - 300 μm in length. The oncomiracidium had 3 ciliary zones, the anterior, the middle and the posterior regions of the body. The cilia were densely packed as can be seen in Figure

5.6 and were borne on epidermal cell plates (Figure 5.7). The ciliary pattern of the oncomiracidium from the dorsal view is shown in Figure 5.8. The whole anterior region (head region) was covered by cilia. The cilia extended as far as the anterior eyespots, whilst a narrowed ciliated band covered only half of the dorsal surface of parasite round the middle region. On the posterior region, two-thirds of the posterior dorsal surface was covered with cilia. The cilia pattern on the ventral surface was different from that on the dorsal surface as shown in Figure 5.9. Cilia on the antero - ventral region extended along the entire length of the anterior region (head part) except for around the mouth region. In the middle ciliated region, the cilia were distributed around the whole middle region of the ventral surface and then spread almost to the beginning of the posterior region (haptor part). In the posterior part of the ventral surface, however, cilia were not found because of the haptor opening.

A pair of adhesive gland duct openings were found on the head region of the oncomiracidium, near the apex. Some exhausted larvae on the bottom of the container or larva which had attached themselves to the container were found to have a swollen anterior head region as shown in Figure 5.10. It seemed that the oncomiracidia were able to attach to the surface of the glass container using this area.

The oncomiracidium of *E. hippoglossi* has 4 eyespots with pigmented cups and lenses as shown in Figure 5. 11. The smaller anterior pair faced posterio -laterally and the larger, posterior pair faced antero- laterally. Figure 5.12 shows that live oncomiracidia had the crystalline lenses appearing a blue-green colour. The posterior pair of eyes were found to be highly mobile in the horizontal plane.

The posterior third of the oncomiracidium consisted of the haptor. The unflattened haptor was cup - shaped and bore ten pairs of sclerites. The three pairs situated

medianly comprise the anterior hamuli, posterior hamuli and accessory sclerites. The posterior hamuli and accessory sclerites were almost the same shape as those of the adult. The anterior hamuli were clearly different from those found on the adult parasite. The anterior hamuli increase in length with age and become more markedly curved. There were fourteen sclerites situated marginally (marginal hooklets), five pairs distributed symmetrically, one pair situated at the top of the haptor anterior to the accessory sclerites and the last pair located between the posterior hamuli (Figure 5.13). The edges of the haptor were folded ventrally in the free swimming stage parasite as shown in Figure 5.14. The marginal sclerites were seen along the edge of the folded haptor.

After the parasite attached to the bottom of the container by the adhesive glands, the haptor unfolded and the arranged marginal hooklets were clearly seen. At this stage, some of the epidermal ciliary plates bearing the densely packed cilia remain on the middle and the posterior regions of the dorsal surface (Figure 5.15).

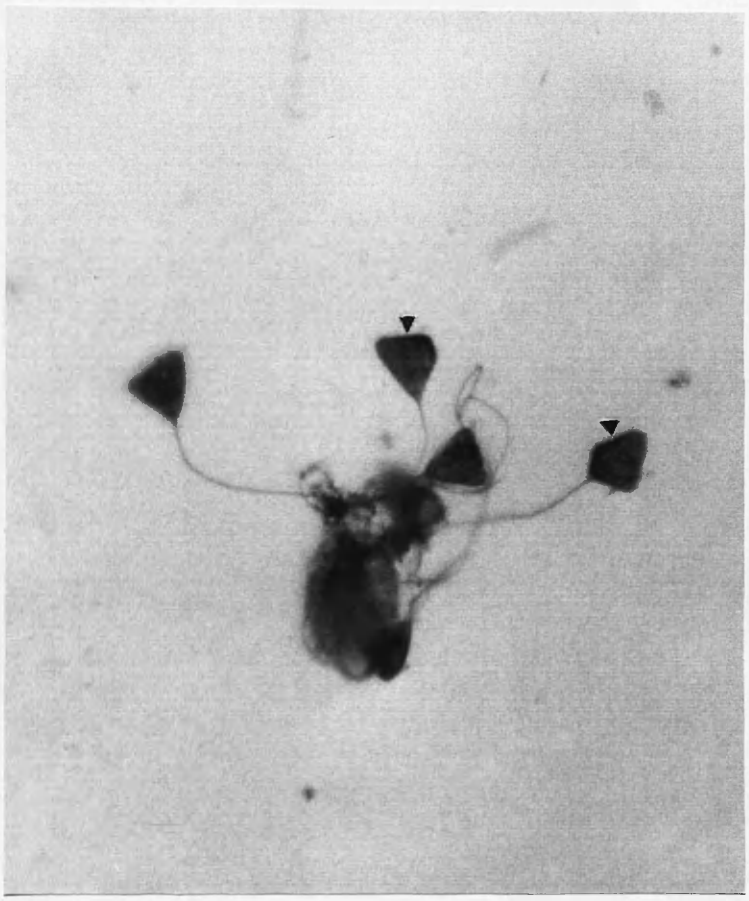


Figure 5.2. Four eye spots (arrows) are clearly seen in three eggs which are attached on the substrate by their appendage around 7 days before hatching. (x 4 objective).

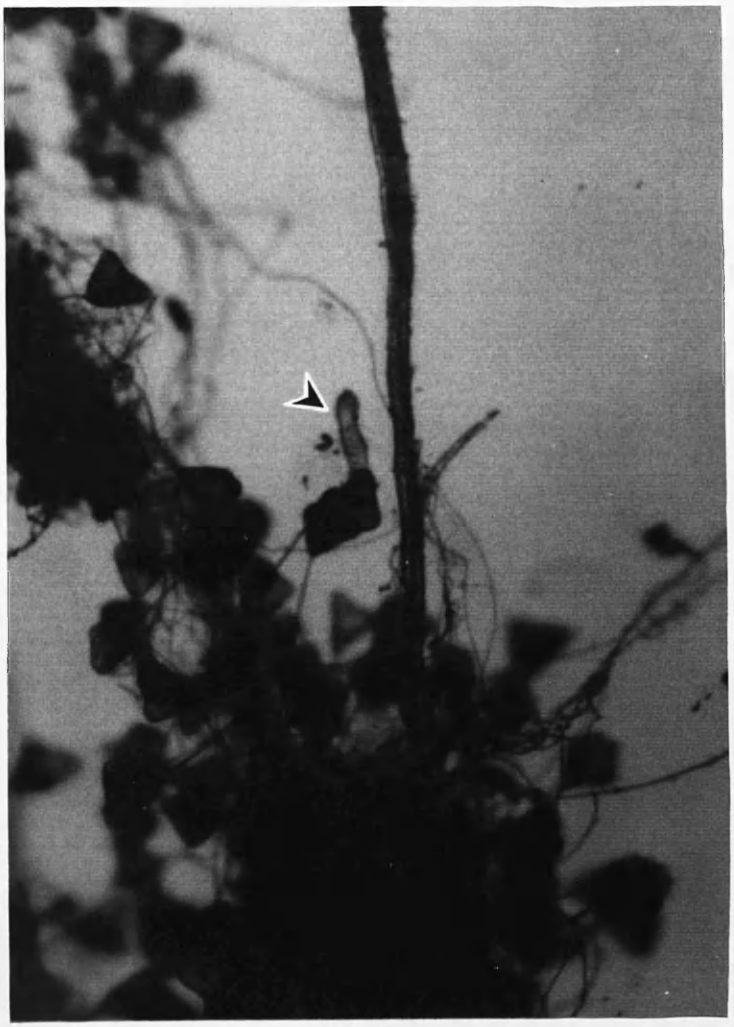
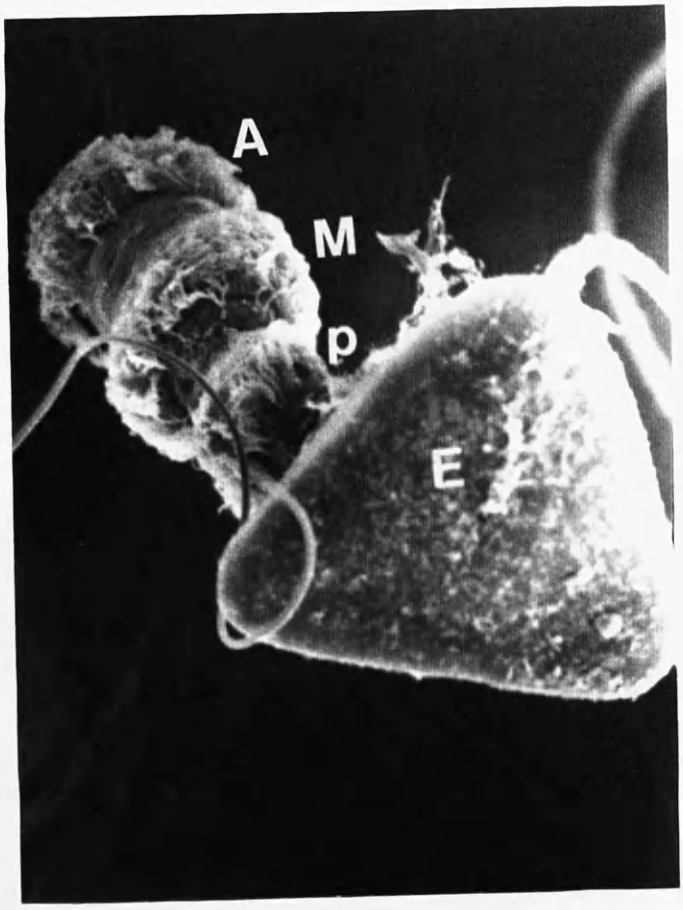


Figure 5.3. The oncomiracidium (arrow) remains attached to the egg by its posterior region. The haptor region is trapped by the partially closed operculum of the egg. (x 4 object).


A
M
p
E

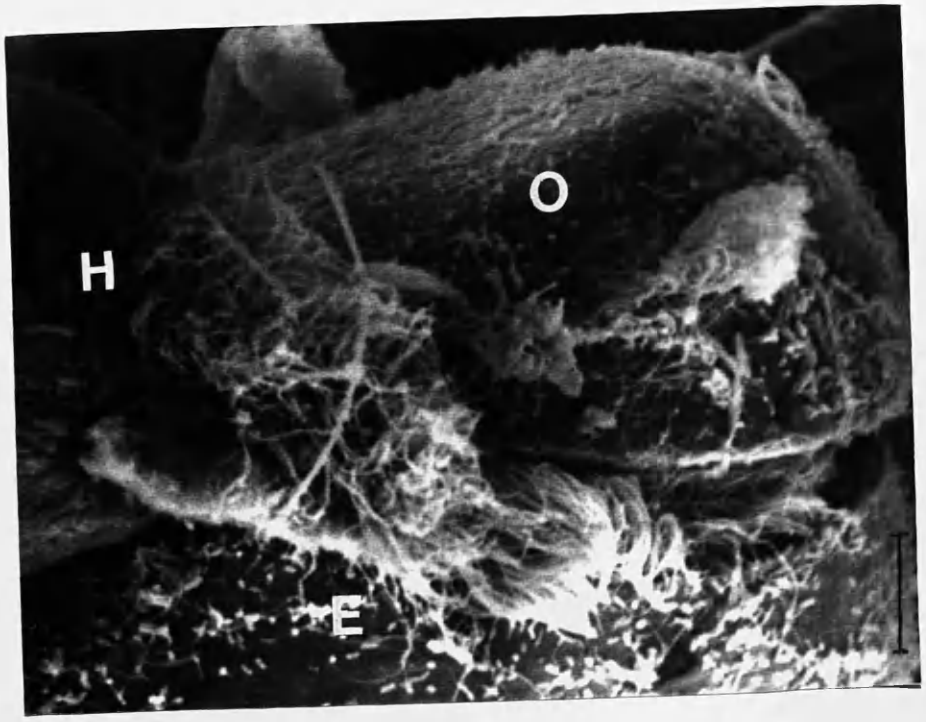

O
H
E

Figure 5.4. Scanning electron micrograph of a hatching oncomiracidium. The larva has emerged from the egg but remains attached to the egg by its posterior (haptor) region. (Scale bar = 100μm).
(**A**: anterior region, **M**: middle region, **P**: posterior region, **E**: egg).

Figure 5.5. Close up of the trapped posterior region of the oncomiracidium. Note the entire haptoral region lies between the egg and the partially closed operculum. (Scale bar = 10 μm).
(**O:** operculum, **H:** trapped haptor with cilia, **E:** egg).

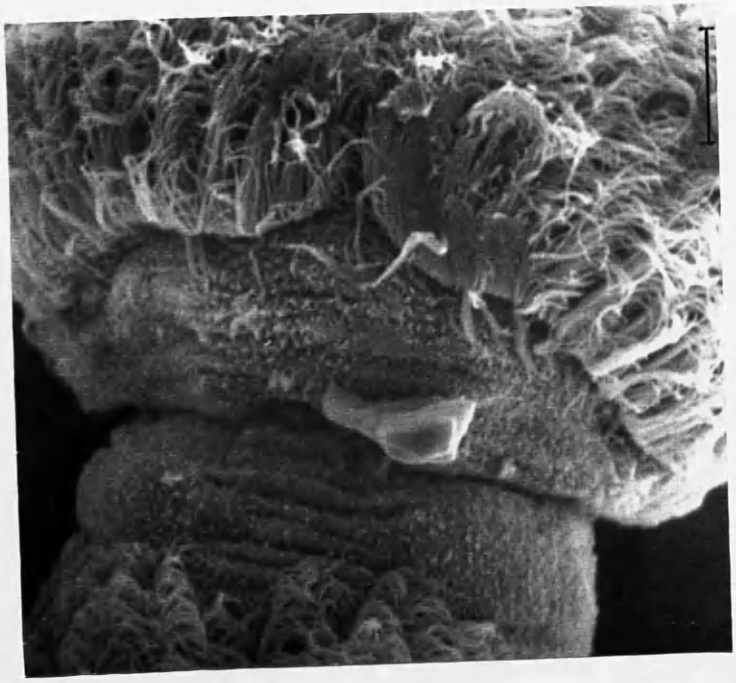
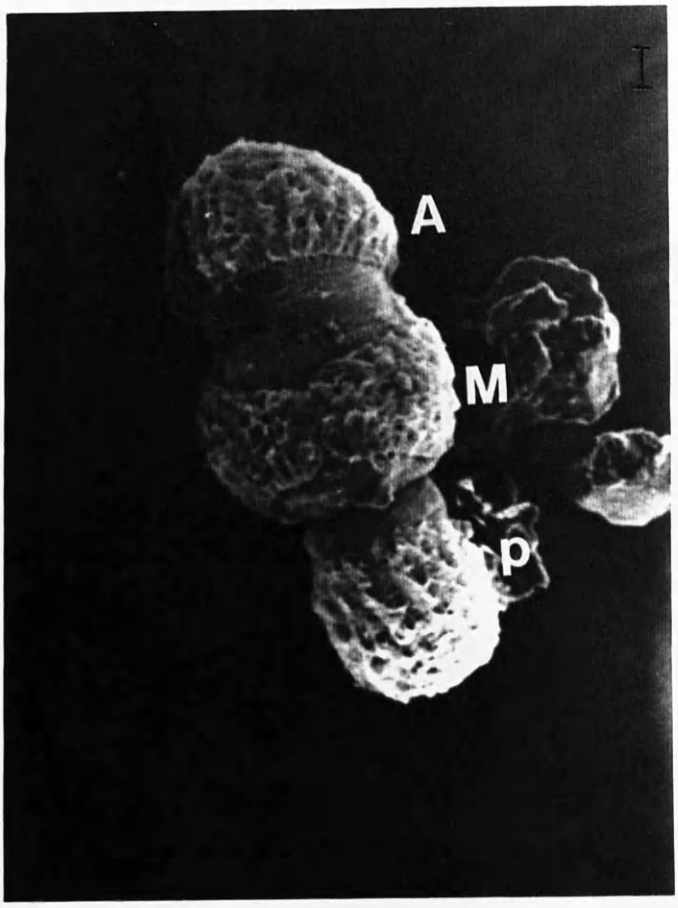


Figure 5.6. Scanning electron micrograph showing a dense covering of cilia on the anterior region of the oncomiracidium.(Scale bar = 10 μm)

Figure 5.7. The middle region of oncomiracidium showing the edge of the epidermal plates on which the cilia are densely borne. (Scale bar = 10 μm).


A
M
p

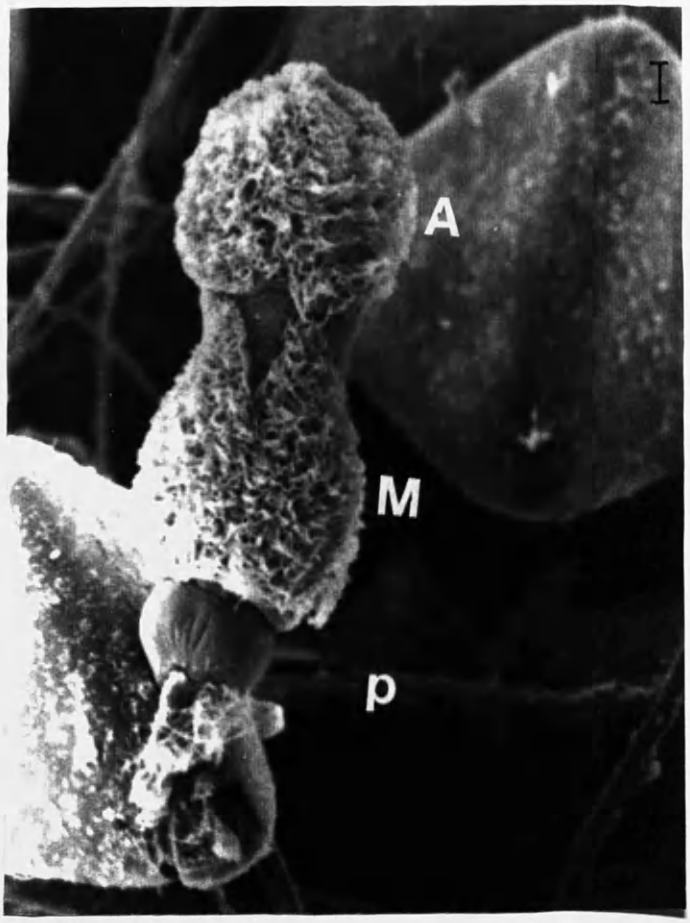

A
M
p

Figure 5.8. SEM showing the distribution of the cilia from the dorsal view of an oncomiracidium. Note the three ciliated zones on the anterior, the middle and the posterior regions. (Scale bar = 10 μm).
(**A:** anterior zone, **M:** middle zone, **P:** posterior zone).

Figure 5.9. SEM showing the distribution of the cilia from ventral view of an oncomiracidium. Note the ciliated zones on the anterior, the middle and the posterior regions. (Scale bar = 10μm).
(**A:** anterior zone, **M:** middle zone, **P:** posterior zone).

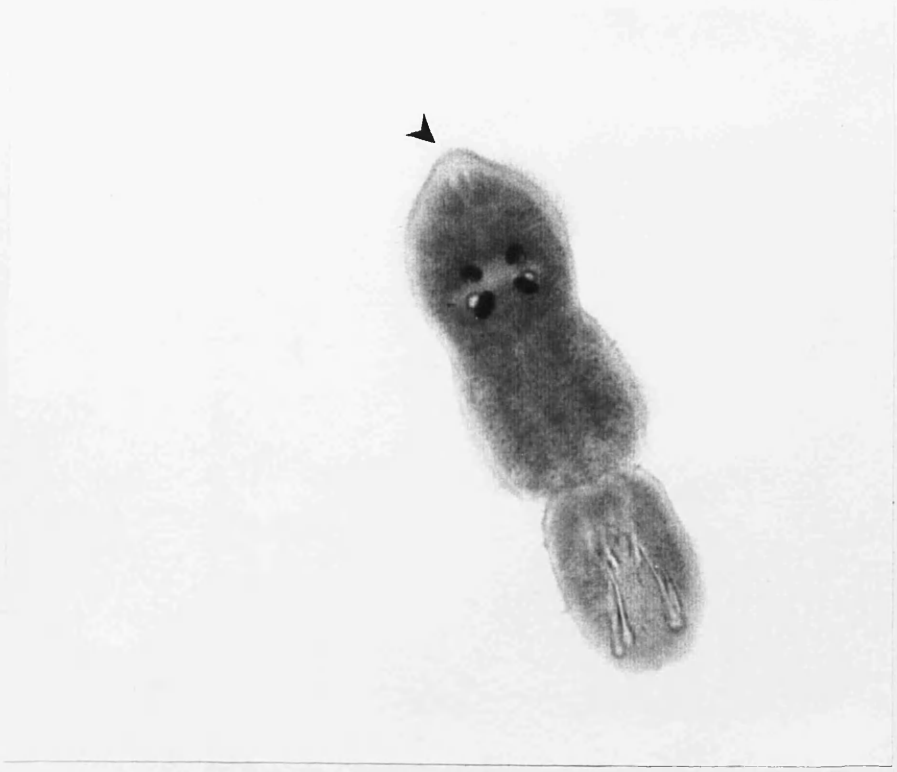


Figure 5.10. Light micrograph of the oncomiracidium showing gland opening ducts (arrow) evident with Mayer's paracamine staining. Note the swollen area on the head region. (x 10 objective).
(**A:** anterior region, **M:** middle region, **P:** posterior region).

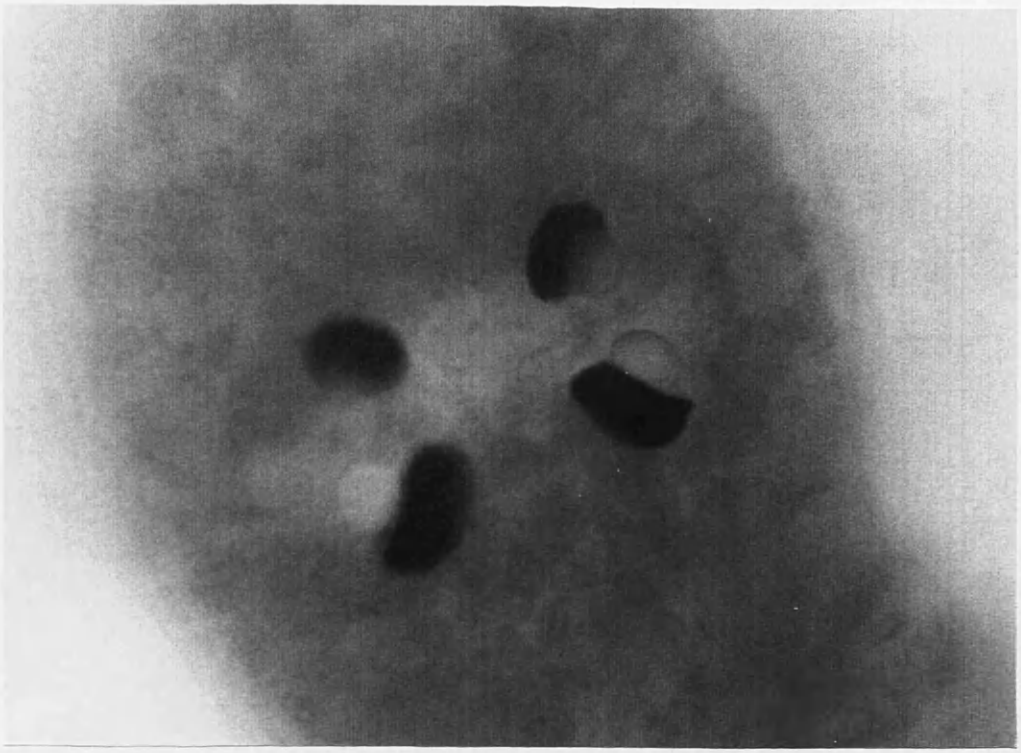


Figure 5.11. Light micrograph showing the anterior region of the oncomiracidium with Mayer's paracarmine staining. There are four lensed eyes with pigmented cups. Note the smaller anterior eyes face posterio-laterally and the larger posterior pair faced antero-laterally. (x 100 objective).

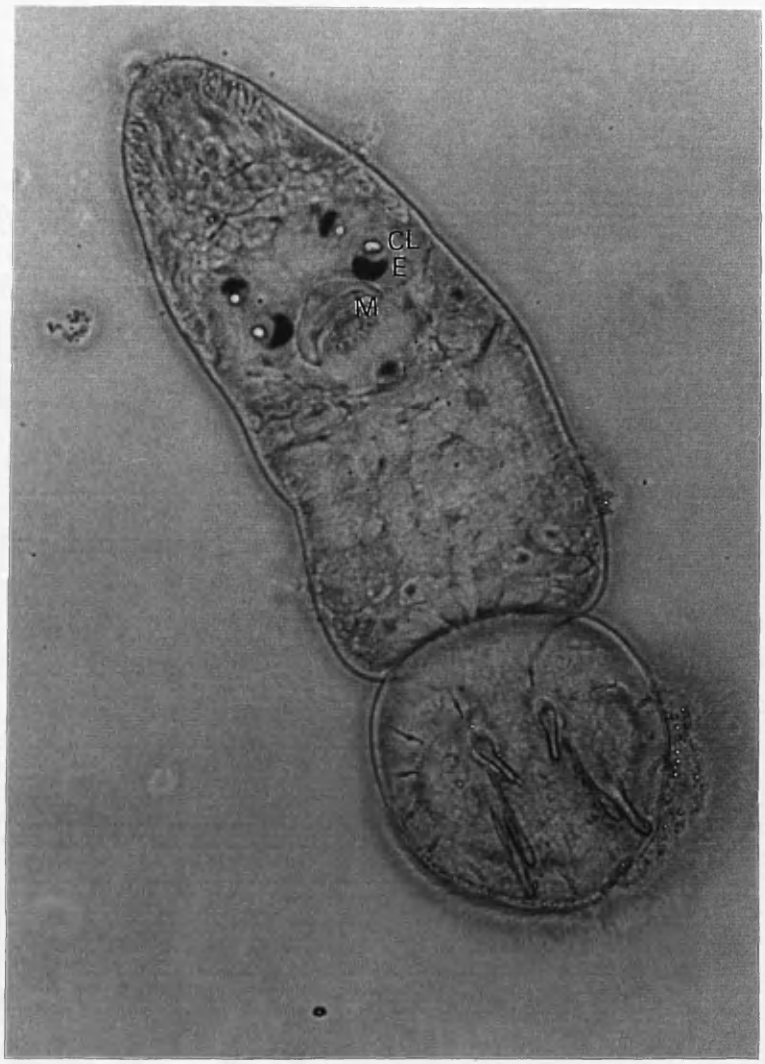


Figure 5.12. Live oncomiracidium of *E. hippoglossi* showing crystalline lenses. Note they appear blue-green and are highly mobile. (x 40 objective). (**E:** eyes, **CL:** crystalline lenses, **M:** mouth).

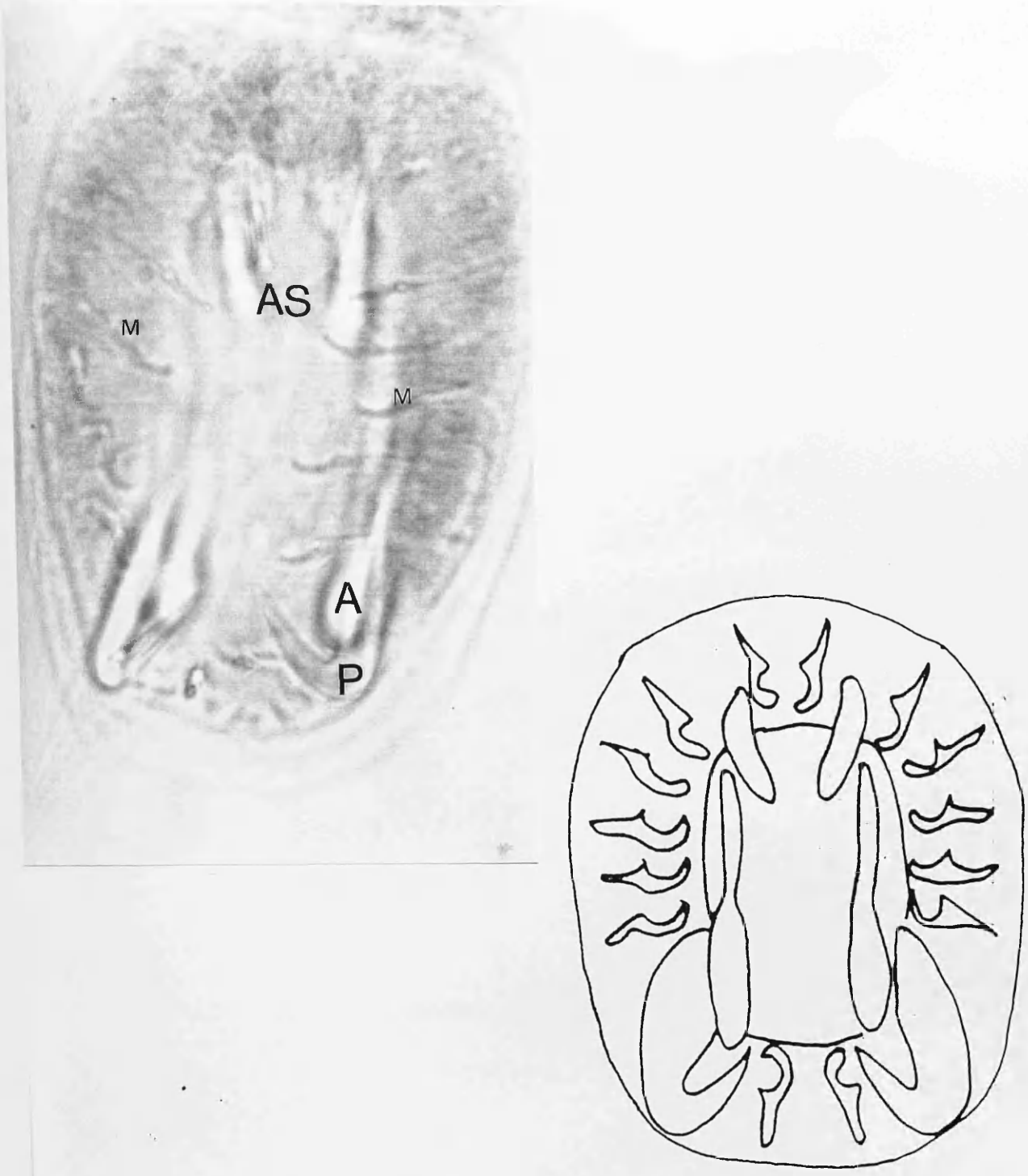
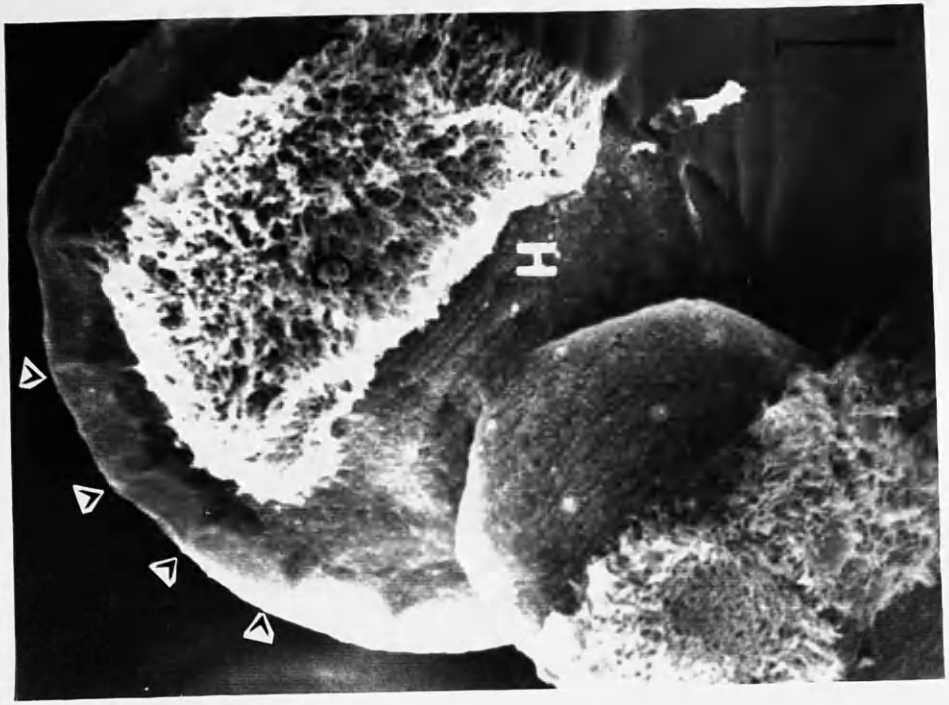


Figure 5.13. (a) Light micrograph of the haptor of *E. hippoglossi* stained in Mayer's Paracarmine. Ten pairs of sclerites can be seen on the haptor of the Oncomiracidium. (b) Diagram of haptor. Note three pairs of medianly situated hamuli and marginally situated seven pairs of marginal hooklet. (**A:** anterior hamuli, **P:** posterior hamuli, **AS:** accessory sclerites and **M:** marginal hooklets). (x 100 object).


H

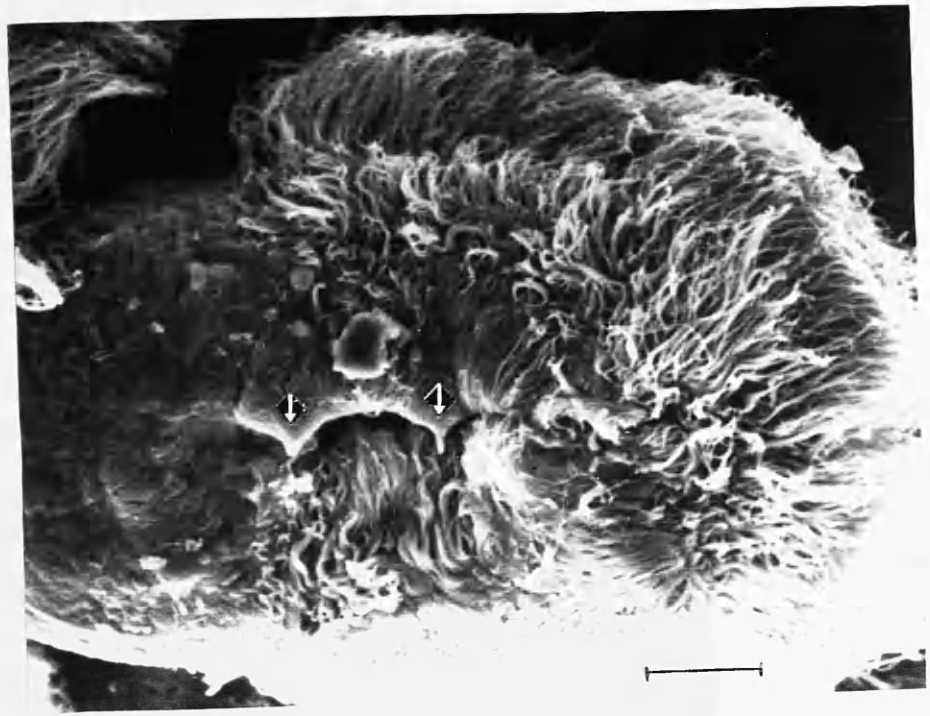


Figure 5.14. Scanning electron micrograph showing the folded haptor with cilia. Note the marginal hooklets (arrows) are clearly seen near the opening area of haptor. (Scale bar: 10 μm)

Figure 5.15. Scanning electron micrograph showing the dorsal view of the unfolded haptor bearing distinct cilia. Note the marginal hooklets (arrows) arranged around the margin of the haptor. (Scale bar = 10 μm) (**M:** marginal hooklets, **C:** cilia, **H:** haptor).

5.3.2 Ciliary epidermal plates of *E. hippoglossi* and *E. soleae*

5.3.2.1 *E. hippoglossi*

The epidermal ciliary plates of the oncomiracidium of *E. hippoglossi* were found on 3 main regions of the body surface, the anterior (zone I), the middle (zone III) and the posterior (zone V) as shown in Figure 5. 16.

The total number of epidermal cell plates on the anterior region was 27 (both ventral and dorsal surfaces). Amongst these, 15 cell plates were located dorsally and the rest of the cell plates (12 cells) were on the ventral surface of the oncomiracidium. The dorsally situated epidermal ciliary plates were almost rectangular in shape and larger than those on the ventral surface.

There was a total of 20 epidermal cell plates in the middle region (zone III) of the parasite (both ventral and dorsal surfaces). The dorsal area had 6 elongated rectangular cells while the ventral area had 8 smaller but densely packed plates. Six epidermal plates were laterally situated on the middle region.

In the posterior region (zone V), the haptoral part of the dorsal surface had 8 very obvious epidermal cell plates; these covered three quarters of the posterior region, whilst those on the ventral surface of the haptor had 9 epidermal cells which were not clearly observable due to the opening part of the haptor.

A scanning electron micrograph of the epidermal cell plates and sensilli on the middle region of the ventral surface of *E. hippoglossi* is shown in Figure 5.17.

5.3.2.2 *E. soleae*

The distribution of the epidermal ciliary plates of the oncomiracidium of *E. soleae* was similar to that of *E. hippoglossi*. The oncomiracidium had epidermal ciliary plates on 3 main parts of the body surface, the anterior, the middle and the posterior as shown in Figure 5.18.

The total number of epidermal ciliary plates on the anterior region was 27 cells, 11 on the dorsal, 6 on the ventral surface and 10 cells laterally situated (Zone I). On the dorsal surface, the epidermal plates covered the whole part of the anterior pair of eyes on the head region. The distance between the anterior plates and those on the middle region was closer than that of *E. hippoglossi*.

There were 20 ciliary plates on the middle region, 6 cells were situated dorsally, 8 cells were ventrally situated and 6 cells were laterally situated (Zone III).

On the haptoral region, 9 ciliary plates were very clearly seen on the dorsal surface while 4 less clearly seen cells were found on the ventral surface (Zone V).

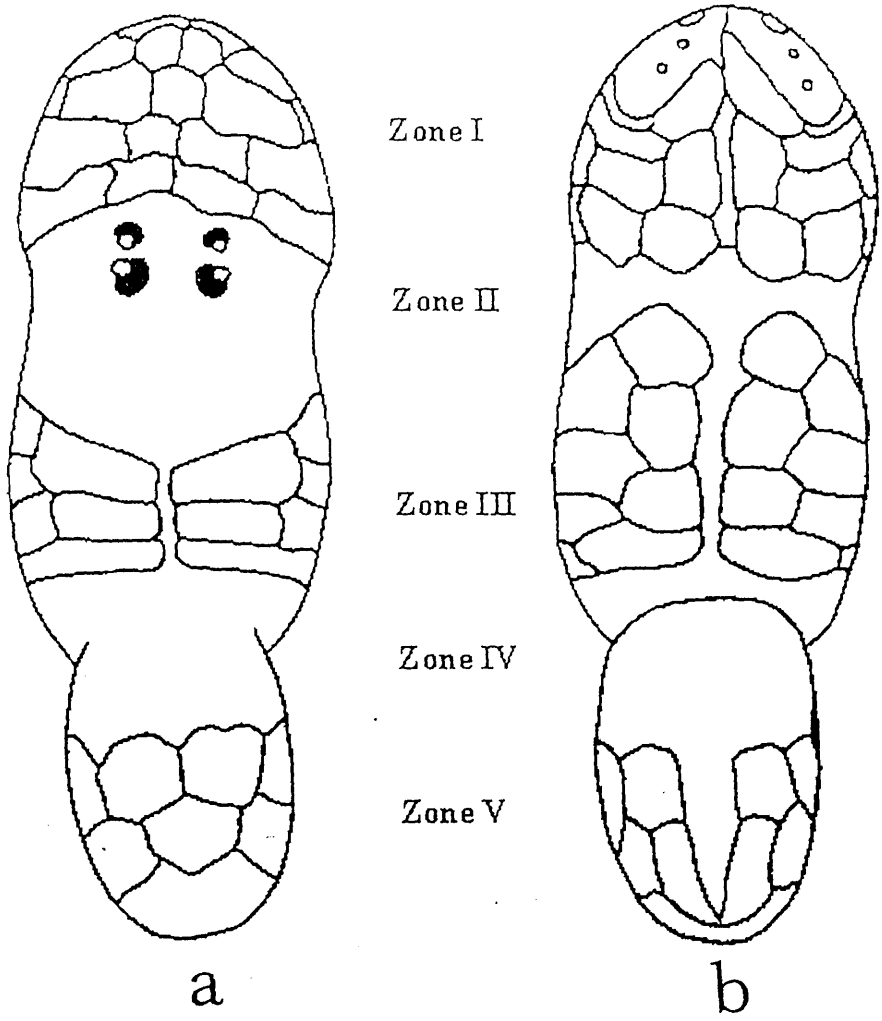


Figure 5.16.The arrangement of the epidermal cell plates of the dorsal (a) and the ventral (b) surfaces of the oncomiracidium of *E. hippoglossi*.

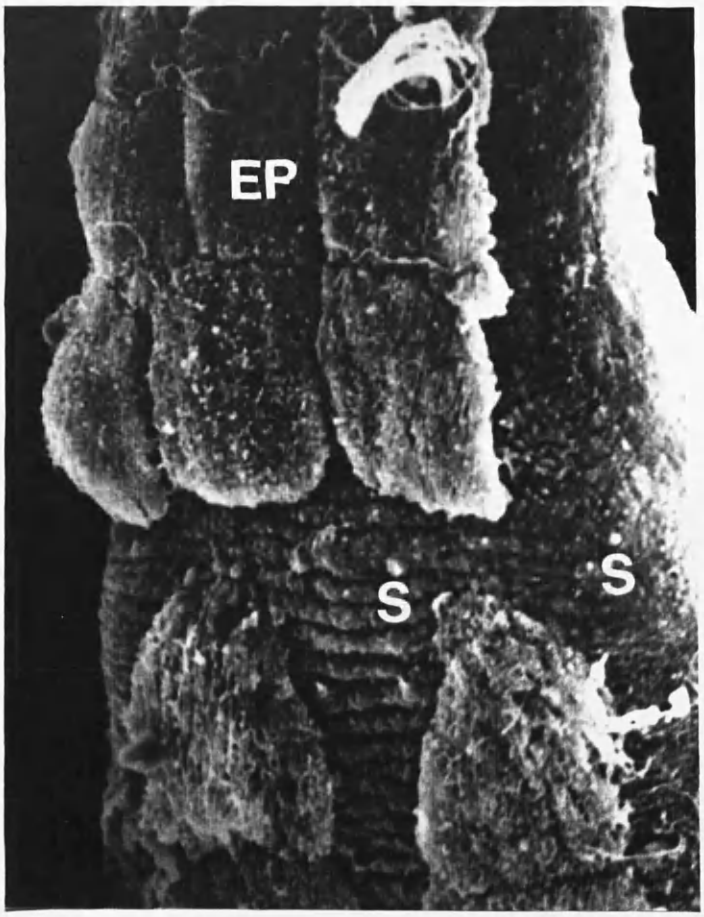


Figure 5.17. Scanning electron micrograph showing the epidermal cell plates (EP) and the sensilla (S) on the ventral surface of the oncomiracidium of *E. hippoglossi*. (Scale bar = 10 µm).

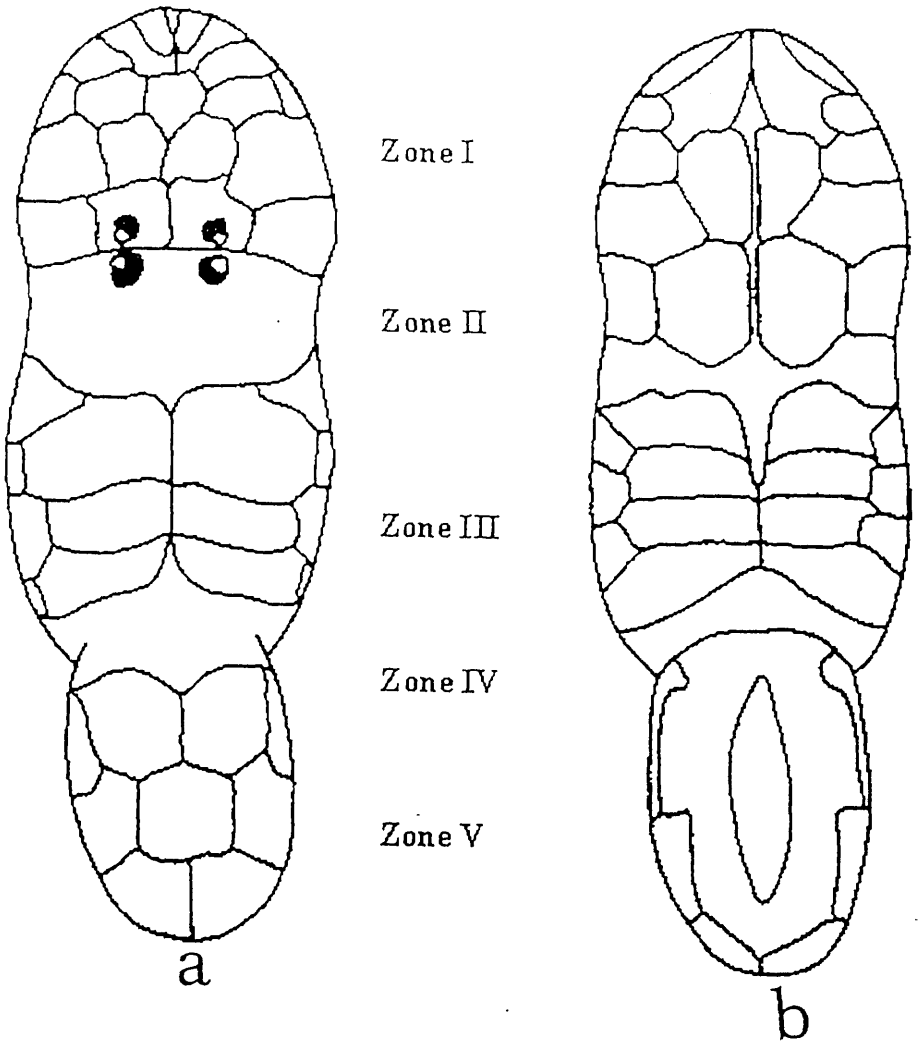


Figure 5.18. The arrangement of the epidermal cell plates of the dorsal (a) and the ventral (b) surfaces of the oncomiracidium of *E. soleae*.

5.3.3 Host finding behaviour

5.3.3.1 The swimming speed and movement pattern in 150 cm glass tubes

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 downwardly swimming behaviour. 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 Figure 5. 19. 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 ± 0.10 cm/sec. (3.1 ± 1.8 sec/cm) throughout the experiments. The average swimming speed of parasites in the first 3 minutes as they headed downwards was 0.30 ± 0.12 cm/sec (3.3 ± 1.88 sec/cm) and that of the parasites between 3 and 6 minutes was 0.32 ± 0.12 cm/sec (3.1 ± 1.92 sec/cm). The average swimming speed of the parasites after 6 min. was 0.37 ± 0.20 cm/sec (2.7 ± 2.2 sec/cm) (Figure 5. 20).

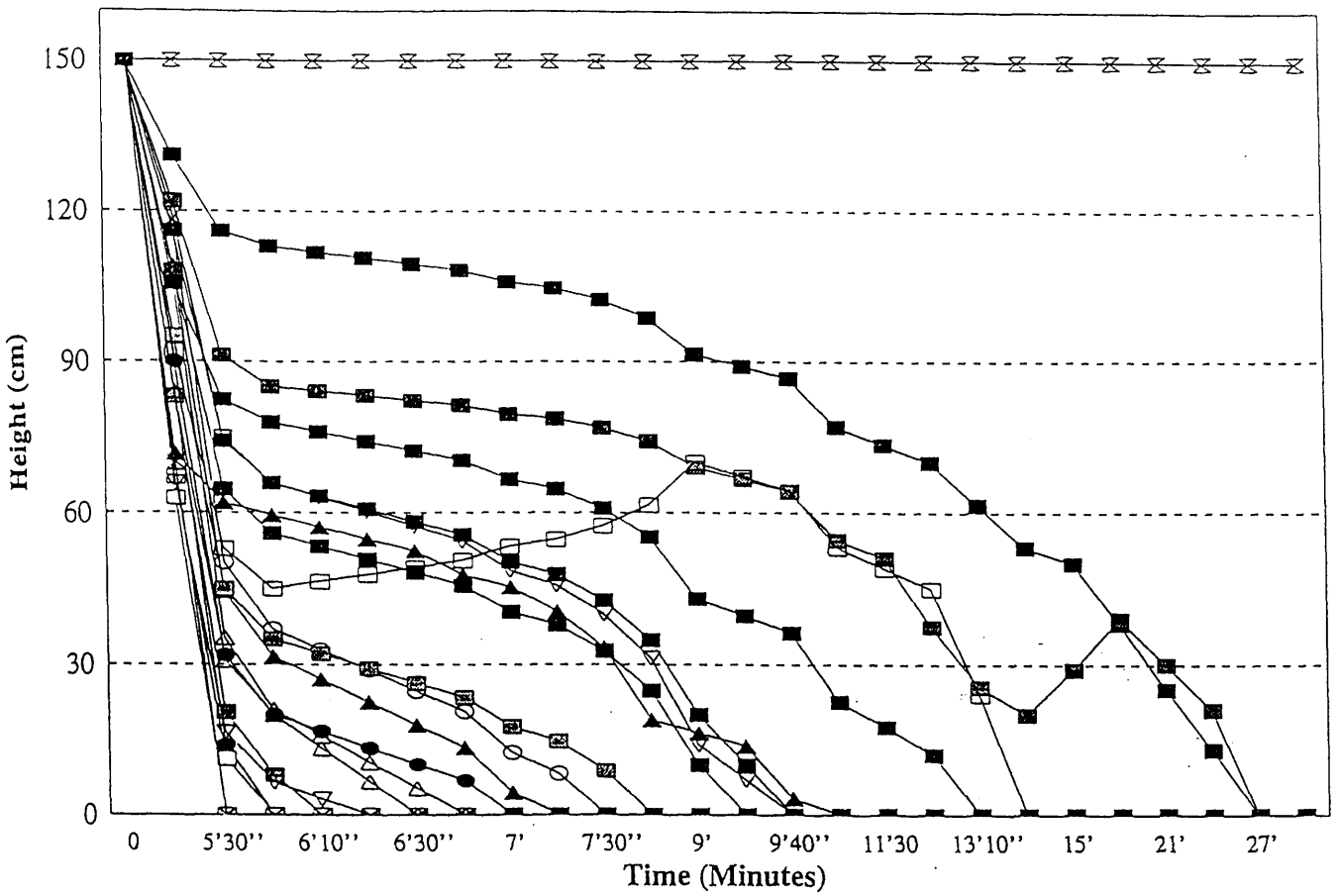


Figure 5.19. Swimming pattern and geotactic response of newly hatched oncomiracidia (n = 21). Note one parasite on the top of the graph did not move at all for 30 minutes while most parasites swam downwardly.

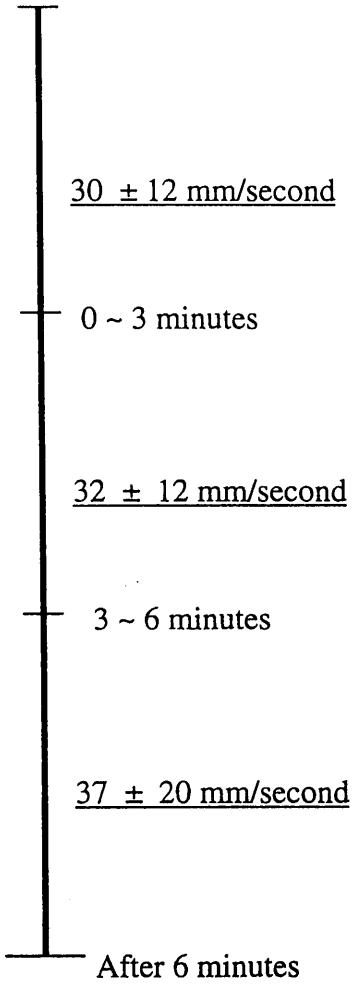


Figure 5.20. Average swimming speed of oncomiracidia at different times after introduction to the 150 cm glass tube.

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 ± 1.9 cm. The average swimming speed upwards was faster, 0.46 ± 0.11 cm/sec (2.2 ± 0.82 sec/cm) than downward swimming speed, 0.37 ± 0.20 cm / sec (2.7 ± 2.2 sec/cm) within the bottom 10 cm of the vessel.

5.3.3.2 Responses to variable distance and pressure

The up and down swimming behaviour within 10 cm of the bottom of the glass tube as described in paragraph 5.3.1 was consistent in all oncomiracidia tested. Therefore, this result needed to be confirmed by determining whether this behaviour would be influenced by different water depths or under different water pressures. Confirmation of this behaviour would suggest that it may be one of the essential methods for host searching of the oncomiracidium.

In the 10 cm glass tube, a scattered pattern of distribution was observed after 5 minutes. After 30 minutes, 4 of the 5 parasites were on the bottom of the glass tube, while one parasite was around 8 cm. After 2 hours from the start of the experiment, the distribution of the parasites was very similar to that after the examination at 5 minutes. There was no significant change at 6 hours. After 12 hours however, all the parasites were seen to have ceased moving and were located on the bottom of the glass tube; they were moribund (Figure 5. 21a).

In the 30 cm glass tube, the location of the parasites found to show a very similar pattern to those in the 10 cm glass tube up to 6 hours. At 12 hours, however, 2 parasites were seen still moving around the top and the middle part of the glass tube while 3 of the parasites were on the bottom (Figure 5.21b).

In the first 5 minutes in the 50 cm glass tube all of the 5 parasites moved very slowly. At 30 minutes and 2 hours, 2 parasites were on the bottom, 2 had moved around 15 cm and only one parasite moved about the top of the glass tube. After 6 hours, 2 parasites were seen on the bottom and one of the parasites around 15 cm moved to 10 cm and the other upward to the top of the glass tube. At 12 hours, 3 parasites were on the bottom of the glass tube, one was around 15 cm and the last one was still at the top of the glass tube (Figure 5. 21c).

In the 70 cm glass tube, the parasites continued to move downwards and upwards for 6 hours. However, by 12 hours, (the next observation), 3 parasites were seen on the bottom, one was seen around 15 cm and one was observed to be still on the top of the glass tube. The parasite at the top had apparently not moved from the beginning of the experiment (Figure 5. 22a).

In the 130 cm glass tube, after 5 minutes, one parasite was seen on the bottom of the glass tube, and a second was 30 cm from the bottom of the tube, while the remaining 3 parasites were still at the top of the tube. After 30 minutes, examination showed all the parasites to be scattered the full length of the glass tube (from bottom to top). When it was checked 2 hours later, 2 parasites were on the top of the glass tube, one of which was originally there, while the other seemed to have moved up from a lower position (90 cm). At 6 hours, the top parasites were still there but the parasites on the bottom were seen to be moving up and down within 10 cm of the

bottom. After 12 hours, all the parasites were found to be moribund on the bottom of the glass tube (Figure 5.22b).

Finally, in a repeat of the 150 cm glass tube, within 5 minutes, 2 parasites were seen 15 cm from the bottom, one parasite had not moved from the top and the other 2 parasites were seen moving towards the bottom of the glass tube. At 30 minutes, all the parasites were near the bottom of the glass tube and moving up and down around 10 cm in height. When these were checked over a further 2 hours to 12 hours, all the parasites were found not to move at all (Figure 5.23).

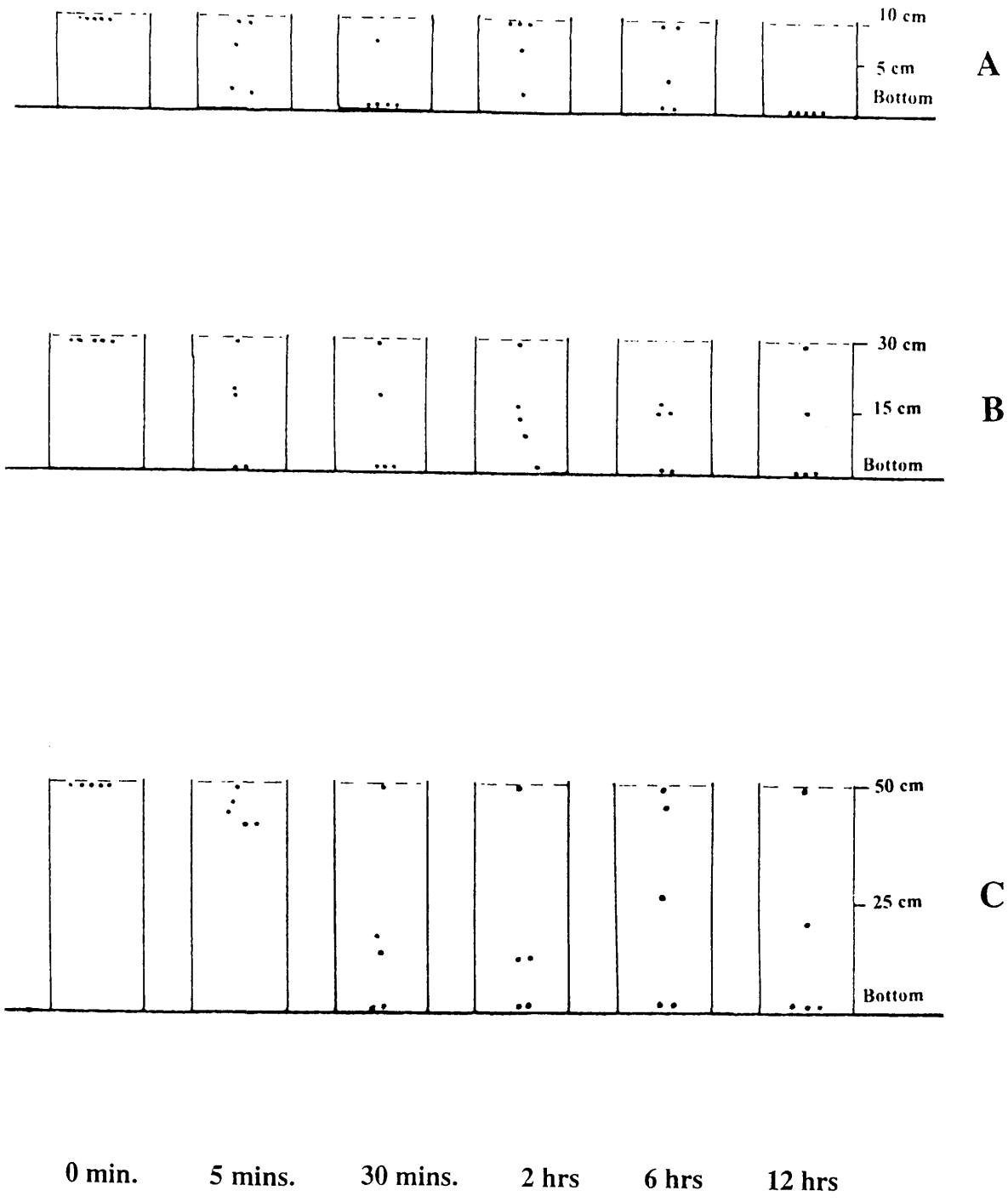


Figure 5.21. Results of the experiment to determine the swimming response of *E. hippoglossi* oncomiracidia to variable distance and pressure. (A: 10 cm, B: 30 cm, C: 50 cm)

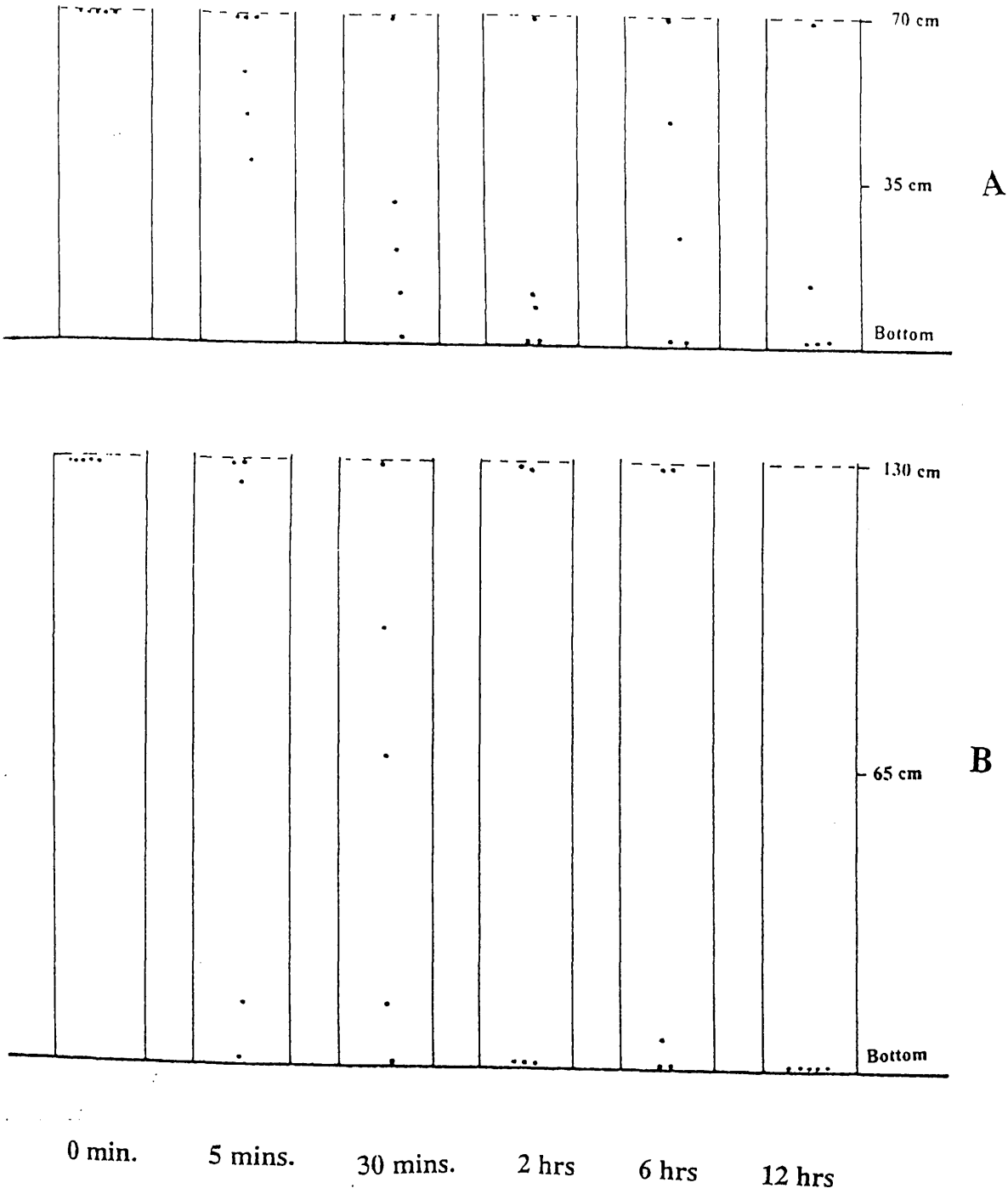


Figure 5.22 Results of the experiment to determine the swimming response of *E. hippoglossi* oncomiracidia to variable distance and pressure. (A: 70 cm, B: 130 cm)

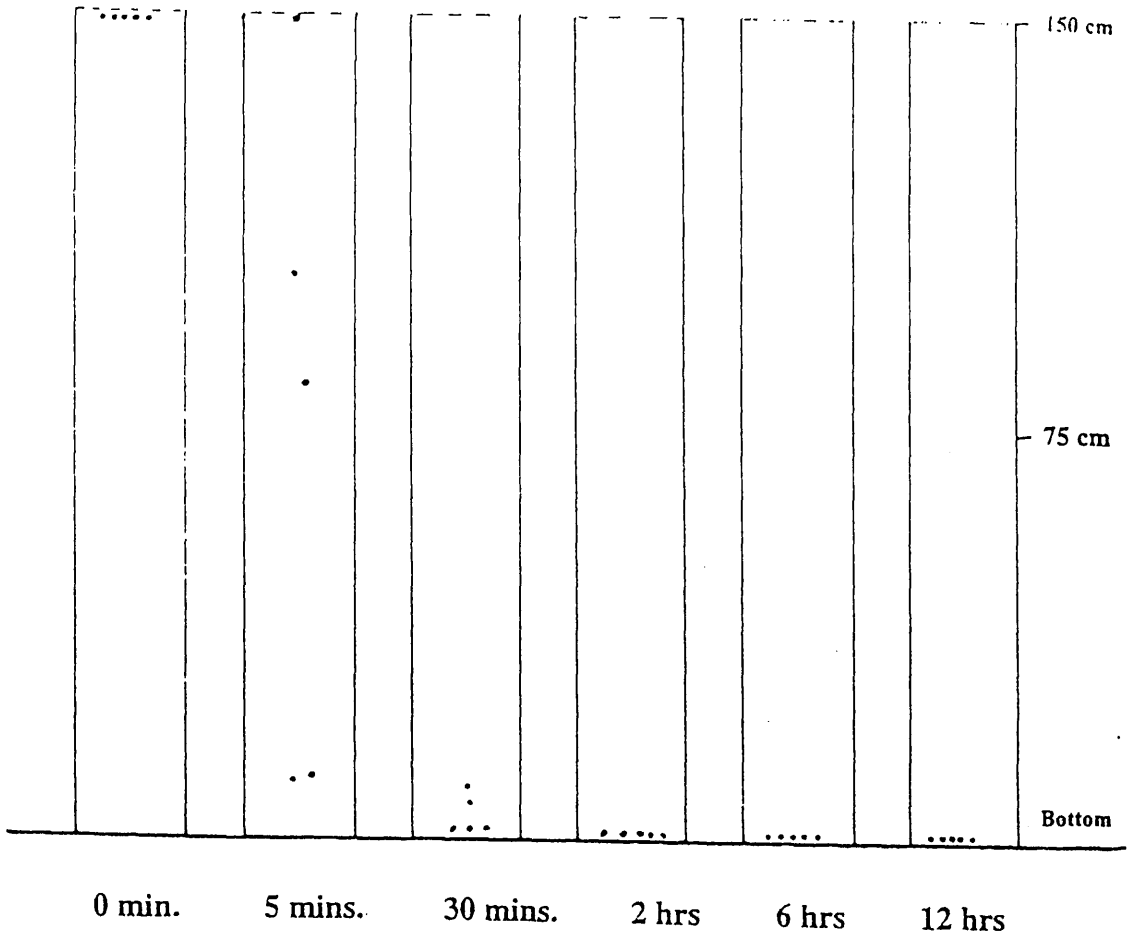


Figure 5.23 Results of the experiment to determine the swimming response of *E. hippoglossi* oncomiracidia to variable distance and pressure. (150 cm)

5.3.4 Two - armed - chamber trials

The response of the oncomiracidia of *E. hippoglossi* to the different stimuli is given in Table 5.2. 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 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.

Table 5.2 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 %)

5.4 DISCUSSION

5.4.1 Egg development and hatching

E. hippoglossi eggs hatched 25-27 days at 12 °C after incubation in the present study. Monogenean eggs appear to hatch spontaneously when their development is completed. The development time of monogenean eggs is highly dependent on water temperature (Kearn, 1970; Ogawa, 1988). It seems that the egg developing rate is strongly related to water temperature. Only one temperature, 12°C, was used in the present study, therefore it was not possible to determine optimal development time of *E. hippoglossi* at various temperatures.

Kearn (1974a) observed that *E. hippoglossi* hatched during the first few hours of darkness in contrast to the hatching of its close relative *E. soleae* which occurred in the light. He suggested that Atlantic halibut might have diurnal feeding habits, perhaps resting at night. Thus, *Entobdella* spp. oncomiracidia may hatch successfully in either light or dark probably depending on the behaviour of their hosts.

Egg hatching may also be chemically stimulated by a component of host skin mucus or by water turbulence, both indicative of the presence of a host fish. Eggs of *Acanthocotyle lobianchi* Monticelli, 1888, *Leptocotyle minor* Monticelli, 1888, *Hexabothrium appendiculatum* (Kuhn, 1829) Nordm, 1840, *Dactylogyrus nobilis* Long and Yu, 1958, *Urocleidus adspectus* Mueller, 1936 and *Pseudodactylogyrus bini* Kikuchi, 1929 all required the presence of host mucus or urea to hatch (Macdonald, 1974; Kearn and Macdonald, 1976; Cone, 1979; Llewellyn and

Macdonald, 1980; Chan and Wu, 1984; Shaharom-Harrison, 1986; Whittington, 1987).

In the present study, no hatching trigger for eggs of *E. hippoglossi* was determined, even though the eggs of many monogenean parasites have been shown to need stimuli for hatching. The eggs of *E. soleae* can also be induced to hatch by mucus from its normal host *Solea solea*, but also by mucus from plaice, halibut, ray and whiting (Kearn, 1974c). Kearn also tried to hatch the eggs of *E. hippoglossi* using halibut mucus and sole mucus but he found that neither mucus could induce the eggs of *E. hippoglossi* to hatch.

In the case of *E. soleae*, the lack of specificity seems to be disadvantageous for the parasite because any fish mucus might induce the hatching of the eggs. However, lack of specificity might give a greater chance of finding a host considering the short longevity of oncomiracidia or the specific host may not be the dominant fish species in the vicinity of the hatching eggs so that the oncomiracidia would not find it. In the case of *E. hippoglossi*, its host, the Atlantic halibut, is a very active predator and a deep sea dwelling fish which may not congregate with other fishes in the natural environment. Bovet (1959) reported that water turbulence may play a part in the hatching of *Diplozoon paradoxum* Nordmann, 1832 and Ktari (1969) found that larvae of *Microcotyle salpae* Parona and Perugia, 1890 hatched when the eggs were disturbed by a water jet. It is likely that these stimuli might also be experienced in the natural environment, perhaps caused by the movement of fish hosts.

With regard to the environment of the natural habitat of Atlantic halibut, it would seem that the water current is one of the most important cues for hatching of *E. hippoglossi* oncomiracidia. However, more precise investigations are needed.

5.4.2 Morphology of the oncomiracidium

The morphology of the oncomiracidium of *E. hippoglossi* bears a general resemblance to the oncomiracidium of *E. soleae* (Kearn, 1963b) and *Benedenia seriolae* (Kearn *et al.*, 1992a). There are three zones of cilia based on the arrangement of epidermal ciliary plates, a ventrally sited mouth, four conspicuous eyes with lenses and a cup shaped haptor bearing 14 peripherally located marginal hooklets and three pairs of median sclerites, namely accessory sclerites, anterior hamuli and posterior hamuli.

Most oncomiracidia are at least partly covered externally with cilia, except those which are ovoviviparous in which embryos escape from the egg capsules into the uterus having no cilia.

The ciliated epidermal plates of *E. hippoglossi* are distributed in three zones, an anterior, a middle and a posterior. Owen (1970) reported that *Discocotyle sagittata* had flattened ciliated epidermal cells on the three areas of the dorsal and the ventral surfaces. The antero-lateral ciliated region was made up of a row of five cells elongated in a dorso-ventral direction. The six various shaped cells were in the lateral areas while the posterior areas consisted of three cells. Owen (1970) found that the ciliated cells enlarged as the larva approached the end of the active swimming stage and then they separated from their neighbours. In the present study using SEM it was also found that the epidermal ciliary plates elongated and separated from neighbour cells on *E. hippoglossi* larva. Tinsley and Owen (1975) found 64 discrete epidermal cells arranged in a consistent symmetrical pattern and two distinct types of sensillae on the oncomiracidium of *Protopolystoma*

xenopodisa. In the present study the *E. hippoglossi* oncomiracidium was found to have 27 ciliary plates in the anterior (dorsal, 15; ventral, 12), 20 in the middle region (dorsal, 6; ventral, 8 and lateral, 6) and 17 in the posterior region (dorsal, 8; ventral, 9). The cells on the dorsal surface were usually larger than those on the ventral surface. It was very difficult to count the cells on the posterior ventral part due to the haptoral opening in the present study. The number of epidermal ciliary plates on the anterior region of *E. soleae* was the same (27) as that of *E. hippoglossi*. Those on the middle region numbered 20, also the same as in *E. hippoglossi* although the epidermal plates of the two species were clearly different in form. However, on the posterior region of *E. soleae* there were only 13 ciliary plates compared to the 17 on *E. hippoglossi* found in the present study.

Kearn (1963b) also reported ciliary plates on the oncomiracidium of *E. soleae*, although he suggested that there was a possibility of losing cells before counting. He found 20 cells from the anterior, 16 cells from the middle area and 9 from the posterior area suggesting that there was indeed a loss of cells in his study. Lambert (1980) found that 3 species of Capsalidae, *Benedenia monticelli* Pauna and Perugia, 1895, *Entobdella soleae* and *Trochopus pini* Beneden and Hesse, 1863 had a total of 64 ciliary plates on the oncomiracidium. The cells were also distributed in the 3 main regions, 27 cells on the anterior region, 20 cells on the middle region and 17 cells on the haptoral region. When comparing previous studies of other capsalids with the present study, the number of ciliary plates on the anterior region and the middle region were the same but the posterior region for *E. soleae* was different. The reason for these differences may be the lack of clarity of the ciliary plates on the posterior region which may be due to the haptoral opening or to technical difficulties.

Details of the pattern of ciliation are less known for marine species than for freshwater species. This lack of balance is due to technical difficulties in investigating ciliated epidermal cells. When treatment with silver nitrate was applied to oncomiracidia living in sea water, it was unsuccessful because of the dark dense precipitate which was formed as it reacted with the sea water. In the present study three techniques were tried. The first method was rinsing of oncomiracidia in distilled water prior to treat with silver nitrate but it was found that the cilia plates were barely visible. The second one used 0.36 M $MgCl_2$ to avoid precipitation, but this method also found that the cilia plates were not sufficiently clear. The third method used only silver nitrate without any prior treatment, but the seawater was (almost completely) removed. Using this method, it was quite hard to recognize the oncomiracidia in the solution but the result was successful.

Most polyopisthocotylean oncomiracidia have a single pair of eyes. However, Llewellyn (1972) pointed out that, although most polyopisthocotylieans have only a single pair of pigment cups without special lenses, two of them, *Discocotyle sagittata* (Leuckart, 1842) Diesing, 1850 and *Diplozoon paradoxum* are, neverthless, positively phototactic. Additionally, species of *Diclidophora*, which apparently lack eyes entirely, have been shown to exhibit circadian hatching rhythms (Macdonald, 1975) and perhaps may have unpigmented photoreceptors. Macdonald (1977) observed *Diclidophora melangi* had no eyes but at least a pair of hairs situated on each side of the mouth which had a sensory function.

Most monopisthocotyleans have eyes with crystalline lenses. Kearn (1963c) described the oncomiracidium of *Capsala martinieri* Price, 1938. It had two pigmented pairs of eyes and body pigment which consisted of small yellowish

brown granules. The granules were distributed continuously over the whole body and the same granules were found in the pigmented cups of the eyes. Kearn *et al.* (1992a) found conspicuous eyes with lenses in oncomiracidia of *B. seriolae*. The oncomiracidia had lenses associated with the large eyes (posterior eyes) which frequently have a shallow median furrow. They suggested that this structure was due to the lenses being secreted in two separate halves. In the present study, the oncomiracidium of *E. hippoglossi* also had highly mobile eyes with developed lenses. It may be that they have the most important role in finding a host. The lensed eyes would have directional sensitivity to light, thus helping to orientate the free swimming larva or, at least, respond to changes in illumination such as shadowing by a passing host. Lyons (1972) reported eye movements in the oncomiracidium of *E. soleae* and Kearn and Baker (1973) using electron microscopy revealed the presence of fibres near the outer surface of the eyes.

Little work has been done on the glands in the oncomiracidia of monogenean parasites. Kearn (1974b) described glandular systems of the oncomiracidia of *E. soleae*, *E. hippoglossi* and *E. diadema*. He found that there were three different kinds of gland cells with ducts opening on the margins of the head region of the oncomiracidium of *E. hippoglossi*. The distribution of the head glands of *E. soleae* and *E. diadema* resembled that of the head glands of *E. hippoglossi*. While the oncomiracidium of *E. hippoglossi* had 9 pairs of body gland cells, that of *E. diadema* had 8 pairs and the oncomiracidium of *E. soleae* had only 4 pairs. He suggested that the head glands secreted adhesive materials to attach the oncomiracidium of *E. soleae* to the host surface. In the present study it was found that the oncomiracidia of *E. hippoglossi* attached themselves on to the bottom of the

container using the head region. The head glands of the oncomiracidium of *E. hippoglossi* were very conspicuous and their secretion was likely to be involved in the attachment of the parasite larvae to the host until the haptor opened.

Skin parasites on an active fish are more exposed to powerful water currents than parasites on a slow moving or sedentary fish. Therefore, the attachment organ of a skin parasite is related to the parasite size, its site of infection and the behaviour and swimming speed of the host. With increasing size of the parasite, its resistance to water flow is likely to increase, requiring a more powerful attachment organ (Kearn, 1976). Monogenean parasites have developed for this purpose a posterior attachment organ in the form of a haptor armed with hooks, clamps or suckers and sometimes an anterior adhesive organ. The subclass Monopisthocotylea possess a single unit haptor with one or two pairs of hamuli and twelve to sixteen marginal hooks. The subclass Polyopisthocotylea have a haptor which is well developed and bears clamps and sucker. Kearn (1963b) reported that the oncomiracidium of *E. soleae* possesses fourteen marginally situated marginal hooklets, which had already reached their definitive size, and a pair of anterior and a pair of posterior hamuli and accessory sclerites. He suggested that one pair of marginal hooklets became the accessory sclerites. In the present sudy, three pairs of medianly situated sclerites, the anterior hamuli, the posterior hamuli and the accessory sclerites, and seven pairs of marginal hooks were found in the oncomiracidium of *E. hippoglossi.* The findings from the present study, the shape, number and arrangement of marginal hooklets and three pairs of medianly located hamuli were similar to those of *E. soleae.* The oncomiracidium of *Capsala martinieri* had fourteen marginal hooklets and a single pair of sclerites situated medianly (Kearn, 1963c). Kearn (1964) found that the

posterior hamuli and marginal hooklets ceased their growth, while the accessory sclerites and the anterior hamuli continued to grow throughout the life of *E. soleae*.

5.4.2 Host finding behaviour

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. Poulin, Curtis and Rau (1990) found that the copepodids of *Salmincola edwardsii*, an ectoparasite on brook trout, *Salvelinus fontinalis* Mitchill, 1814, moved very actively when visual and mechanical cues were produced by potential fish hosts. Due to its strong positively phototactic response (Wootten, Smith and Needham, 1982; Bron, Sommerville, Jones and Rae, 1993), the copepodids of the salmon louse, *Lepeophtherius salmonis* Krøyer, 1837 distributed near the surface of water during the day and spread out into deeper layers at night (Heuch, Parsons and Boxaspen, 1995). It seemed that they might have a better chance of locating and infecting a suitable host (Wootten *et al.*, 1982; Bron *et al.*, 1993).

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. 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. Despite different water pressure and swimming distance, all parasites swam downwards and showed a similar behaviour of upwards and downwards swimming after arriving at the bottom of the test vessel. For all the experimental distances, 10 to 150 cm, the parasites moved within 10 cm length from the bottom of the glass tube after arriving. This may suggest that the oncomiracidia of *E. hippoglossi* do not swim higher than that 10 cm distance due to their host habitat. 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 exposed 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 20°C, 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* Beneden and Hesse, 1863 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 sinking passively.

Mackerel, *Scomber scombrus* L., 1758, gill parasites, *Kuhnia scombri* Kuhn, 1829 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 behaviour has a stronger influence than phototactic behaviour 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. Third, they attach on to the host temporarily by their anterior adhesive gland organ. Then the haptor opens and attaches to the host firmly by means of hooks.

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.

Chapter 6

THE HOST/PARASITE INTERFACE

6. HOST AND PARASITE INTERFACE

6.1 INTRODUCTION

Many species of parasites affect marine organisms in such a way as to make them commercially less valuable and some are capable of causing mass mortalities. Generally, mass mortalities caused by parasites have not been frequently observed, but there are several examples of mass mortalities caused by parasites. When the gill monogenean, *Nitzschia sturionis* (Abildgaad, 1794) Krøyer, 1852 was transferred with the sturgeon *Acipenser stellatus* into the Aral sea, the parasite killed the local sturgeon, *A. nudiventris*, which were not previously infected with this monogenean (Petrushevski and Shulman, 1961). Becheikh, Rousset, Maamouri, Ben-Hassine and Raibaut (1997) reported that the copepod parasite *Peroderma cylindricum* Heller, 1865 attached to the skin of pilchard, *Sardina pilchardus* Walbaum, 1792 then inserted the cephalic region into the anterior part of the host's kidney. This infection induced mortality mainly in young pilchards aged 1 to 2 years. *Lepeophtheirus salmonis* commonly occurs on salmonid fishes, causing high mortality (Cusack, 1995; Johnson, Blaylock, Elphick and Hyatt, 1996).

Among invertebrate hosts, many cases of mortality were reported from oyster, *Crassostrea virginica* (Chintala, Ford, Fisher and Ashton-Alcox, 1994; Andrews, 1996; Oliver, Fisher, Burreson, Ragone-Calvo, Ford and Gandy, 1996), scallop, *Argopecten irradians* (Chu, Burreson, Zhang and Chew, 1996) and giant clam, *Tridacna gigas* (Boglio and Lucas, 1997).

Weight loss or retardation of growth may be a result of parasite infection. Khan and Lee (1989) found that fish infected with young parasites ate more food and gained

more weight than uninfected fish for the first few weeks after infection, but, over a 16 month period, condition factors were lower than in controls in Atlantic cod. The brown sole, *Limanda herzensteini*, in Japan showed a reduced growth rate and weight loss when infected with the copepod parasite *Haemobaphes diceraus* (Nagasawa and Maruyama, 1987). Bower and Boutiller (1989) reported that when the crustacean parasite *Sylon hippolytes* occurred in the prawn, *Pandalus platyceros*, the gonad weight of infected prawns was 50 % that of uninfected prawns.

Some parasites may affect the consumability of their hosts. Rohde (1976) reported that all large kingfish, *Seriola grandis*, at the southern end of the Great Barrier Reef were infected with a myxozoan, which makes the flesh tasteless and inedible. Also, the myxosporean parasite *Kudoa thyrsites* (Gilchrist) is well recognised as a cause of flesh liquefaction in several fishes (Whitaker and Kent, 1991; Langdon, Thorne and Fletcher, 1992).

Halibut culture has been developing very rapidly and, therefore, it is important to know more precisely how *E. hippoglossi* may harm the host fish. There is a possibility of infection of juvenile fish in the hatchery with potentially serious consequences. The previous study, Chapter 3, has shown that *E. hippoglossi* infections only occurred on mature halibut. However, heavier infections are always treated in anticipation of more severe effect as the broodstock have a high price value. Aquaculture systems will give a greater chance of exposure of juvenile halibut to mature halibut with the potential of epizootics in valuable growing stages. Therefore, this study set out to investigate whether *E. hippoglossi* parasitizes only mature halibut because the parasites have no chance to meet juveniles due to difference of habitats between mature halibut and juvenile halibut in fish farms, or whether the parasite only infects mature fish due to some specific reason. Since

juveniles are generally more susceptible to ectoparasites, the study also investigated the pathological effects of the adult parasites on experimentally infected juvenile fish. The host and parasite interactive mechanism was investigated using histopathology and SEM of infected skin.

6.2 MATERIALS AND METHODS

6.2.1 Parasite infection

6.2.1.1 Infection conditions

Parasites were collected from a heavily infected mature halibut. Sampled parasites were brought into the University of Stirling and kept at 12°C in a constant temperature room to collect parasite eggs over a period of 2 days. Only healthy parasites were collected from the spawning container. These parasites were used to infect 4 juvenile halibut which came from Ardtoe and they did not infect the parasite before held in a 1 m^3 culture tank held at the marine research station at Machrihanish. The fish were about 30 cm in standard length and were held at a water temperature of 12°C , the water being on a gentle flow through.

Twelve parasites were used to artificially infect each fish. The parasites were carefully removed from the container using rounded forceps and then placed on the skin of the fish. Each fish was clearly identifiable from its skin pigmentation. Fish 1 had the same colour on both surfaces and one eye on each side. Fish 2 had the darkest dorsal surface amongst the 4 fish. Fish 3 had dark and white marking on its dorsal surface and fish 4 had completely white skin on both surfaces, dorsal and ventral. The 4 infected fish were kept together with other 4 uninfected fish in the

same tank to check whether the parasite could transfer to uninfected fish or not. The uninfected fish were from the same stock and were the same size as the infected fish. The 4 uninfected fish were not identifiable from each other.

6.2.1.2 Susceptibility of juvenile fish

The parasites were experimentally placed on the 4 juvenile halibut, but the twelve parasites were placed on a different area on each fish. The twelve parasites were placed on the posterio - ventral area of Fish 1, 12 parasites were placed on the posterio - dorsal region of Fish 2. On Fish 3, 12 parasites were placed on the mid - dorsal region and, finally, 12 parasites were placed on the mid - ventral region of Fish 4, which had unpigmented dorsal and ventral surfaces. The movement of the parasites was monitored after infection, and the susceptibility of the juvenile halibut to adult parasites was checked every 2 weeks for 8 weeks. The 4 uninfected fish were checked at the same time as the infected fish to investigate whether any parasites transferred to other fish.

The adult parasites on the dorsal surface were counted and mapped for their location. Parasites on the ventral surface were checked using a specially designed 'see-through container' which was designed for checking the underside of the flatfish. This was made from a normal feed container from which the base was cut and then a piece of perspex was sealed on the bottom of the container using silicon adhesive. The size of the container was 70 cm x 40 cm x 11 cm.

When the experiment was terminated after 8 weeks, all the 8 fish were checked under the dissection microscope to see if they carried oncomiracidia or not.

6.2.2 Pathology

6.2.2.1 Histology

The infected juvenile fish were killed after 8 weeks and prepared for histology. The fish skin was cut into blocks of 4 cm^2 (2 cm x 2 cm), fixed and processed for histology. Exactly the same area of the skin from uninfected juvenile fish was used as a control. Blocks were fixed in 10 % neutral buffered formalin for a minimum of 24 hours. All the procedures for histology were followed as described in Chapter 2. The sections were stained in haematoxylin and eosin.

6.2.2.2 SEM

Infected fish skin with attached parasites was dissected after 8 weeks infection and then put into 1 % glutaraldehyde at 4°C for 3 days. They were then moved into 3 % glutaraldehyde at 4°C for a week. During the fixation, the parasites detached from the infected skin naturally. All the procedures followed were as described in Chapter 2.

6.3 Results

6.3.1 Susceptibility of the juvenile halibut to the parasite

Almost all of the parasites moved to the front part of the fish whether they were placed on the dorsal or ventral surface of the fish. Observation during the first 2 hours showed that the parasites usually followed a straight line on the upper part of the fish (near the base of the dorsal fin). After only 2 hours observation time, the parasites on Fish 1 moved to the front region of the ventral surface of the fish and

then they settled there. No parasites were found infecting the dorsal surface, even though the pigmentation was the same as both surfaces. On Fish 2, the parasites rapidly moved to the front region of the dorsal surface of the fish then, moving around, they settled on the front area of the ventral surface of the fish. The parasites placed on Fish 3, also moved to the front area of the fish except for one parasite, which stayed in the same area in which it had been placed initially, in the middle area.

On Fish 4, all 12 parasites moved to the front part of the dorsal surface of the fish but later 3 parasites settled on the dorsal head region of the fish whilst the other parasites moved to the ventral surface.

Fish 1

On Fish 1, 3 parasites were found attached on the ventral surface of the fish after 2 weeks infection. Two of them were near the dorsal fin base and one parasite was on the operculum of the fish. When they were checked 4 and 6 weeks post infection, the parasites were found in almost the same sites as they had settled at 2 weeks post infection. After 8 weeks post infection, only two parasites were found on the operculum of the ventral surface of the fish. None of the parasites were found to have moved to the dorsal surface (Figure 6.1).

Fish 2

On Fish 2, four parasites were found to have moved to the ventral surface of the fish, 2 of them were near the dorsal fin base and another 2 were found on the operculum of the fish after 2 and 4 weeks post infection. When they were checked after 6 weeks, one parasite on the operculum of the fish had detached while the 2 on

the dorsal fin base were still there. After 8 weeks post infection, the 2 parasites on the dorsal fin base were still attached while one parasite on the operculum was detached (Figure 6.2).

Fish 3

The parasites were placed on the middle region of the dorsal surface of Fish 3 and when they were checked after 2 weeks, 4 of them were found on the ventral surface and one parasite was still in the same area as it had been placed initially. After 4 weeks' infection, a total of 5 parasites was found on the ventral surface of the fish, the parasites from the dorsal surface had seemingly moved to the ventral surface. They were all settled near the dorsal fin base. When they were checked at 6 weeks, the parasite number and location was almost the same as week 4. No parasites were found when they were checked after 8 weeks post infection. It appears that all the parasites had detached from the host fish (Figure 6.3).

Fish 4

On the unpigmented Fish 4, seven parasites were found on the front part of the ventral surface after 2 weeks infection. When they were checked 4 weeks after infection, a total of 5 parasites were found on the ventral surface. Two of them were found near the dorsal fin base and another 3 were on the operculum of the ventral surface. At 6 weeks, two parasites were found near the ventral fin base and one parasite was found near the mouth on the ventral surface. At 8 weeks, only one parasite was found near the mouth part of the ventral surface and no parasite was found on the dorsal surface (Figure 6.4).

When the 4 uninfected fish were checked carefully, no transferred parasites were found. The 8 weeks period was sufficient time for the hatching of the eggs of this parasite at the holding temperature, however, no oncomiracidia were found either on the fish infected with parasites or on the uninfected fish.

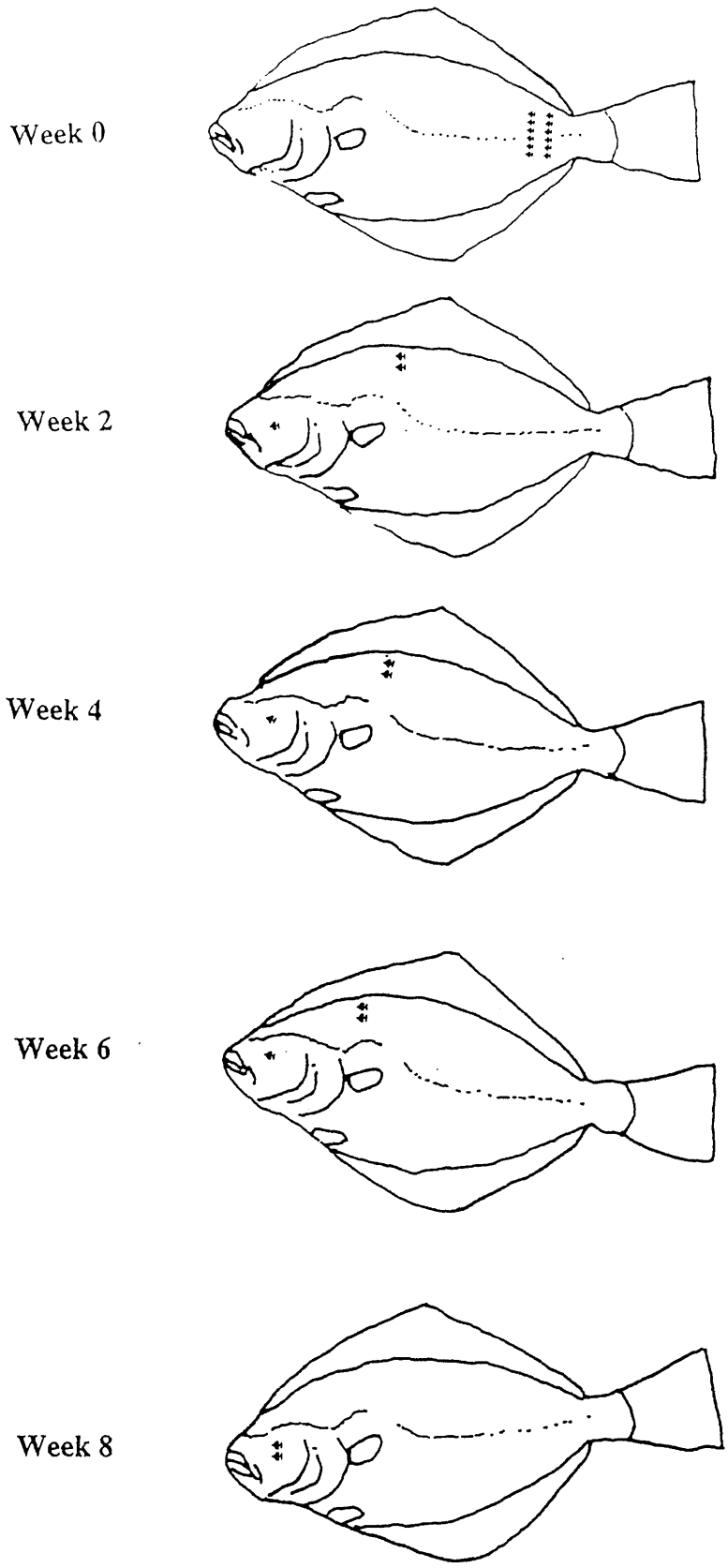


Figure 6.1 The distribution of adult parasites on juvenile Fish 1 during the 8 weeks of experimental period. ♠ sign represents the parasites. (ventral surface).

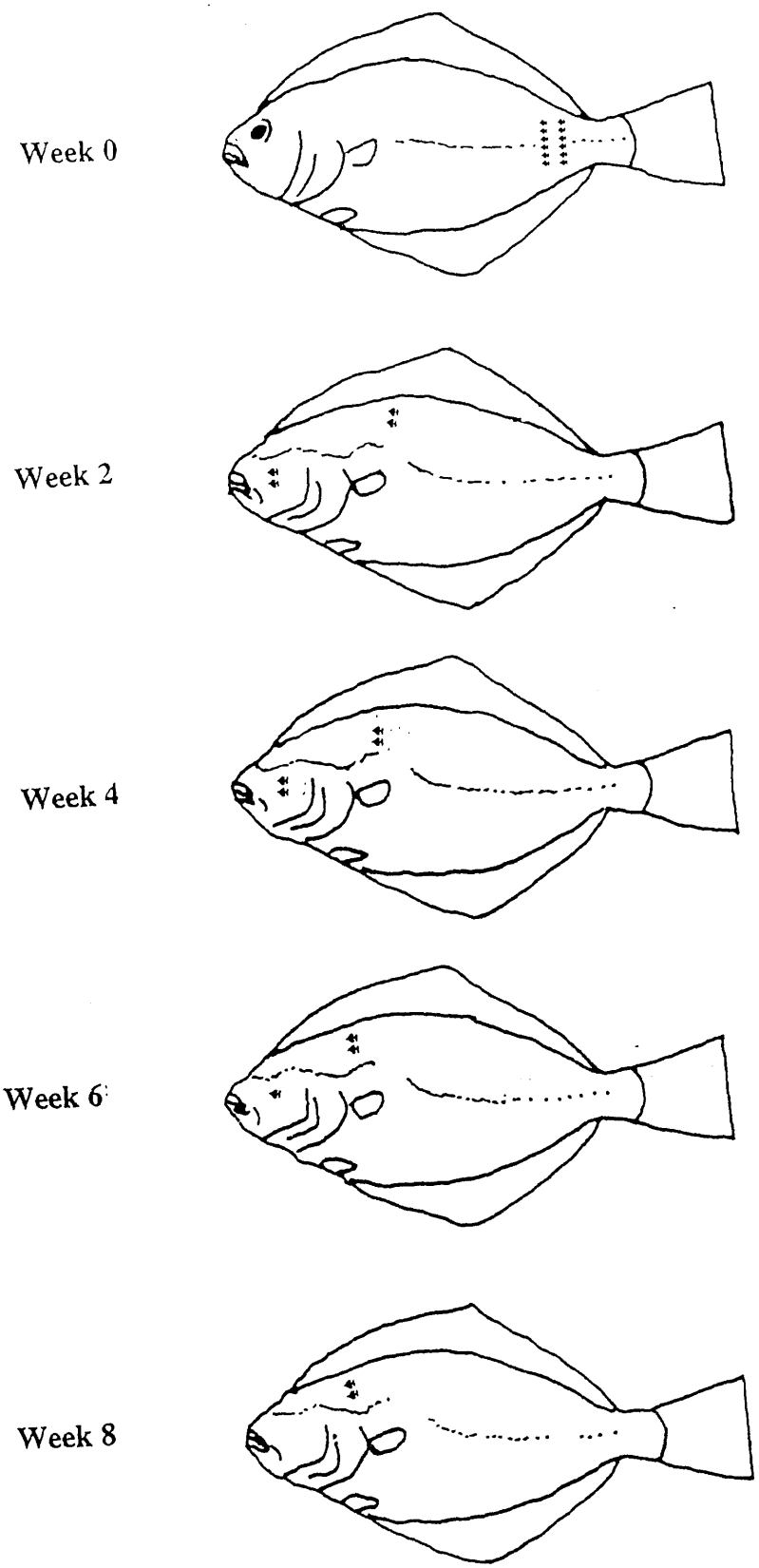


Figure 6.2. The distribution of adult parasites on juvenile Fish 2 during the 8 weeks of experimental period. ♠ sign represents the parasites. (dorsal and ventral surface)

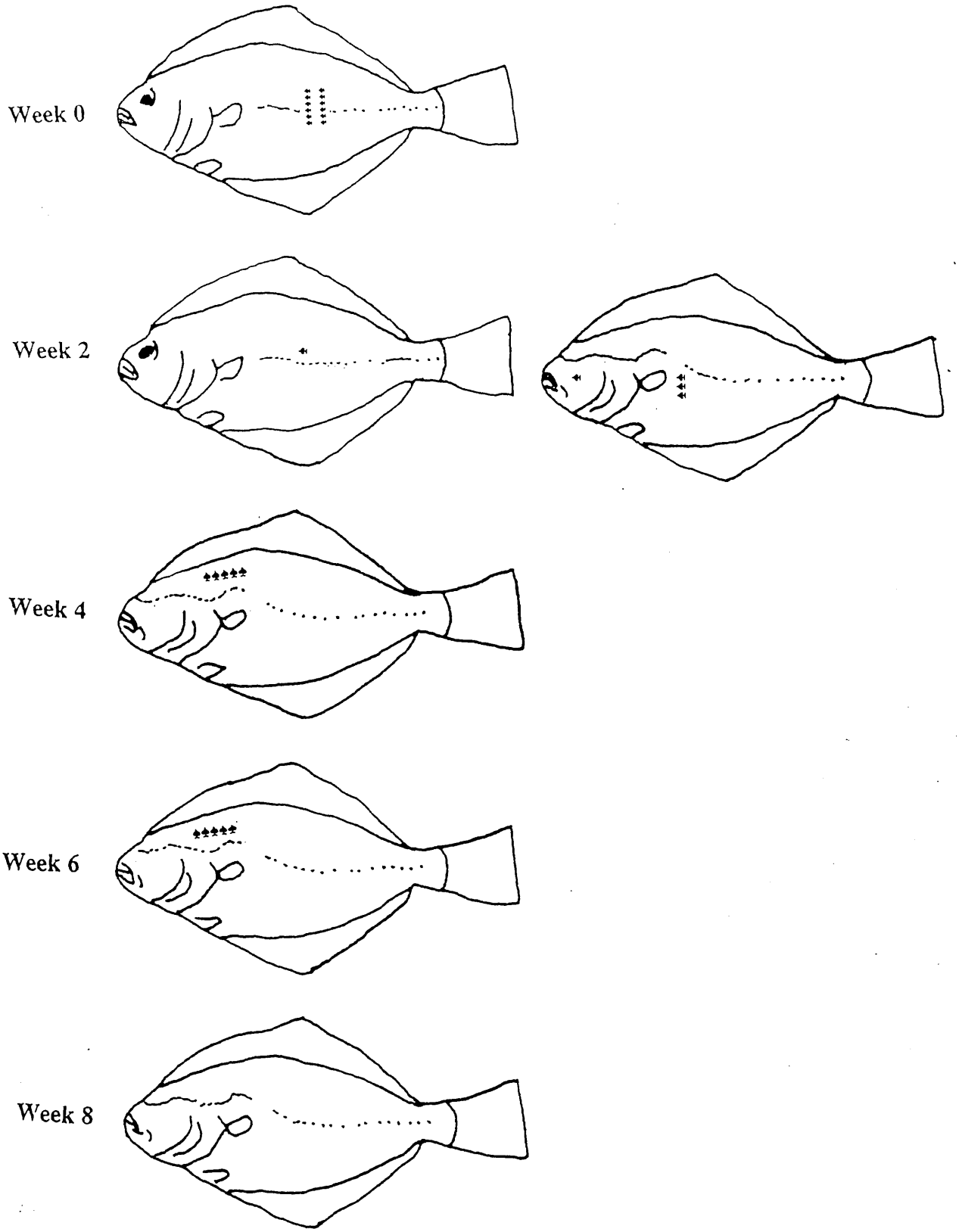


Figure 6.3 The distribution of adult parasites on juvenile Fish 3 during the 8 weeks of experimental period. ♠ sign represents the parasites. (dorsal and ventral surface).

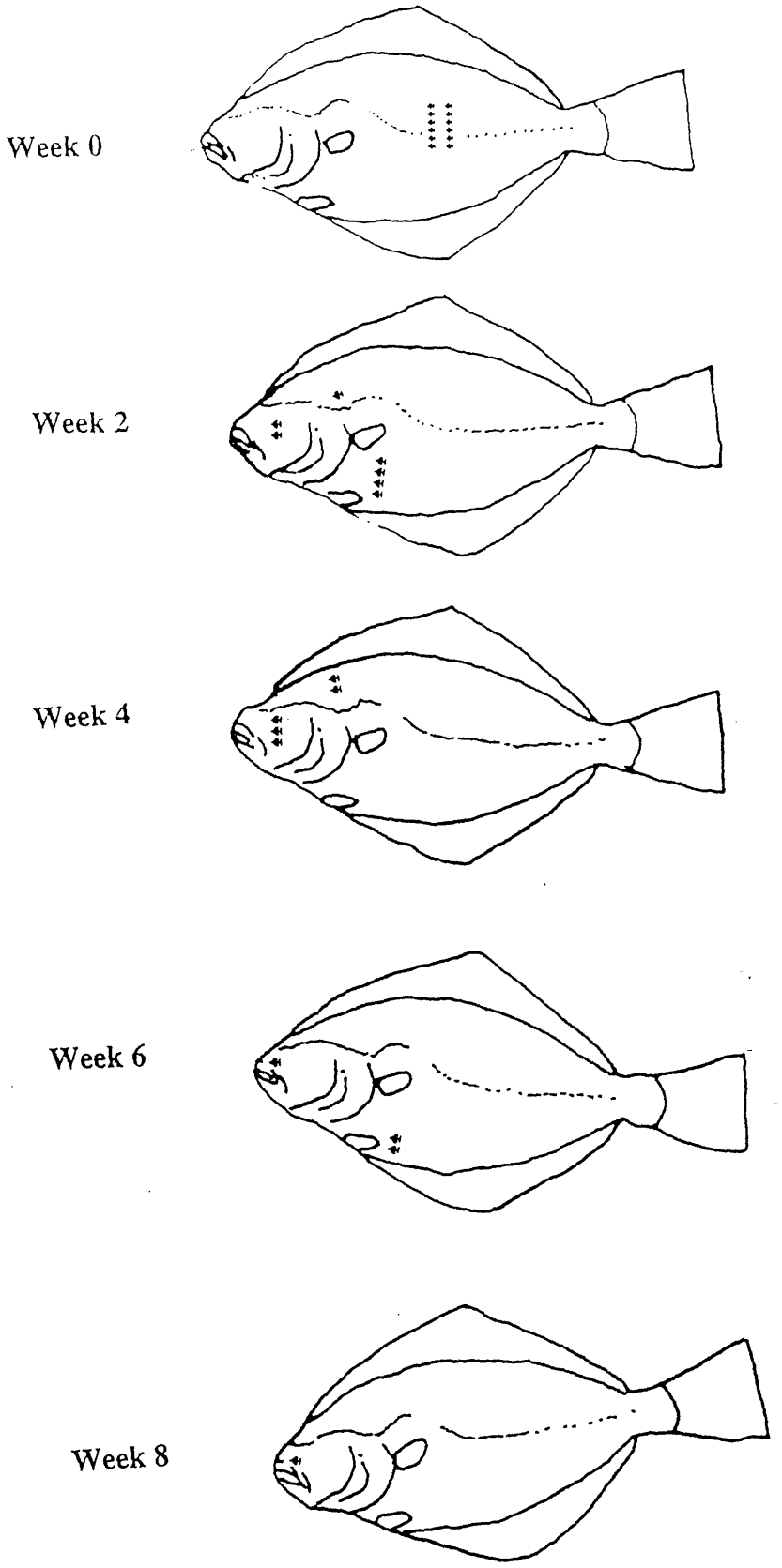


Figure 6.4 The distribution of adult parasites on juvenile Fish 4 during the 8 weeks of experimental period. ♠ sign represents the parasites. (ventral surface).

6.3.2 pathology

6.3.2.1 Clinical signs

There were not many differences between the infected fish and the uninfected fish. However, haemorrhages were evident in the infected fish skin. The infected fish reduced their appetite, the same symptom reported from growing halibut farms. One parasite settled on the antero-ventral surface of the juvenile fish made a marked foot print on the fish skin while other parasite infected areas had no such marked signs of infection.

6.3.2.2 Histopathology

Slight but significant changes in infected tissue were observed when compared with the normal histological sections. A mass of sloughed necrotic epithelial cells mixed with mucus and debris was found on the surface of infected tissue in the area left vacant by the attached parasite. This was apparently due to the action of the parasite because it was not found on the normal tissue. Superficial erosions were also evident on the infected tissue (Figure 6.5). Figure 6.6 shows two hook - shaped holes found on the epidermis of infected tissue. The distance of the two holes was 1.5 mm. This distance was similar to the anterior hamuli distance of the parasite. A high power view of these holes is shown in Figure 6.7. The holes penetrated the epidermis. The depth of the one hole was 120 μm while the other hole was 150 μm deep. These depths were almost the same as the length of the hook of the anterior hamuli (the sharp tip from the end of hamuli sheath). The mucous cells around the holes were oval shaped rather than circular as is the normal shape of a mucous cells.

The infected tissue showed marked hyperplasia. The epidermal layer of infected tissue was almost twice as thick as that of the normal tissue. Furthermore, the mucous cells were irregularly distributed in the epidermis of the infected tissue while mucous cells were regularly arranged in the normal tissue (Figure 6.8).

6.3.2.3 SEM findings

Worms were attached primarily to the head region of the ventral surface of the fish. The SEM of the infected skin area from which the parasites were removed is illustrated in Figure 6.9. The area of attachment by the posterior haptor of the parasite was markedly swollen. The swollen area shows the print of the marginal membrane, the papillae and a disruption of the epithelium suggesting a deep lesion. The marginal membrane seems to assist attachment of the parasite, ensuring a firm grip by means of the creation of an area of reduced pressure between the parasite haptor and the fish surface. This was supported by the observation that, when the parasites were removed from the fish skin, a " released vacuum sound " was clearly heard. The marginal membrane appeared as a flap surrounding the whole of the marginal edge of the posterior haptor. A closer examination of the print of the marginal membrane is shown in Figure 6.10. It can be seen that the print of the membrane was regularly marked by bands of ridges and, inside the membrane, the skin was highly swollen.

The papillae also made deep indentations in the fish skin. It seems that the papillae might act as a grip for enhancing the attachment of the parasite. Figure 6.11 shows a closer examination of halibut skin, deeply indented by the papillae. The papillae

were larger in the posterior two-thirds of the haptor and were generally regularly arranged in rows in the anterior to posterior direction. However, papillae in adjacent rows had alternating positions (Figure 6.12). This arrangement possibly affords a better grip during locomotion or attachment. The papillae were variable in size and the shapes of the papillae on the haptor were not identical. Figure 6.13 shows cone shapes, some markedly pointed at the apex, while others were bicuspid and tricuspid at the papilla apex. Usually, the cone shaped papillae were found around the margin, with bicuspid and tricuspid shapes distributed in the centre area of the haptor and on the sheath of the anterior hamuli. These papillae in the area between the sclerites were not regularly arranged (Figure 6.14). Figure 6.15 shows the papillae on the sheath of the anterior hamuli; the tricuspid shapes of the papillae are arranged in such a way that it seems likely that these papillae support the holding capacity of the anterior hamuli.

A lesion was found near to the centre of the attachment area (see Figure 6. 9). The lesion appeared to be deeply ingressed into the juvenile halibut skin as shown in Figure 6.16. The location of the lesion suggested that it was not produced by the anterior hamuli but by the accessory sclerites. Unfortunately the other side of the haptor print was covered by mucus obscuring the area under the other accessory sclerite. The accessory sclerites are located in the centre of the haptor, whilst the anterior hamuli are on the posterior margin of the haptor. The comparison of shapes of the anterior hamuli and the accessory sclerites is shown in Figure 6.17. The accessory sclerites are sharper and more robust than the anterior hamuli. The location and sharpness of the accessory sclerites support the conclusion that the lesion in the area of the haptor attachment is made by the accessory sclerites.

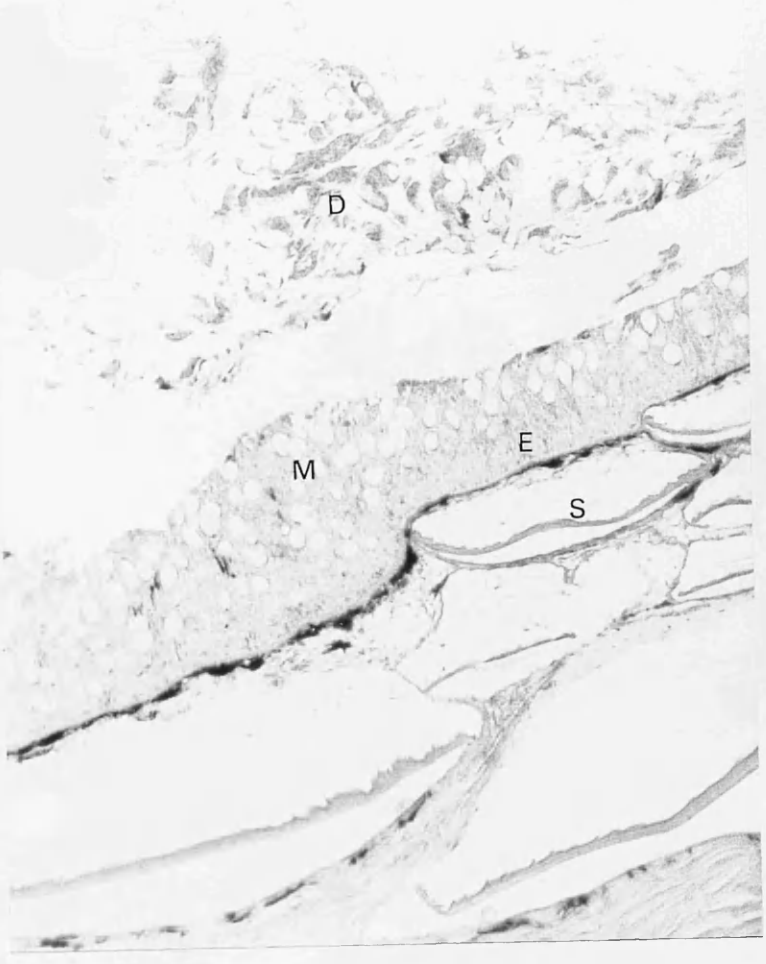


Figure 6.5 A mass of sloughed necrotic epithelial cells mixed with mucous and debris are found on the surface of Atlantic halibut skin infected by *E. hippoglossi*.
(**D**: debris, **E**: epidermis, **M**: mucous cell: **S**: scale). (x 4 objective)

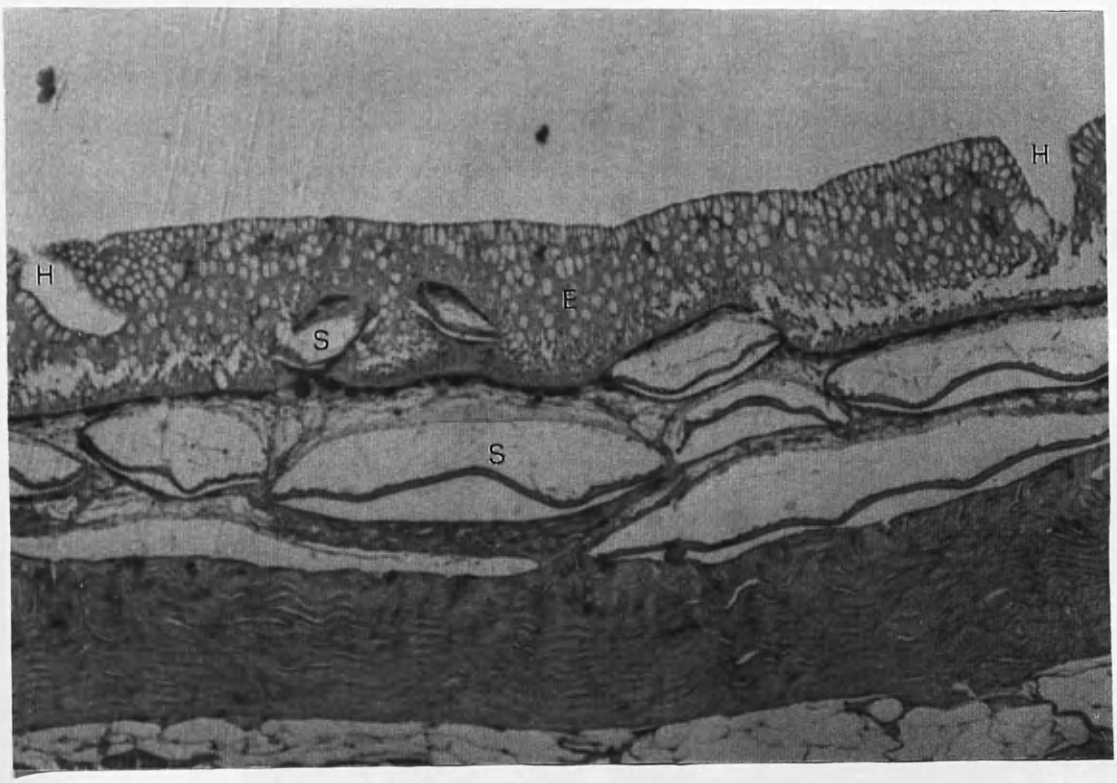


Figure 6.6. Two holes were found on the epidermal layer of infected juvenile halibut. The distance between the two holes was 1.5 mm which was the same as the distance between the anterior hamuli of the parasite. (**E**: epidermis, **H**: holes, **S**: scale: **D**: dermis). (x 4 object)

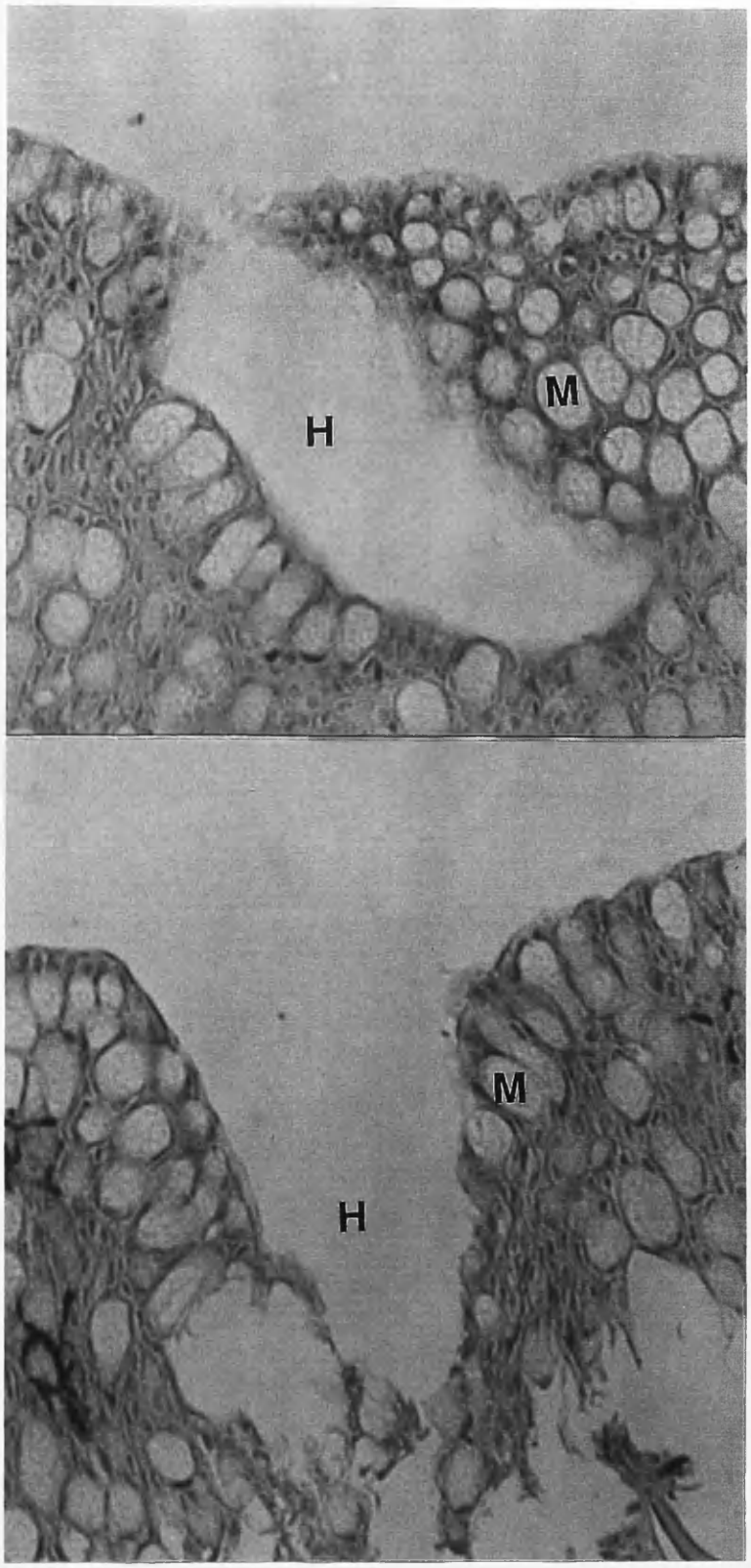


Figure 6.7. Close examination of the two holes on the epidermal layer. Note the shape of the mucous cells is oval and the mucous cells are very densely packed near the hole.
(**E**: epidermis: **M**: mucous cell: **H**: hole). (x 40 objective).

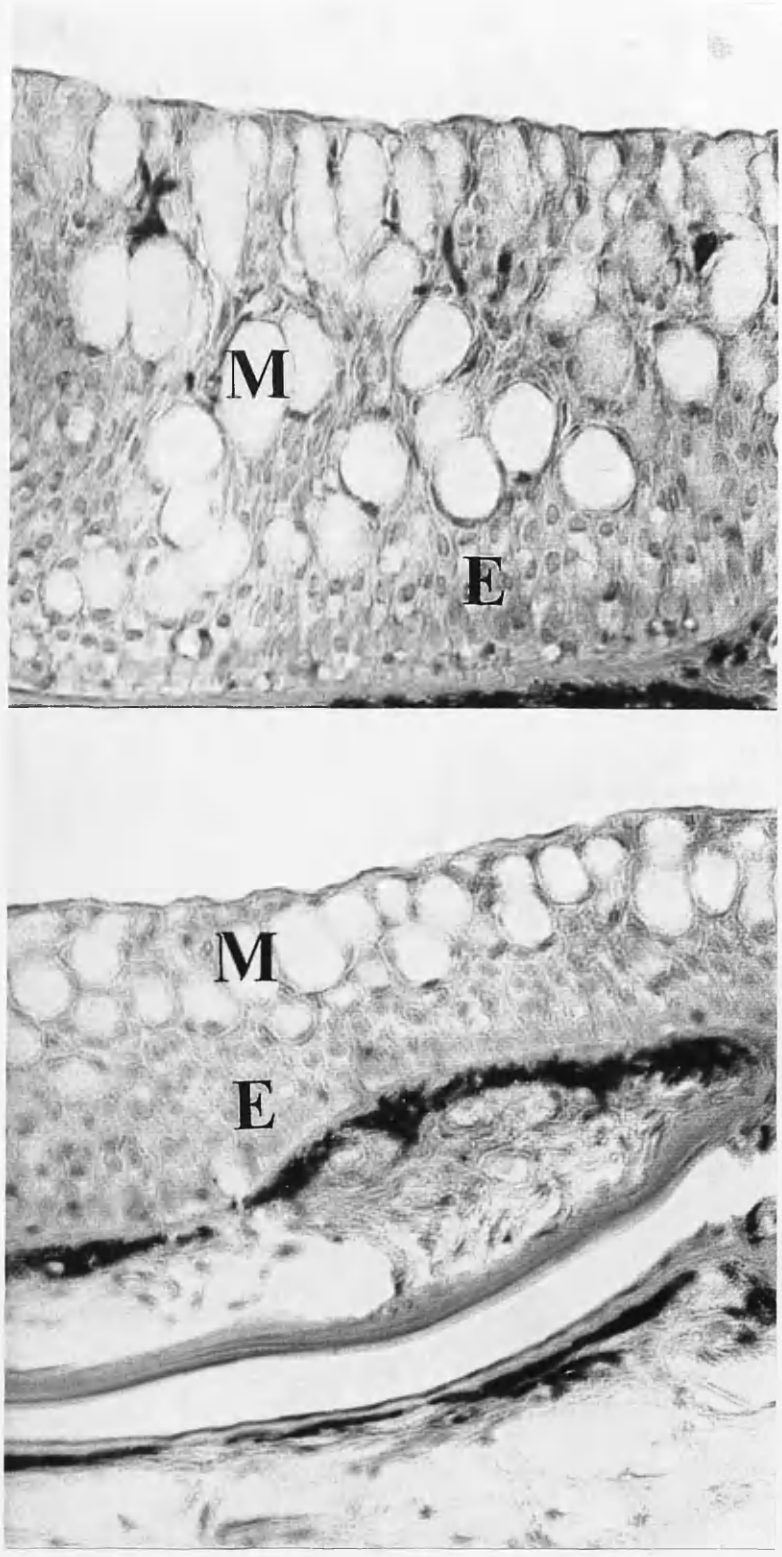
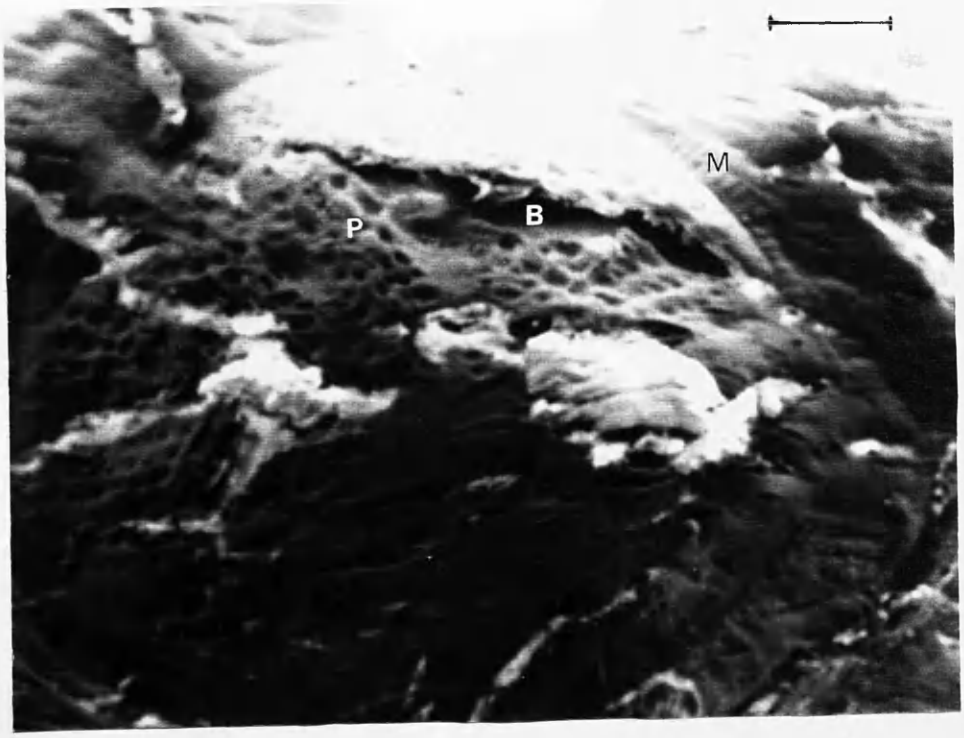


Figure 6.8. Infected tissue was hyperplastic and the thickness of the epidermal layer was almost twice that of normal tissue. Note irregularly distributed mucous cells in the epidermis of the infected tissue (a) and regularly arranged mucous cells in normal tissue (b).
(**E**: Epidemis, **M**: mucous cell) (x 40 objective)


M
P
B

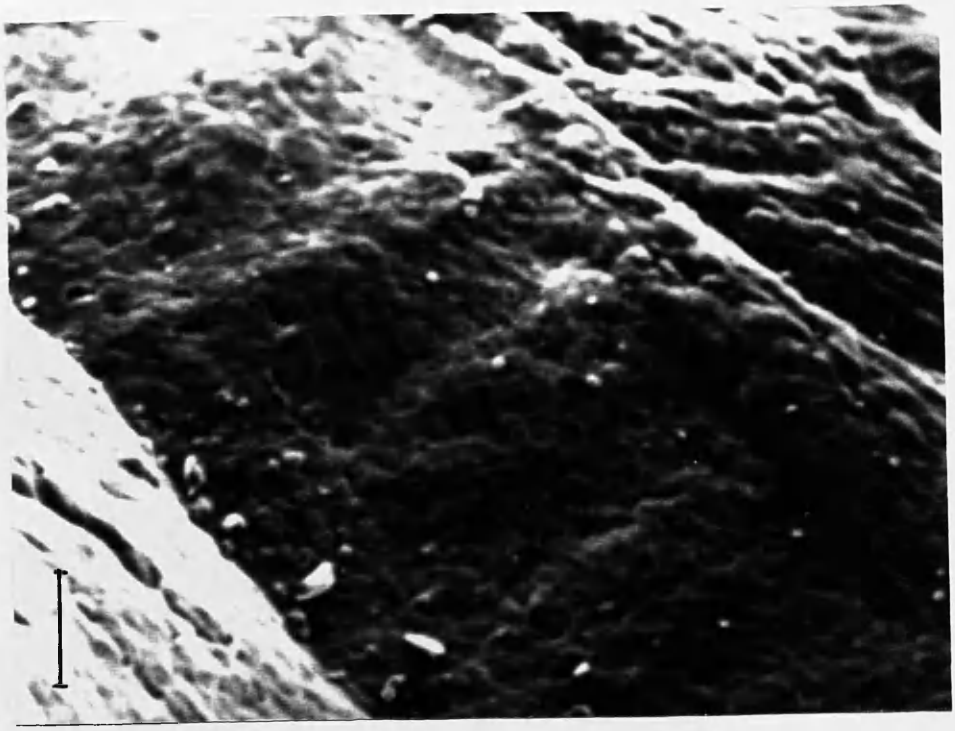


Figure 6.9. Scanning electron micrograph of the attachment area of *E. hippoglossi* clearly showing the print of the marginal membrane and papillae. Note the attachment site is highly swollen and there is an elongated breach in the epidermis.
(**P**: papillae print, **M**: membrane print, **B**: breach).
(Scale bar = 100μm).

Figure 6.10. Scanning electron micrograph (higher power) of the marginal membrane print, regularly marked by bands of ridges.
(Scale bar = 10 μm).

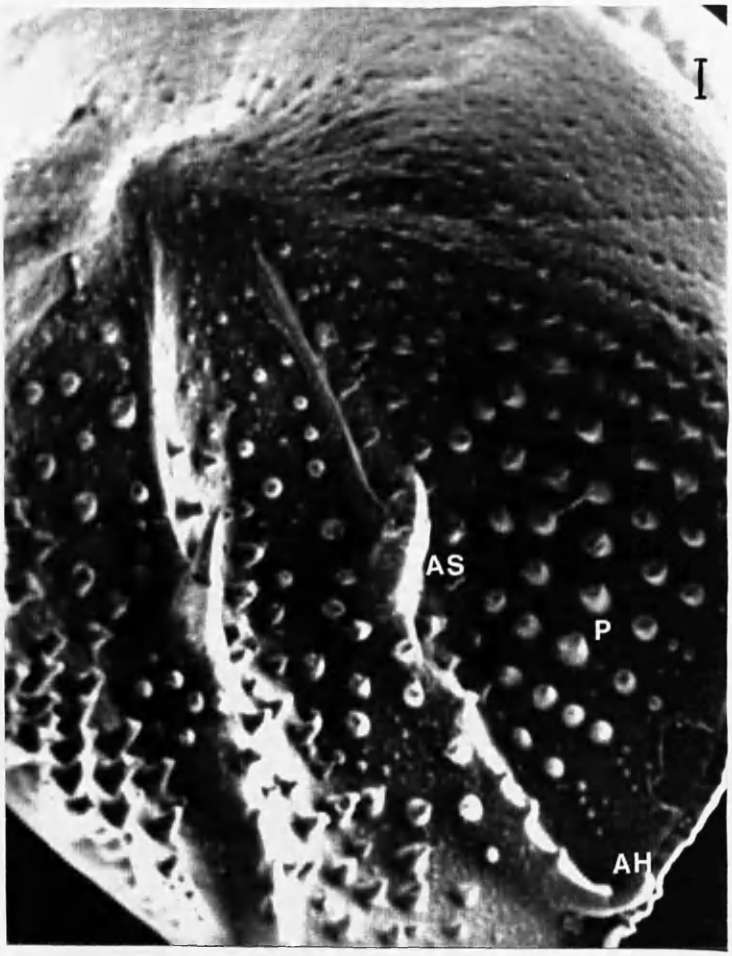
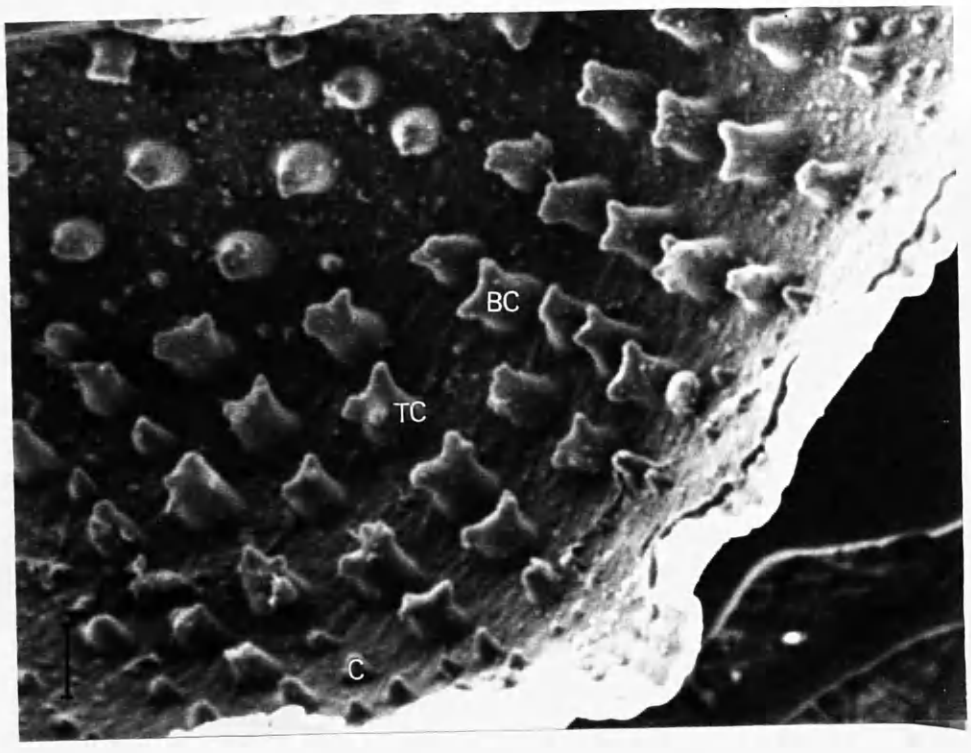

AS
P
AH

Figure 6.11. Scanning electron micrograph showing skin deeply indented by papillae on posterior haptor. Note the size and depth of indentation are different. (Scale bar = 10 μm).

Figure 6.12. Scanning electron micrograph of the arrangement of papillae on the posterior haptor. Note the papillae were larger in the posterior, two-thirds of haptor and were regularly arranged in rows in the anterior to posterior direction and adjacent rows with alternating positions (Scale bar = 100 μm).


BC
TC
C

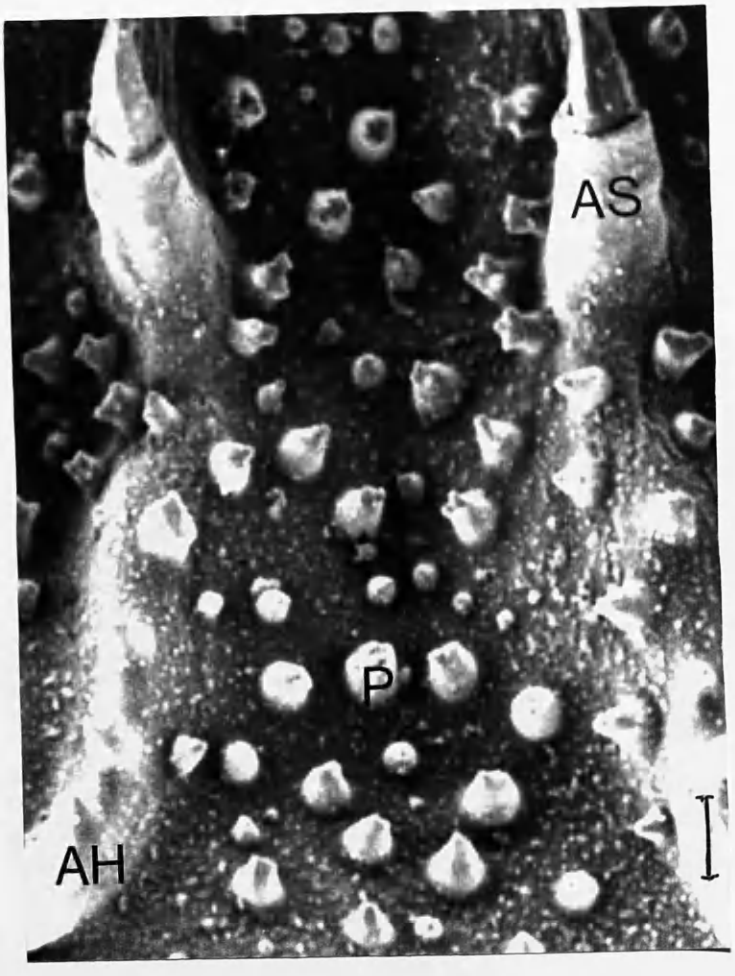
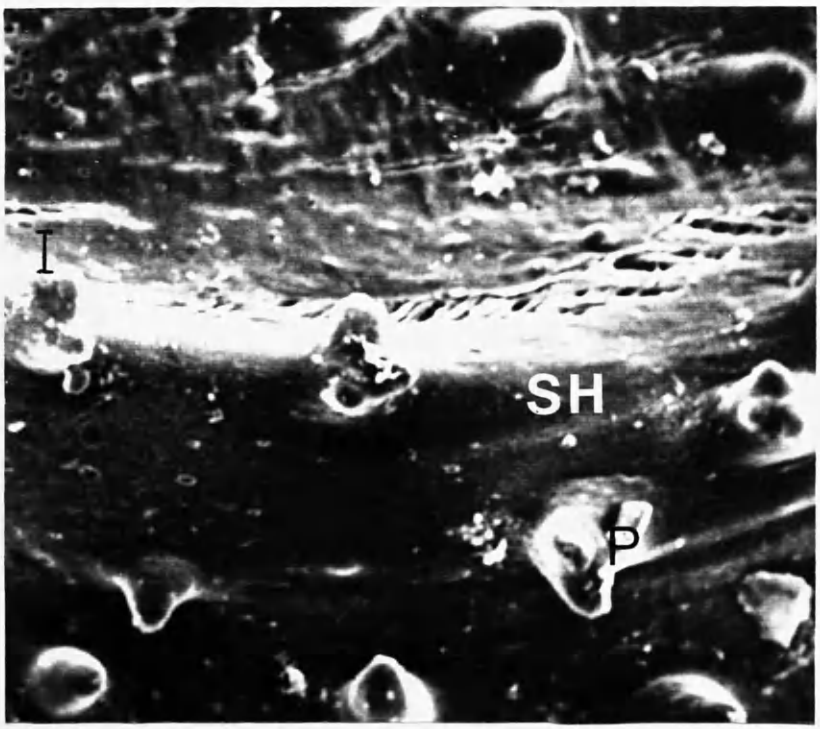

AS
P
AH

Figure 6. 13. Scanning electron micrograph of different shapes of papillae of posterior haptor of *E. hippoglosssi*. Note cone, bicuspid and tricuspid shapes of papillae.
(**C**: cone shape papilla, **BP**: bicuspid shape papilla, **TP**: tricuspid shape papilla).
(Scale bar = 100 μm).

Figure 6.14. Scanning electron micrograph showing bicuspid and tricuspid shapes of papillae between sclerites. Note the papillae are bigger than those in the marginal area.
(**AS**: accessory sclerite, **AH**: anterior hamuli, **P**:papillae)
(Scale bar = 100 μm).


I
SH
P

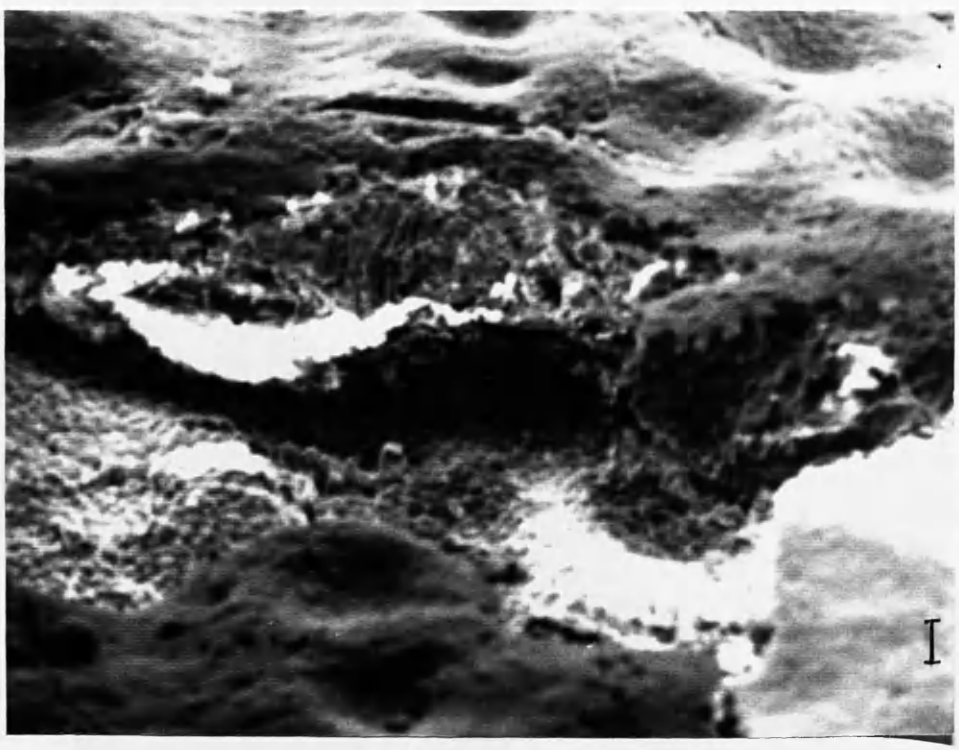

I

Figure 6.15. Scanning electron micrograph showing bicuspid and tricuspid shapes of papillae on the sheath of the anterior hamuli.
(**SH:** sheath, **P**: papilla)
(Scale bar = 10 μm)

Figure 6.16. Scanning electron micrograph showing the deep lesions on the infected tissue of juvenile halibut. Note lesion is deeply ingressed into the juvenile halibut skin.
(Scale bar = 10 μm)

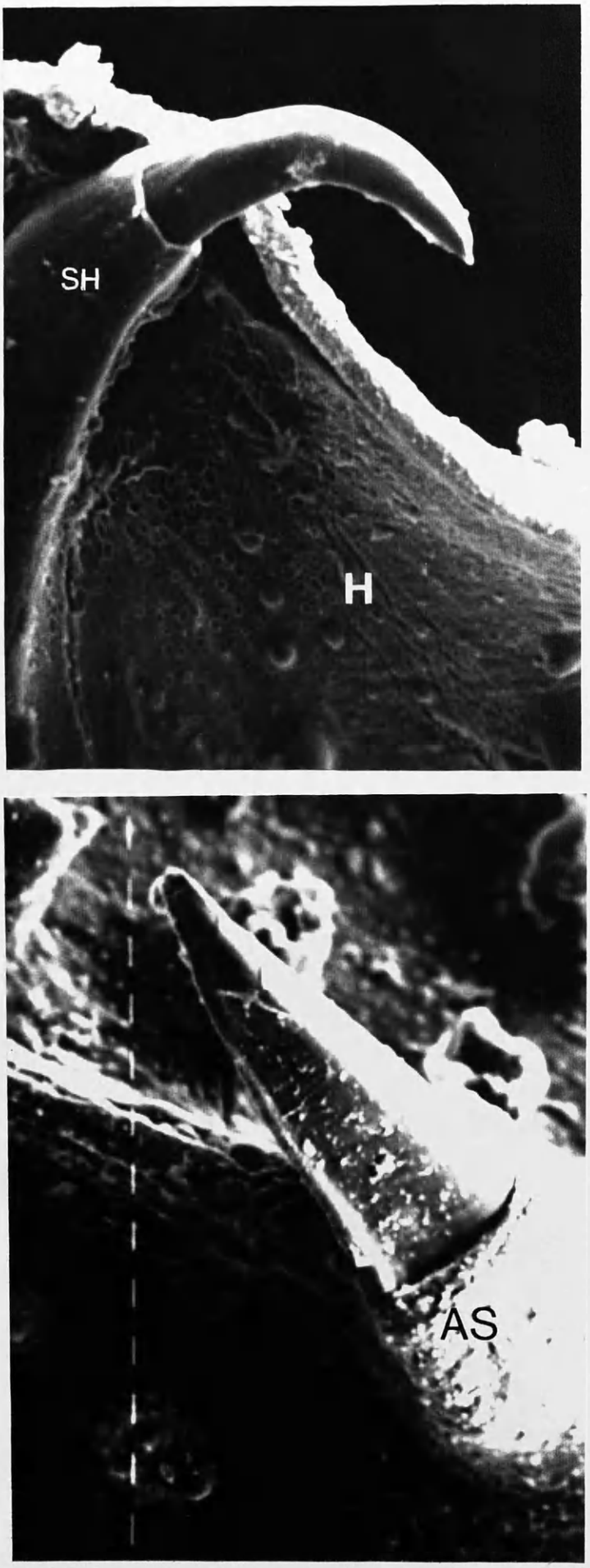

SH
H
AS

Figure 6.17. Scanning electron micrograph showing the shape of the anterior hamuli (a) and the accessory sclerites (b) of *E. hippoglossi*. Note the accessory sclerites are sharper and thicker than the anterior hamuli.
(**SH**: sheath, H: haptor, AS: accessory sclerites)
(Scale bar = 10 μm).

6.4 DISCUSSION

6.4.1 Movement of adult *E. hippoglossi* on juvenile halibut

Kearn (1976) suggested that the body surface of fishes is especially important as a habitat for skin parasites. In some species of parasites, the infective stages attach to the target area and further development occurs there. In other cases, larvae migrate to the target site from the attachment area after infection has taken place and in some cases, the juveniles and adults occupy different attachment sites. For instance, the larvae of the copepod *Caligus diaphanus* on its fish host *Trigla lucerna* lived on the gill filaments but adults occupied the inner wall of the opercular cavity. The larvae were never found in the mouth cavity and the adult females were never found on the gills (Rohde, 1980). The differences in their distribution must be due to migration, but the stimuli directing the larvae and inducing final settlement are unknown.

In the present study, the adult parasites of *E. hippoglossi* moved to the anterior region of the ventral surface of fish, whether they were placed initially on the dorsal or the ventral surface and they settled on the anterior ventral region of fish. The reason why adult *E. hippoglossi* moved to the anterior region of the ventral surface is not clear.

Kearn (1984) found that the oncomiracidia of *E. soleae* migrated from the dorsal surface of *Solea solea* to the ventral surface with a speed of approximately 1 cm per day. Finally, when they reached the ventral surface, they became widely distributed. He suggested that the advantages for juvenile parasites moving onto the lower surface were to have better opportunity for exchange of spermatophores and to facilitate the laying of eggs into the sand of the seabed. In the present study, adult

parasites migrated to the ventral surface very rapidly within 2 hours after attachment. Kearn (1988) has shown for adult *E. soleae* that experimentally disorientated worms re-orientate themselves readily in the absence of a water current and migrate to the ventral surface of the host, *Solea solea*. He suggested that the scales of the fish provide tactile cues for orientation of this parasite.

In the present study, the cues that cause *E. hippoglossi* to migrate to the antero-ventral surface are not clear. It is assumed that the migration of *E. hippoglossi* might relate to several factors, *i.e.* strength of light or pigmentation of host (background colour), aggregation of parasites, resistance of parasite against current and the quantity or quality of mucus (food). However, the light or background colour was not related to the parasite migration. Even though Fish 1 had the same pigmentation on both sides and Fish 4 had no pigmentation, experimentally placed parasites moved very actively and still preferred the anterior region of the ventral surface.

Due to the differences of structure of dorsal and ventral surfaces of flat fish, the physical water current may differentially affect parasite attachment on the host surface. Examination of the effect of water current was determined on artificially infected halibut placed in an experimental container with a very low water flow rate and trickled air supply. Within 2 hours almost all parasites placed n the dorsal surface migrated to the ventral surface. The quantity or quality of mucus on the dorsal and the ventral surfaces of halibut is presently unknown. It is therefore suggested that the quantity or quality of available food caused the migration of adult *E. hippoglossi* to the ventral surface of halibut. However, more precise studies are needed to investigate the reason for this migration on *E. hippoglossi* onto the antero-ventral surface and what cues cause the parasites to move to this location.

6.4.2 Susceptibilty of the juvenile fish

Host specificity in the Monogenea is a characteristic feature of this group. Among the monogeneans, it is very common for parasites to prefer a certain age group of host. For instance, Llewellyn (1962) showed that *Gastrocotyle trachuri* Beneden and Hesse, 1863 and *Pseudaxine trachuri* Parona and Perugia, 1890 were most common on young fish and much less frequent on 2-3 year old fish, *Trachurus trachurus* L.. Paling (1965) found that the infection intensity and frequency of *Discocotyle sagittata* increased with the age of its host, *Salmo trutta*.

In Chapter 2 of this study, it was noted that *E. hippoglossi* has not been found on juvenile halibut. Also the results of the present study showed that *E. hippoglossi* did not survive and did not establish a population on the juvenile halibut, even though they survived for over 8 weeks on the juvenile halibut.

There are no references to experimental infections of parasites which only occur on a certain age of host. However, differential mortality after infection leads to host specificity in many cases. For instance, *Gyrodactylus* spp attach themselves readily to different hosts such as fish and frogs, but subsequent development is poor and all worms die after short periods (Lester, 1972; Lester and Adams, 1974). *Gyrodactylus salaris* Malmberg, 1957 can infect several fish species, but seems to be unable to reproduce on non-salmonid fishes (Bakke, Harris, Jansen and Hansen, 1992). Kearn (1967b) investigated experimentally the ability of the oncomiracidia of *E. soleae* to attach to the scales of other flatfish hosts. Almost all oncomiracidia of *E. soleae* found the scale of sole, *S. solea* which is the specific host of the parasite. Kearn also experimentally infected alien hosts with the adult parasites but the parasites became

detached after only 24-30 hours, even though they could survive 2-6 days in a glass dish at 14-17°C. He suggested that some changes occur when parasites near maturity so that they can no longer attach to the skin of the alien fishes. Alternatively, the parasite was unable to maintain its hold on the skin of these fishes because of mechanical difficulties or because some substance produced by the fish skin repelled the parasite.

Buchmann and Uldal (1997) investigated the host susceptibility of 4 salmonids (rainbow trout, *Oncorhynchus mykiss*, brown trout, *Salmo trutta*, a Baltic and an Atlantic strain of *Salmo salar*) using *Gyrodactylus derjavini*. The parasite populations increased significantly faster and to a higher level in rainbow trout compared to the other salmonids, with brown trout exhibiting an intermediate position. They suggested that the host epidermis was responsible for the varying degrees of resistance. For example, the anatomy of the host epidermis could differ among salmonid species and provide microhabitats better suited for certain parasites than for others or defence mechanisms and specific immune response of the fish host could influence resistance to parasite invasion.

In the present study, the parasites placed on the juvenile halibut laid eggs but oncomiracidia were not found on the skin of the fish. The findings of the present study suggest that there may be differences in the epidermis of mature and juvenile halibut. On the other hand, mature halibut might have a particular attractant to the parasite or provide proper nutrition for the parasite. However, more detailed studies of the susceptibility of mature fish are needed for *E. hippoglossi*.

6.4.3 Pathology

During the present study, many haemorrhagic lesions were found around the parasite infection area. Even though infected and uninfected halibut were clinically similar, it was reported by the farm that, after chemical treatment or after removal of the parasites manually, the appetites of the infected fish were increased.

Mechanical action by ectoparasites injures skin or gills by boring, pressure, penetration or sucking activities (Körting, 1975). The attachment organs of skin-parasitic monogeneans are varied, including the use of hooks, suckers and cement or combinations of these. The post- oncomiracidia of many skin parasitic monogeneans and the skin inhabiting early larvae of some gill monogeneans mostly rely on marginal hooklets for attachment (Kearn, 1967a, 1968). Lester (1972) has shown that the marginal hooklets were used by adult *Gyrodactylus alexanderi* and have a major part to play in attachment of this parasite. According to Cone and Odense (1984), the 16 marginal hooks of *G. salmonis* Yin and Sproston, 1948 lodge deeply into host epidermis and cause extensive fin damage and skin discoloration.

In the present study, *E. hippoglossi* seemed to attach itself to the skin of the halibut by a suction pressure generated between the haptor and the skin of the fish. This was achieved with the aid of the accessory sclerites which are inserted in the epidermis of the fish skin. Evidence was found in this study with histopathology that the pair of anterior hamuli acted by insertion into fish skin to a depth of 120-150 µm which is almost the same as the size of the sharply hooked tips of the anterior hamuli. The depth of the holes and their appearance suggested a pressure wound which eventually punctures. Kearn (1964) described the foot print of *E. soleae* on the skin

of the common sole, *S. solea*. The foot print showed the contact points of the accessory sclerites, but he suggested that they acted not as hooks but as props. In the case of *E. hippoglossi* in the present study, evidence from both histology and SEM clearly showed evidence that the accessory sclerites acted as hooks and made very deep wounds in host skin.

Williams, Ellis and Spaull (1973) studied the suction pressure of *Pseudobenedenia nototheniae* Johnston, 1931 by the haptoral musculature on the host skin. They claimed that this sucking action is necessary when the host swims, thereby creating strong currents which might tend to dislodge the parasites.

Oliver (1977) described effects due to *Diplectanum aequans* (Wagener, 1857) Diesing, 1858 on *Dicentrarchus labrax* L., 1958 using histological methods and SEM. He observed hyperplasia of the gills and haemorrhages where the hooks of the parasites were inserted. Kearn (1963d) found *E. soleae* changed its site frequently on the host and thus may inflict many feeding and attachment wounds. The epidermis is apparently replaced rapidly so that feeding wounds were rarely seen on unpopulated parts of the fish. In the present study, such feeding wounds were not evident in the sections of skin examined and a thorough histological examination would be useful in further work. However, regarding the findings from the footprint of the parasites, especially the suction activity of the posterior haptor and effect of the accessory sclerites, it would appear that *E. hippoglossi* damage halibut skin.

Histopathological examination in the present study revealed that sloughed necrotic epithelial cells and debris on the surface of the infected fish might be due to the penetration activities of the parasite, including feeding. The irregular arrangement of mucous cells in the epidermis and the proliferation of the epidermal layer might be due to the irritation caused by the parasite and the attempted recovery activity of the

skin. Urawa and Kusakari (1990) found that the ectoparasitic flagellate *Ichthyobodo* sp. induced increasing secretion of mucus followed by epidermal erosion and ulcers of the skin of the flounder, *Paralichthys olivaceus*. The heavy infection caused hyperplasia of the malphigian cells and depletion of mucous cells followed by loss of osmotic balance. They suggest that these might be cause of the mortality in the fish. On the other hand, Cusack and Cone (1986) reported that the epidermis of fry infected by *Gyrodactylus salmonis* was thinner and had fewer goblet cells than those of uninfected fish.

In summary, the adult parasites of *E. hippoglossi* migrated to the ventral surface of the host body very rapidly, but the cues or reasons for this are not clear. However, it is assumed that some factors affected the parasite migration, such as quantity or quality of food, aggregation of parasites and resistance to current movement on the host surface. From the present study, it seems likely that the food quantity or quality is the most important reason for *E. hippoglossi* migration.

The adult *E. hippoglossi* could survive on the juvenile halibut for at least 8 weeks. However, the reasons for the detachment of these adult parasites from the juvenile fish was not clearly shown whether they died naturally or whether their detachment was related to the juvenile host.

SEM and histology studies revealed that not only the anterior hamuli but also the accessory sclerites, marginal membrane and papillae were involved in the attachment of *E. hippoglossi*. The anterior hamuli penetrated host skin with the sharp tip of the hook and the accessory sclerites acted as a grip not as a prop. The different size, different arrangement and different shape of the papillae on the haptor might provide more holding capacity to the parasite on the host skin. However, whether the different shapes of the papillae may be due to their being in different

stages of development or they may have different functional significance is not clear. It was not clear from this study but further histology and TEM studies of papillae would be useful.

Chapter 7

MUCOUS CELL DISTRIBUTION ON HALIBUT SKIN

7. MUCOUS CELL DISTRIBUTION ON HALIBUT SKIN

7.1 INTRODUCTION

Fish skin has many functions such as protection from physical and chemical aggressions, osmoregulation, facilitating locomotion, disease resistance, and social relations (Van Oosten, 1957; Noakes, 1973; Burton, Burton and Idler, 1984; Hara, Macdonald, Evans, Marui and Akai, 1984; Pottinger, Pickering and Blackstock, 1984). Kearn (1976) suggested that the body surface of fishes as a habitat for parasites was especially important. Fish respond to a variety of environmental and pathogenic agents by mucous secretions and fish mucous contains a variety of chemical substances, including nucleic acids, free proteins, glycoproteins and mucopolysaccharides (Askawa, 1970). There are contrasting reports on the influence that parasitic infections have on the density of mucous cells in the skin epidermis of fishes. Some researchers (Bauer, Musselius and Strelkov, 1969; Rogers and Gaines, 1975; Ahmed, 1976) have reported that mucification increases during ectoparasitic infections. Other researchers (Hines and Spira, 1974; Logan and Odense, 1974; Paperna, 1980; Roubal, Bullock, Robertson and Roberts, 1987) have reported that infections decrease mucous cell concentrations.

In the previous studies reported in Chapter 3 and Chapter 6, it was found that *E. hippoglossi* migrated to the antero-ventral surface of fish skin whether they were placed on the dorsal surface or on the ventral surface. It was suggested that *E. hippoglossi*

preferred this specific site of its host for certain reasons such as food sources, the advantage of mate location, the avoidance of predators etc. In the previous chapter 6 some possible factors related to migration of *E. hippoglossi* to the ventral surface were dismissed. Among those, the food quantity or quality was most likely related to parasite migration in the previous study. Therefore, the present study investigated the possible relationship between site preference of the parasite and mucous cell density, mucous cell size and epidermis thickness. Mucous cell density, mucous cell size and epidermis thickness were compared in the different sexes (male and female) and different ages (mature and juvenile) of Atlantic halibut and also compared for six different regions on the same host fish.

7.2 MATERIALS AND METHODS

7.2.1 Skin sampling

The fish skins were obtained from mature male, female and juvenile halibut which were freshly dead. The skin samples were taken from 6 parts of each fish, fixed and processed for histology as described in Chapter 2. The sampling areas of the fish are shown in Figure 7.1.

7.2.2 Histology

Alcian blue / PAS staining method for mucous cells was used as described in Chapter 2.

7.2.3 Analysis

7.2.3.1 Mucous cell size (area), mucous cell number and epidermal thickness

The number of mucous cells were counted within a 2.5 mm^2 (50 X 50 µm) square box. The square box was made under a drawing tube on a Leitz Wetzlar SH Lux compound microscope. Then the slide and square box were overlapped under magnification at x 4. Counting areas were randomly selected at each side of the fish (10 replicates). Each side had 3 slides each and therefore the slides were also randomly selected.

The area of mucous cells and the thickness of the epidermal layer were measured using a KS 300 Imaging System (Kontron Elektronik).

7.2.3.2 Statistics

The Non – parametric Dunn's test was used for comparing mucous cell size, epidermal thickness and mucous cell number for the 6 sites: front dorsal; middle dorsal; rear dorsal; front ventral; middle ventral; rear ventral. These sites were compared for each fish: male; female; juvenile. All findings were considered significant at values of $P<0.05$.

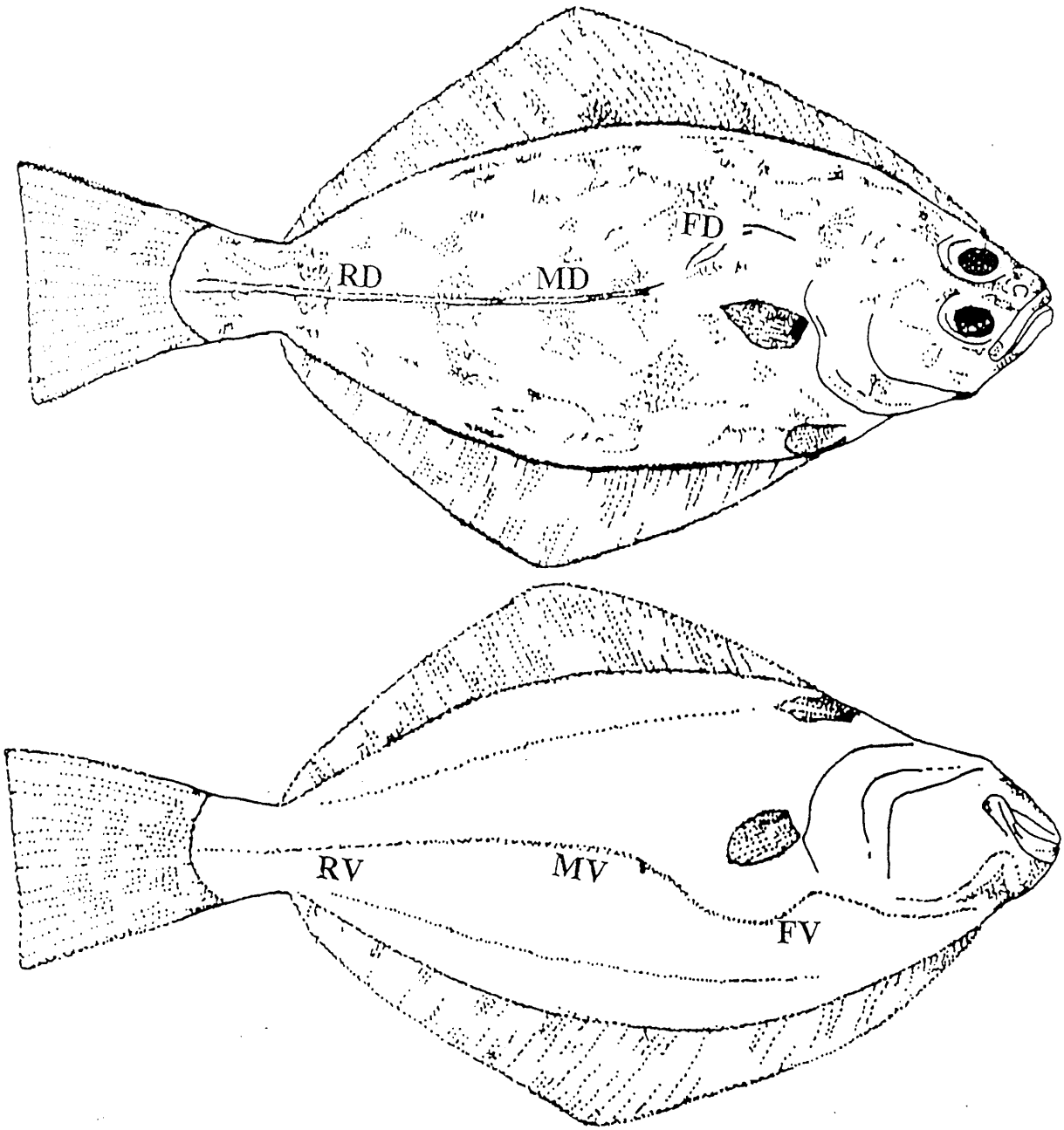


Figure 7.1 Six skin sampling areas of the host: juvenile, male and female Atlantic halibut, *H. hippoglossi*
(**FD**: front dorsal, **MD**: female middle dorsal, **RD**: rear dorsal, **FV**: front ventral, **MV**: middle ventral, **RV**: rear ventral).

7.3 RESULTS

7.3.1 Mucous cell size

The average size of mucous cells as measured by area in histological section from the juvenile, male and female halibut, respectively are shown in Figure 7.2. From the female halibut, the mean size of the mucous cells on the front dorsal region was significantly greater than that from the other regions on the dorsal surface ($P < 0.05$). The average mucous cell size on the antero- dorsal region was 189. 52 ± 59.21 μm^2 while those on the mid-dorsal region and the postero-dorsal region were 147.1 ± 59.35 μm^2 and 154.64 ± 65.03 μm^2, respectively, from the female host. There was no significant difference between the mucous cell size on the middle and the posterior dorsal regions ($P>0.05$).

On the ventral surface of female halibut, the mucous cell size pattern was the same as that on the dorsal surface. The average mucous cell size from the antero- ventral region was 186.01 ± 62.69 μm^2, which was significantly bigger than that of the mucous cells on the mid-ventral region (146.62 ± 62.04 μm^2) ($P<0.05$). However, there was no significant difference between the mucous cell size on the front region and that on the rear region (159.68 ± 47.84 μm^2) on the ventral surface of female halibut.

From the male host, the average mucous size on the antero- dorsal region was 148.22 ± 64.53 μm^2 while those on the mid-dorsal region and the postero- dorsal region were 88.18 ± 35.29 μm^2 and 117.75 ± 78.40 μm^2, respectively. The mucous cell area from

the front dorsal region was statistically significantly different from that from other regions on the dorsal surface ($P<0.05$).

The pattern of mucous cell size on the ventral surface showed the same as on the dorsal surface. The average mucous cell size from the antero- ventral region (186.01 ± 62.69 μm^2) was significantly bigger than that on the mid-ventral (146.62 ± 62.04 μm^2) and the postero-ventral (159.68 ± 47.84 μm^2) regions ($P<0.05$).

The average mucous cell size on the dorsal surface from the juvenile host showed a totally different pattern from that of male and female adult halibut. The mucous cell size on the rear region (160.32 ± 58.65 μm^2) and on the middle region (108.44 ± 45.08 μm^2) was significantly bigger than that on the front region (83.13 ± 34.60 μm^2) ($P<0.05$), of the dorsal surface of juvenile halibut.

On the ventral surface, the mucous cell area on the front region (174.22 ± 51.85 μm^2) was significantly bigger than that on the middle region (108.45 ± 45.08 μm^2) and on the rear region (129.76 ± 44.93 μm^2) *i.e.* it showed the same pattern as the male and female adult hosts.

Comparing all the hosts, the mucous cell size on the antero- dorsal region of the female host was significantly bigger than those from male and juvenile hosts, respectively. Also the mucous cell size of the other regions of the female host were significantly bigger than those from the male host ($P<0.05$), except for the mid-ventral region. However, there was no significant difference between male and juvenile fish and there was no significant difference between the female and juvenile hosts (except the antero-dorsal region).

7.3.2 Mucous cell number

The average numbers of mucous cells in the skin of 6 areas of female, male and juvenile halibut are shown in Figure 7.3. In the female host, the antero- dorsal region (37.05 ± 10.74) and the mid-region (26.3 ± 13.30) had significantly higher numbers of mucous cells than the rear dorsal region (12.05 ± 6.30) ($P<0.05$).

The mean number of mucous cells from the different areas of the ventral surface was almost the same on the female host (25.25 ± 9.43, 27.45 ± 15.21 and 25.6 ± 10.88, front, middle and rear regions, respectively).

In male halibut, the mean number of mucous cells on the antero-dorsal (25.95 ± 7.42) was significantly more than that on the mid-dorsal (12.10 ± 3.35) and the postero-dorsal (6.24 ± 3.75) regions ($P<0.05$).

The mean number on the antero-ventral (36.75 ± 11.32) was significantly more than that on the postero-ventral (15.95 ± 6.52) region ($P<0.05$). However, there was no significant difference between the mean number on the middle ventral (21.35 ± 5.23) and on the front and rear regions from the ventral surface.

From the juvenile fish, the mean number of mucous cells on the antero-dorsal region (20.4 ± 3.47) was more than that on the mid-dorsal (14.3 ± 7.15) and the postero-dorsal (10.2 ± 2.44) regions, even though there were no significant differences statistically.

On the ventral surface of juvenile halibut, the mean mucous cell number on the antero-ventral region (47 ± 7.09) was significantly more than that on the mid-ventral (19.9 ± 3.73) and the postero-ventral (14.5 ± 3.62) regions ($P< 0.05$).

When comparing the mean mucous cell number of the same regions on the different fish, there was no significant difference between male and female halibut comparing the 6 sites. However, there was a significant difference in the mean number of mucous cells from the mid-dorsal region and the mid-ventral region between female and juvenile fish ($P<0.05$).

7.3.3 Epidermal thickness

Generally the epidermal layer on the front region was thicker than that on the middle and rear regions from both dorsal and ventral surfaces. The epidermal thickness of male, female and juvenile hosts is shown in Fig. 7.3. From the female host, the antero-dorsal region (148.54 ± 62.41 μm) was significantly thicker than the other regions (119.51 ± 17.12 μm and 120.64 ± 23.65 μm, the mid-dorsal region and the postero-dorsal region, respectively).

On the ventral surface, however, the epidermal layer on the middle region was thicker than the other regions, although there was no significant difference between the front (145.10 ± 47.02 μm), the middle (156.04 ± 54.28 μm) and the rear (149.60 ± 31.09 μm) regions.

The results from the male dorsal surface showed the same pattern as the female dorsal surface. The epidermal layer of the antero- dorsal region (145.10 ± 47.02 μm) was significantly thicker than the mid-dorsal (87.89 ± 24.81 μm) and the postero- dorsal (63.90 ± 22.88 μm) regions ($P < 0.05$).

The findings from the ventral surface of the male fish showed a slight difference from the same surface of the female host where there was no difference in epidermal thickness on the ventral surface. The male showed a gradation from the antero- ventral region (141. 74 ± 47.60 μm) which was significantly thicker than that on the middle (91.79 ± 18.55 μm) and the rear (79.44 ± 9.49 μm) regions ($P<0.05$).

From the juvenile halibut, the antero-dorsal region (119.39 ± 21.44 μm) was significantly thicker than the mid- dorsal (69.01 ± 4.26 μm) and the postero- dorsal (79.17 ± 7.15 μm) regions ($P<0.05$).

From the ventral surface of the juvenile fish, the antero-ventral region (126.24 ± 25.78 μm) was also significantly thicker than the mid-ventral (70.71 ± 12.83) and the postero-ventral (87.25 ± 8.84 μm) regions ($P<0.05$). It was also found that there was a significant difference between the epidermal thickness of the mid-ventral region and the postero- ventral region ($P<0.05$).

Comparing the different host fish, the epidermal thickness on the antero-dorsal region of the female was significantly thicker than that from the juvenile fish but there was no difference between female and male fish. The epidermal thickness on the other regions of the female were also significantly thicker than the same regions of the male host on both the dorsal and ventral surfaces ($P<0.05$).

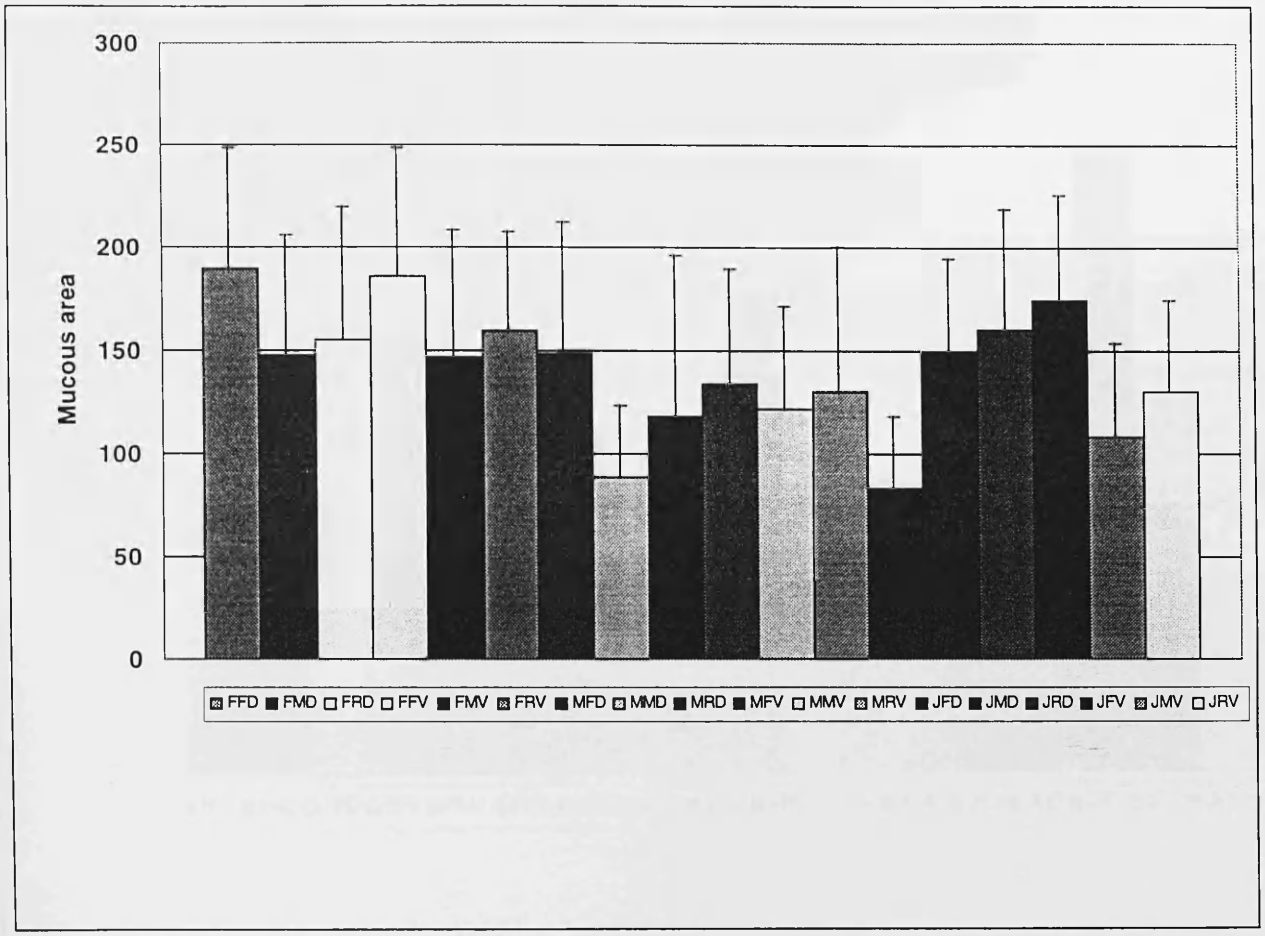


Fig. 7.2 Mucous cell size from different sites of juvenile, male and female Atlantic halibut, *Hippoglossus hippoglossus*. (FFD: female front dorsal, FMD: female middle dorsal, FRD: female rear dorsal, FFV: female front ventral, FMV: female middle ventral, FRV: female rear ventral, MFD: male front dorsal, MMD: male middle dorsal, MRD: male rear dorsal, MFV: male front ventral, MMV: male middle ventral, MRV: female rear ventral, JFD: juvenile front dorsal, JMD: juvenile middle dorsal, JRD: juvenile rear dorsal, JFV: juvenile front ventral, JMV: juvenile middle ventral, JRV: juvenile rear ventral)

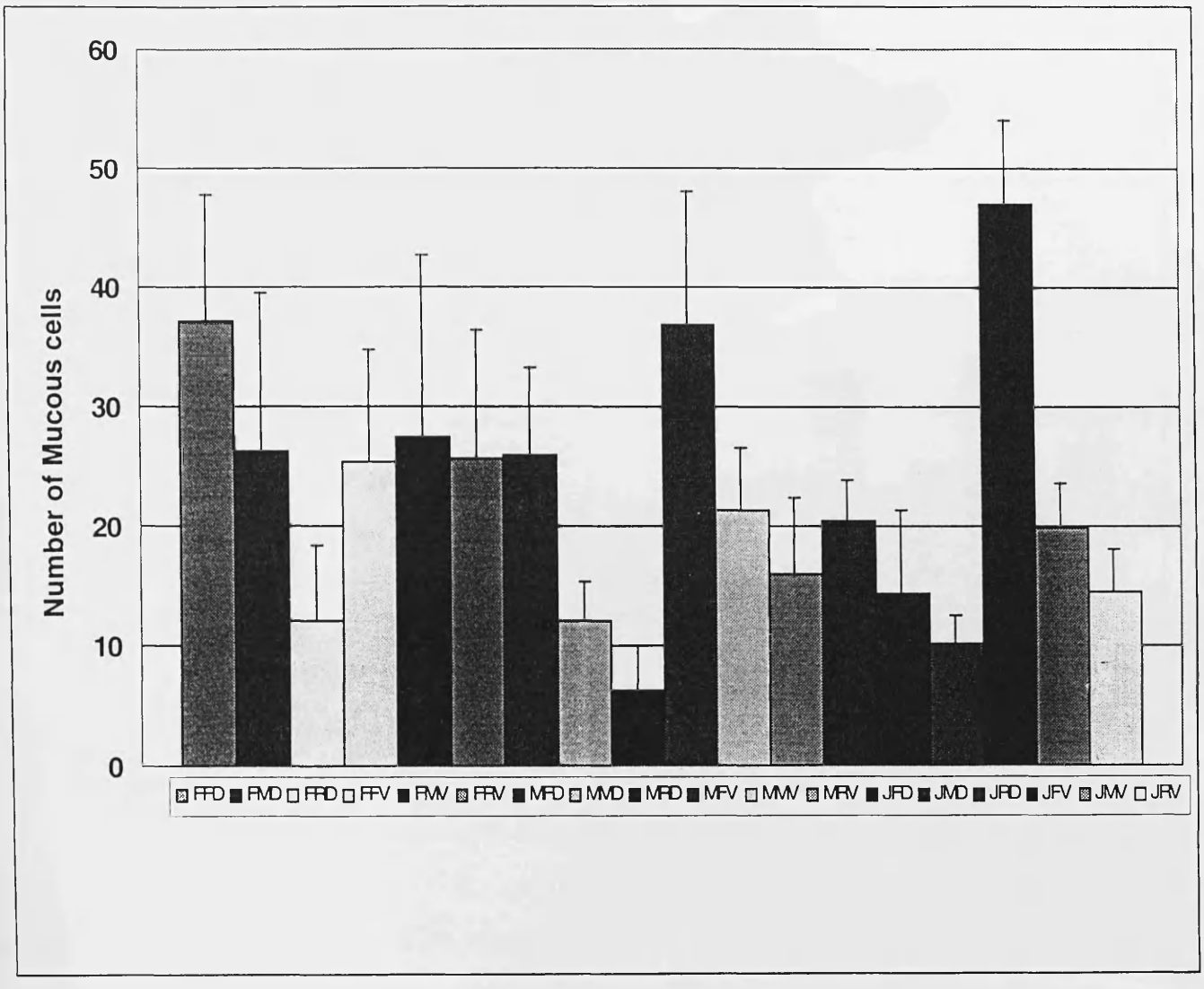


Figure 7.3. Number of mucous cells in the skin at different sites on the surface of juvenile, male and female Atlantic halibut, *Hippoglossus hippoglossus*. (FFD: female front dorsal, FMD: female middle dorsal, FRD: female rear dorsal, FFV: female front ventral, FMV: female middle ventral, FRV: female rear ventral, MFD: male front dorsal, MMD: male middle dorsal, MRD: male rear dorsal, MFV: male front ventral, MMV: male middle ventral, MRV: female rear ventral, JFD: juvenile front dorsal, JMD: juvenile middle dorsal, JRD: juvenile rear dorsal, JFV: juvenile front ventral, JMV: juvenile middle ventral, JRV: juvenile rear ventral)

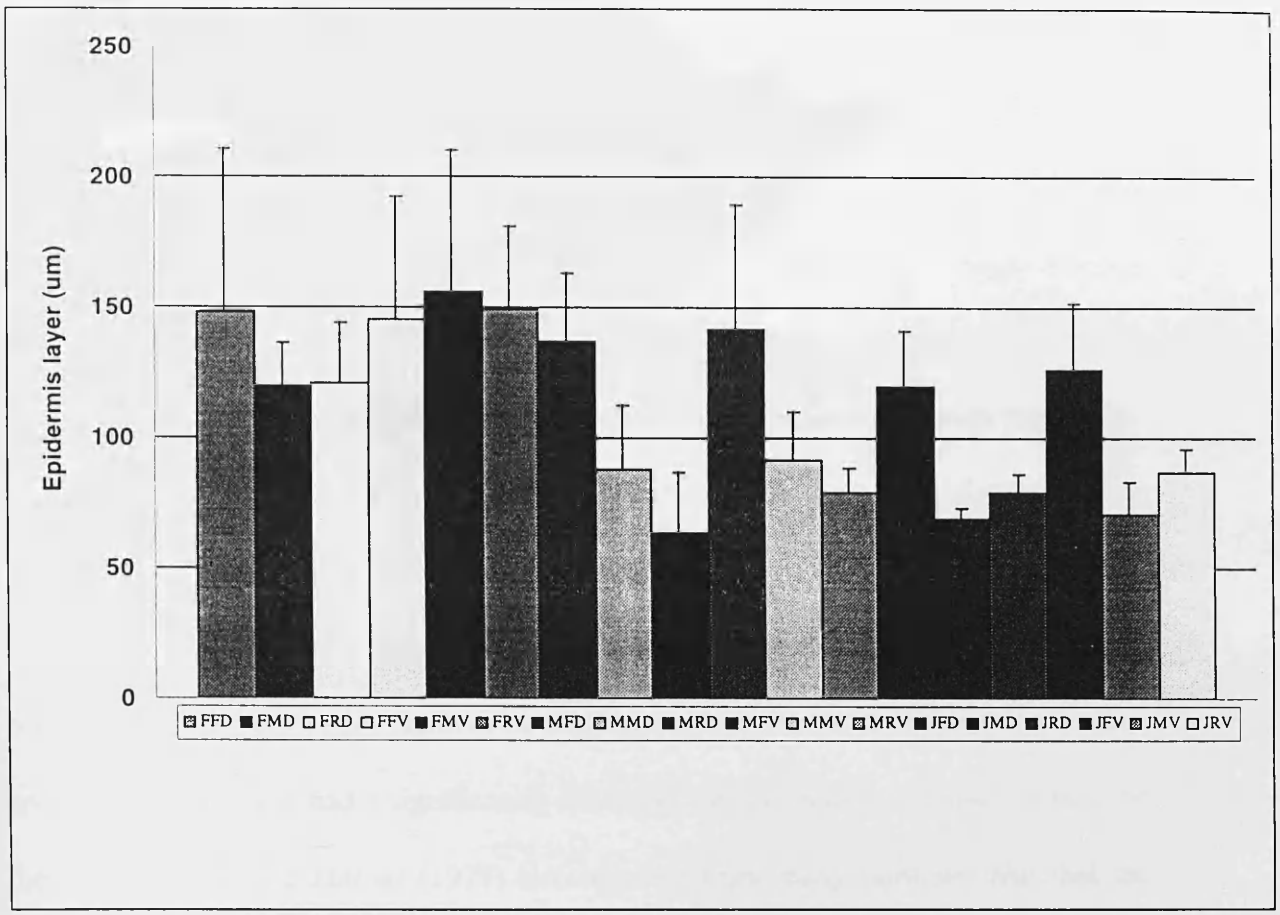


Figure 7.4. Epidermal thickness of different sites of juvenile, male and female Atlantic halibut, *Hippoglossus hippoglossus*. (FFD: female front dorsal, FMD: female middle dorsal, FRD: female rear dorsal, FFV: female front ventral, FMV: female middle ventral, FRV: female rear ventral, MFD:male front dorsal, MMD: male middle dorsal, MRD: male rear dorsal, MFV: male front ventral, MMV: male middle ventral, MRV: female rear ventral, JFD: juvenile front dorsal, JMD: juvenile middle dorsal, JRD: juvenile rear dorsal, JFV: juvenile front ventral, JMV: juvenile middle ventral, JRV: juvenile rear ventral)

7.4 DISCUSSION

Results from the present study showed that there was quite a regular pattern of mucous cell distribution and thickness of epidermis in the Atlantic halibut skin. Usually, the anterior region had a greater density of mucous cells compared to other regions and mature fish had more mucous cells than immature fish. Female fish had larger mucous cells than male fish and the ventral surface had more dense and bigger mucous cells than the dorsal surface. Exceptionally, the mean size of mucous cell from the antero-dorsal region of juvenile fish was significantly smaller than those from the mid-dorsal and from the postero-dorsal regions.

Pickering (1974) found that the epidermis of both male and female brown trout, *Salmo trutta* undergoes rhythmical changes in thickness during successive spawning cycles, and that male *S. trutta* had a significantly thicker epidermis than the female for most of the year. Wilkins and Jancsar (1979) also reported from many salmonid fish that the thickness of skin was greater in sexually mature than in immature fish and the male skin was thicker than female skin due to the level of sex hormones. Pickering and Macey (1977) reported that repeated handling, which stressed the fish host, the char, *Salvelinus alpinus* (L.), induced an increase in the number of mucous cells but it did not affect the size of the mucous cells and the thickness of the epidermis. They suggested that some changes would occur in the number or size of superficial goblet cells immediately under stress conditions. Pickering (1974) reported that the mucous cells in the brown trout, *S. trutta*, and the char, *S. alpinus* gradually increased in size from the region of the basal cells to the surface of the epidermis. He found that the mucous cell distribution was

extremely variable depending on species, sex and age of fish. It was found that both species of fish had fewer mucous cells in the fins compared with the rest of the body. The epidermis of the tail fin of the female trout contained approximately 5 times fewer mucous cells than the epidermis of the head. In the trout, usually the head had more mucous cells than the shoulder region, but in the char this distribution was reversed. The concentration gradient of mucous cells from anterior to posterior may ensure an even layer of slime over the body of the fish. As the fish moves forward, the posterior regions of the body would receive mucus not only from the mucous cells in that area but also from the anterior regions. Therefore the anterior region needed a greater concentration of mucous cells than the posterior regions. Wells and Cone (1990) found that a mixed infection of *Gyrodactylus colemanensis* and *G. salmonis* did not influence rainbow trout, *Oncorhynchus mykiss* fry growth or survival but the parasitic infection reduced mucous cell number by 50 % in the epidermis of the fin. They suggested that the reason why the mucous cell number was decreased in infected fish was that surface mucins served as an important food source for these ectoparasites

Considering the results from Chapters 3 and 6 in this study, there is a clear positive relationship between the population structure of *E. hippoglossi* and mucous cell distribution of halibut. The previous results showed that the adult parasites migrated to the antero-ventral surface. The size distribution and the mean size of the parasites correlated with the mucous cell concentration of the halibut skin. It was evident that *E. hippoglossi*, at least the adult, preferred the halibut skin area which had the greatest mucus concentration. It may be that the concentration of mucous cells on the dorsal

surface of juvenile fish might relate to the infection mechanism of *E. hippoglossi* oncomiracidia. Taking into account previous results (Chapters 3 and 6) and the present results, the oncomiracidia of *E. hippoglossi* attach to an area where there is a smaller volume of mucous cells. Alternatively the oncomiracidia may not be selective in their attachment but survival in some areas may be greater than others. For example, if mucus is acting as a defence mechanism, protecting the fish from the parasite then the parasites might survive better in areas where there is less mucus. It is not evident why a thinner epidermis might be an advantage and it may simply be that the epidermal thickness correlates with mucous cell density and size.

After the oncomiracidia have become accustomed to the new environment of the host, they are able to migrate in search of a better environment on the host. This would explain the distribution of the different sizes of *E. hippoglossi* on the halibut skin.

However, more precise studies will be needed, such as the analysis of mucous components and their role in defence against these parasites and cues for migration of parasites to the ventral surface.

Chapter 8

SUMMARY AND CONCLUSIONS

8. SUMMARY AND CONCLUSIONS

The higher intensity of the parasite infection from halibut at Machrihanish was thought to be due to the higher temperature there than that of the halibut culture system of Ardtoe. When parasite samples were taken, the water temperature in Machrihanish was 9.5°C in May whilst at Ardtoe it was 5°C in March.

The findings from the present study show very strong evidence that the parasite population in Machrihanish was actively growing compared to that in Ardtoe. The parasite population in Ardtoe was dominated by bigger, older group, 67.3 % being in the 9-15 mm group.

In comparison, only 29.6 % of the Machrihanish population was in the same size class.

Each species of monogenean has an optimum temperature for the maximum efficiency of reproduction. Pilcher *et al.*, (1989) suggested that the *Diclidophora merlangi* population increased at 7-11°C, with progressively declining reproductive success below 7°C and above 11°C. Ogawa (1988) suggested that increasing water temperatures must be an essential factor to facilitate the growth of the parasite population due to a shorter incubation time of the egg. In the present study, unfortunately, the optimal temperature of *E. hippoglossi* reproduction could not be assessed as a range of temperature was not available. However, monogenean ectoparasites must be affected by the host environment. The optimal temperature for Atlantic halibut was suggested by Bjørnsson and Tryggvadottir (1996) to be around 10°C. Therefore, it seems reasonable to suggest that the optimum temperature for reproduction of *E. hippoglossi* might be around 10°C. This may explain why the

parasite burden of Machrihanish was higher than Ardtoe and the younger parasite groups were found in Machrihanish.

Schram and Haug (1988) suggested that the more active spawning behaviour of males presents difficulty for the settling of oncomiracidia resulting in a greater number of *E. hippoglossi* on female halibut in the wild. In the present study, it was found that the mean intensity of the parasite on the female host was almost 3 times higher than on the male host. However, the greatest frequency of small parasites was found on the male host, not on the female host. It seems that the difference in the population structure on the different host sexes is not only due to the host spawning behaviour. The weight of the female host was 2 - 4 times that of the male in the present study and the surface area twice as large. This might be a reason why the male halibut has a smaller parasite population, the bigger surface of female halibut providing a greater quantity of food and a larger surface for attachment of parasites. However, culture conditions provide different environmental conditions from the natural state, so there might be different opportunities for parasites settling or it may be that the behaviour of host may be affected. Therefore, a comparison of the population structure of parasites from male halibut and female halibut originating in the wild and in culture systems is needed.

In the present study, parasite numbers were almost the same on the dorsal and ventral surfaces. The parasites were found mainly in the front region on both the dorsal and ventral surfaces of halibut. Strong evidence that *E . hippoglossi* migrates over the host surface was found in the present study. In contrast to *E. soleae* from the sole, oncomiracidia of *E. hippoglossi* seemed to invade the posterior part of the halibut, then

migrate from the invading area to the anterior part of the dorsal surface, finally settling on the ventral surface of the host. The results from the parasite measurements on the 4 principal zones in the present study showed that the 3-7 mm size classes were the majority groups of the 3 dorsal zones while 11-13 mm was the dominant size class on the ventral surface. However, the parasites which had undeveloped sexual organs were almost equally distributed on the 4 principal zones (1.4 –1.8 %). It was suggested that the oncomiracidia could invade every part of the host body but for some reason they migrate to the final destination, the ventral surface of the host. Kearn (1984) suggested that the advantages of migration from the upper surface to the lower surface were increasing the opportunity for exchanging spermatophores, having a better position to attache eggs to sand grains and avoiding predators of the parasite. It is assumed that *E. hippoglossi* migrated for the same reasons as *E. soleae*. However, due to differences of host ecology more precise studies are needed.

The act of egg-laying in *E. hippoglossi* appeared to involve muscular effort utilising contraction of the general body musculature as well as of the muscles associated with the reproductive tract. In the present study, the common spawning method of *E. hippoglossi* found was that the eggs attached together by their appendages before being expelled as a chain. The second pattern was the 'egg ball' style; the parasites laying eggs which became attached to a large ball of unidentified material originating from the reproductive system of the parasite. It seems that this style has an advantage. When eggs combine together and sink to the bottom of the sea, the ball is able to resist water currents and to provide a substrate for the eggs. It was presumed that the parasites

might use both methods for laying eggs in different environmental conditions. Because the eggs attaching to the balls developed normally, it suggests that this method can be one of the normal egg laying patterns of *E. hippoglossi* and not only occurring *in vitro*.

In the present study, the egg laying rate of the parasites was clearly dependent on the origin of the parasites. The egg laying rates of parasite from males and females was similar, 2.2 eggs and 2.9 eggs per hour, respectively. However, parasites on the dorsal and ventral surfaces of the host had significantly different egg laying rates, 3.4 eggs per hour and 1.1 eggs per hour, respectively. This could be explained by the fact that the mean length of the parasites on the ventral surface was longer than that on the dorsal. Bigger parasites laid more eggs than the smaller ones. However, there were big differences in the egg productivity of even similar sized parasites on the dorsal and ventral surfaces. For example, a parasite collected from the ventral surface measuring 10 mm in length produced approximately 80 eggs in a 24 hour period, whilst a similar sized parasite from the dorsal surface laid c. 35 eggs over the same time period. This suggested that there were unknown factors which enhanced egg production on the ventral surface of the fish other than worm size. Many factors affect the egg-laying rate of the parasites, especially temperature and parasite intensity. Therefore, parasite intensity could be one of the reasons why the egg production of parasites from the ventral surface was higher than that of parasites from the dorsal surface. However, this requires further investigation.

Generally, the eggs of *E. hippoglossi* and *E. soleae* were similar in shape and size. The main differences were seen in the appendages of *E. hippoglossi,* which carry an

irregular sized buoy-like structure, whilst the eggs of *E. soleae* have appendages with sticky droplets. Many parasites found on bottom living marine teleosts or elasmobranch flat-fishes have sticky materials on their eggs. Sticky material may be of great importance for such parasites since attached sand particles would prevent the eggs being carried upwards. However, the buoy-like structures on the appendage of the eggs of *E. hippoglossi* were totally different in appearance from sticky droplets. It is suggested that these structures are not sticky droplets but a buoy for floating eggs which are entwined together on the bottom of the sea. The structures found on the appendage of the eggs in the present study maybe explained as follows: When the eggs are expelled from the parasites, they then entwine together on the bottom of the sea which is very deep. The deep sea where Atlantic halibut spawning is very calm and with no strong currents, so the sticky droplet is not needed but some structure such as a buoy is needed for preventing them from being covered by mud or particles, thus supporting them by holding them up in the oxygenated medium. When comparing them to the eggs of *E. soleae*, which is most closely related to *E. hippoglossi*, the eggs of *E. soleae* have sticky droplets along the egg filament which adhere to sand grains in the shallow waters where the current may be strong. According to Kearn (1963a) *S. solea* spends large amounts of time partially buried in the sand. This explanation could be tested experimentally by placing eggs in different depths of sand, with different levels of oxygenation.

Monogenean eggs appear to hatch spontaneously when their development is completed. In the present study, *E. hippoglossi* hatched 25-27 days after incubation at 12°C. Kearn

(1974) observed that *E. hippoglossi* hatched during the first few hours of darkness in contrast to the hatching of its close relative *E. soleae* which occurs in the light. Atlantic halibut might have diurnal feeding habits, perhaps with a resting period at night. Thus, oncomiracidia may hatch during the dark period providing greater benefits for attaching to its host. However, in the present study, it was found that the oncomiracidia hatched during the illuminated and the dark period without showing a response to any stimuli for hatching.

The morphology of the oncomiracidium of *E. hippoglossi* bears a general resemblance to the oncomiracidium of *E. soleae* and *Benedenia seriolae*. In the present study it was found to have a total of 64 epidermal ciliary plates. The epidermal ciliary plates on the dorsal surface were usually larger than those on the ventral surface. On the ventral part of the posterior of the oncomiracidia it was very difficult to count the plates due to the haptoral opening.

The number of epidermal ciliary plates on the anterior and the middle regions of *E. soleae* had the same number as *E. hippoglossi* in the present study. However, the epidermal ciliary plates on the posterior region of the two parasites were different, *E. soleae* having 13 plates whilst *E. hippoglossi* had 17 on the same region. Because of the haptor opening, the precise numbers of ciliary plates was difficult to count. It appears that there may be a general pattern in capsalid epidermal ciliary plate distribution. Kearn (1963b) found the same numbers of epidermal plates in the anterior and the middle regions with only minor differences in the posterior region. Details of the pattern of ciliation are less known for marine species than for freshwater species. This lack of balance is due to technical difficulties in investigating ciliated epidermal cells.

When silver nitrate was applied to oncomiracidia living in sea water, it was not successful because of the dark, dense precipitate which formed. In the present study three techniques were tried. The first method was to rinse oncomiracidia in distilled water prior to treatment with silver nitrate, but it was found that the epidermal ciliary plates were barely visible with this method. The second method used 0.36 M $MgCl_2$ to avoid precipitation, but this method resulted in the epidermal ciliary plates not being very clearly seen. The third method used only silver nitrate without any prior treatment, but, prior to treatment, almost all seawater was removed with a pipette. With this method it was quite hard to recognize the oncomiracidia in the solution, but the result was successful.

Observation showed that the oncomiracidia of *E. hippoglossi* attached themselves to the bottom of the container using the head region. The head glands of the oncomiracidium of *E. hippoglossi* were very conspicuous and it was thought their secretion was likely to be involved in the attachment of the parasite larvae to the host until the haptor fully opened and was functional. Kearn (1974) described glandular systems of the oncomiracidia of *E. soleae*, *E. hippoglossi* and *E. diadema*. He found three different kinds of gland cells with ducts opening on the margins of the head region of the oncomiracidium of *E. hippoglossi*. The distribution of the head glands of *E. soleae* and *E. diadema* resembled that of the head glands of *E. hippoglossi*, while the oncomiracidium of *E. hippoglossi* had 9 pairs of body gland cells, that of *E. diadema* had 8 pairs and the oncomiracidium of *E. soleae* had only 4 pairs.

Kearn (1963b) reported that the oncomiracidium of *E. soleae* possessed fourteen marginally situated hooklets, which had already reached their definitive size, and a pair of anterior and a pair of posterior hamuli and accessory sclerites. He suggested that a pair of marginal hooklets became the accessory sclerites. In the present sudy, three pairs of medianly situated sclerites, the anterior hamuli, the posterior hamuli and the accessory sclerites, and seven pairs of marginal hooks were found on the oncomiracidium of *E. hippoglossi*. The findings from the present study, the shape, number and arrangement of marginal hooklets and the three pairs of medianly located hamuli of the oncomiracidium were similar to those of *E. s*oleae.

In order to survive, parasitic organisms must find a suitable host. Therefore, the parasite has evolved very efficient host finding mechanisms. The newly hatched oncomiracidia of *E. hippoglossi* actively swam, rotating about their longitudinal body axes when swimming. It was found that the oncomiracidia continuously swam up and down with an average speed of 0.46 cm/second and 0.37 cm/second upward and downward, respectively. Under different water pressures and swimming distances, the larvae showed similar responses of upwards and downwards swimming. It seems that the downward and upward swimming behaviour is one of the important methods for host finding in oncomiracidia. When oncomiracidia were exposed to separate stimuli in the horizontal choice chamber, the parasites were positively responsive to light and host mucus. However, it was found that the oncomiracidia were more attracted by light rather than by halibut mucus when the parasites were exposed to the two stimuli at the same time. Most of the newly hatched oncomiracidia of *E. soleae* were photopositive.

According to Kearn (1980) the larvae responded immediately, turning into the light path after hatching but, 12 h after hatching, most of larvae showed a photonegative response. He suggested that the changing behaviour of the parasite larvae might be related to the capability of host finding by the oncomiracidium. In the present study, it was found that the larvae of *E. hippoglossi* did not have such strong responses to light stimuli as the larvae of *E. soleae*.

From the findings of the above experiments, a hypothesis might be made as to how the oncomiracidium of *E. hippoglossi* finds its host, Atlantic halibut. First, newly hatched oncomiracidia keep swimming upward and downward within about 10 cm height from seabed until they find a host. Because the host rests on the seabed, so the oncomiracidium does not need to swim upwards to any great extent. Second, when they have found a host, at a very close distance, the larvae recognize their specific host using chemotactic cues which are released from the host skin. Third, they attach to the host temporarily by their anterior adhesive gland organ. During this period the haptor opens and it attachs to the host firmly by means of its haptoral hooks.

However the host finding behaviour of the oncomiracidium is not determined by only one cue or only a single factor in the environment. Therefore, more precise studies are needed in future research.

The adult parasites of *E. hippoglossi* moved to the anterior region of the ventral surface of the fish whether they were placed initially on the dorsal or the ventral surface and they settled on the anterior ventral region of fish. The reason why adult *E. hippoglossi* moved to the anterior region of the ventral surface is not clear. However, it is assumed

that the migration of *E. hippoglossi* might be related to the volume of food, intensity of light, aggregation of parasites and the resistance of the parasite to currents or waves when the host fish moves.

E. hippoglossi did not survive, nor did they establish a further population on the juvenile halibut, even though they survived for over 8 weeks on the juvenile halibut. The reason why the parasites could not survive or detached from the juvenile fish was not clear. However, it is assumed that the immune system of adult and juvenile halibut might be different or the quality or quantity of mucus might be different.

Kearn (1967) investigated experimentally the attachment of the oncomiracidia of *E. soleae* to the scales of other flatfish hosts. Almost all oncomiracidia of *E. soleae* favoured the scale of the sole which is its normal host. Also he experimentally infected alien hosts with the adult parasites but the parasites became detached after only 24-30 hours, even though they could survive 2-6 days in a glass dish at 14-17°C. He suggested that some changes occur when the parasites near maturity so that they can no longer attach to the skin of the alien fishes, or the parasite was unable to maintain its hold on the skin of these fishes because of mechanical difficulties, or some substance produced by the fish skin repelled the parasite.

Many haemorrhagic lesions were found around the parasite infection area. *E. hippoglossi* seemed to attach itself to the skin of the halibut by a suction pressure generated between the haptor and the skin of the fish. This was achieved with the aid of the accessory sclerites inserted into the epidermis of the fish skin. The shape of the accessory sclerites are different from those of *E. soleae*, being sharper and more robust

than those of *E. soleae*. Therefore they may have a different function compared to those of *E. soleae*.

Histopathological examination revealed that sloughed necrotic epithelial cells and debris on the surface of the infected fish might be due to the penetration activities and feeding by the parasite. The irregular arrangement of mucous cells in the epidermis and the proliferation of the epidermal layer might be due to the recovery activity of skin. A similar proliferation was found by Urawa and Kusakari (1990) in the flounder (*Paralichthys olivaceus*) infected with the flagellate protozoan *Ichthyobodo* sp. The heavy infection caused hyperplasia of the malphigian cells and depletion of mucous cells, then loss of osmotic balance, suggesting that it might be a cause of mortality in fish.

The structure of the epidermis of uninfected fish showed that there was a quite regular pattern of mucous cell distribution and thickness of epidermis on the Atlantic halibut skin. Usually, the anterior region in the mature, female host and the ventral surface had a more denser and bigger mucous cell population than on the posterior region, immature fish, male hosts and the dorsal surface. The size distribution and mean sizes of the parasites correlated with mucous cell concentration of halibut skin. It was clear that *E. hippoglossi*, at least as an adult, preferred the halibut skin regions which had the greatest mucous concentration. The oncomiracidia of *E. hippoglossi* attached to an area where there was a smaller volume of mucous cells. Alternatively the oncomiracidia may not be selective in their attachment, but survival in some areas may be greater than in others. For example, if mucus is acting as a defence mechanism protecting the fish

from the parasite, then the parasites might survive better in areas where there is less mucus. After the oncomiracidia have become accustomed to the new environment of the host, they are able to migrate in search of a better environment on the host. However, more precise studies such as analysis of mucous components and their role in defense against these parasites as well as cues for migration of parasites to the ventral surface are needed.

Although there are still many interesting aspects of this host/parasite system to research, this study has contributed to the understanding of this host/parasite interaction, transmission and pathology of *Entobdella hippoglossi* on the Atlantic halibut, *Hippoglossus hippoglossus* in culture systems.

REFERENCES

REFERENCES

Ahmed, A. T. A. (1976). Trichodiniasis of goldfish and other carps. *Bangladesh Journal of Zoology*, **4:** 12-20.

Anderson, M. (1981). The change with host age of the composition of the ancyrocephaline (Monogenea) populations of parasites on thick-lipped grey mullets at Plymouth. *Journal of the Marine Biological Association of the United Kingdom*, **61:** 833-842.

Andrews, J. D. (1996). History of *Perkinsus marinus*, a pathogen of oysters in Chesapeake Bay 1950-1984. *Journal of Shellfish Research*, **15:** 13-16.

Andriyashev, A. P. (1954). Fishes of the Northern seas of the USSR. *Trudy Zoologichskogo Institut Akademiya Nauk SSSR. No. 53* (Translated from Russian by the Israel Program for Scientific Translations, IPST Cat. No. 836).

Arme, C. & Halton, D. W. (1972). Observations on the occurrence of *Diclidophora merlangi* (Trematoda: Monogenea) on the gills of whiting, *Merlangius merlangus*. *Journal of Fish Biology*, **4:** 27-32.

Askawa, M. (1970). Histochemical studies of the mucus on the epidermis of the eel, *Anguilla japonica*. *Bulletin of the Japanese Society of Scientific Fisheries*, **36:** 83-87.

Baer, J.G. & Euzet, L. (1961). Traité de Zoologie. In: *Class des Monogénes* (Ed; Grassé). *Traité de Zoologie*, **4:** 244-325.

Bakke, T. A., Harris, P. D., Jansen, P. A. & Hansen, L. P. (1992). Host specificity and dispersal strategy in gyrodactylid monogeneans, with particular reference to *Gyrodactylus salaris* (Platyhelminthes, Monogenea). *Diseases of Aquatic Organisms*, **13:** 63-74.

Bauer, O. N. (1959). Ecology of fresh water fish parasites. *Izvestiya Gosudarstvennogo Nauchno-Issledovatel'skogo Instituta Ozernogo i Rechnogo Rybnogo Khozyaistva*, **49:** 5-207.

Bauer, O. N., Musselius, V. A. & Strelkov, Yu. A. (1969). *Diseases of pond fishes.* Israel Program for Scientific Translations. Jerusalem.

Becheikh, S., Rousset, V., Maamouri, F., Ben-Hassine, O. K. & Raibaut, A. (1997). Pathological effects of *Peroderma cylindricum* (Copepoda: Pennellidae) on the kidneys of its pilchard host, *Sardina pilchardus* (Osteichthyes: Clupeidae), from Tunisian coasts. *Diseases of Aquatic Organisms*, **28:** 51-59.

Bell, A. S. (1995). *Studies on the biosystematics and biology of Strigeids (Digenea) parasitic in freshwater fish.* Ph.D Thesis, University of Stirling.

Bell, A. S., Gibson,D. I. & Sommerville, C. (1997). Chaetotaxy and surface structures of the miracidia of *Ichthyocotylurus erraticus* (Rudolphi, 1809), *I. variegatus* (Creplin, 18925) and *Apatemon gracilis* (Szidat, 1928) (Digenea, Strigeidae). *Systematic Parasitology*, **36:** 203-212.

Berg, L. & Øiestad, V. (1986). Growth and survival studies of halibut (*Hippoglossus hippoglossus*) from hatching to beyond metamorphosis carried out in mesocosms. *Council Meeting of the International Council for the Exploration of the Sea*, **16:** 1-19.

Bjørnsson, B. (1995). The growth pattern and sexual maturation of Atlantic halibut (*Hippoglossus hippoglossus* L.) reared in large tanks for 3 years. *Aquaculture*, **138:** 281-290.

Bjørnsson, B. & Tryggvadottir, S. V. (1996). Effects of size on optimal temperature for growth and growth efficiency of immature Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture*, **142:** 33-42.

Blaxter, J. H. S., Danielssen, D., Moksness, E. & Øeistad, V. (1983). Description of the early development of the halibut *Hippoglossus hippoglossus* and attempts to rear the larvae past first feeding. *Marine Biology*, **73:** 99-107.

Boglio, E. C. & Lucas, J. S. (1997). Impacts of ectoparasitic gastropods on growth, survival, and physiology of juvenile giant clams (*Tridacna gigas*), including a simulation model of mortality and reduced growth rate. *Aquaculture*, **150:** 25-43.

Bolla, S. & Holmefjord, I. (1988). Effect of temperature and light on development of Atlantic halibut larvae. *Aquaculture*, **74:** 355-358.

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.

Bovet, J. (1967). Contribution à la morphologie et à la biologie de *Diplozoon paradoxum* v. Nordmann, 1832. *Bulletin de la Société Neuchâteloise des Sciences Naturelles*, **82:** 231-245.

Bower, S.M. & Boutillier, J.A. (1989). Extent of castration of prawns (*Pandalus platyceros*) by *Sylon* (Crustacea: Rhizocephala). *Journal of Shellfish Research*, **8:** 467.

Bron, J. E., Sommerville, C., Jones, M. & Rae, G. H. (1993). The settlement and attachment of early stages of the salmon louse, *Lepeophtheirus salmonis* (Copepoda: Caligidae) on the salmon host, *Salmo salar*. *Journal of Zoology* (London), **224:** 201-212.

Buchmann, K. & Uldal, A. (1997). *Gyrodactylus derjavini* infections in four salmonids: comparative host susceptibility and site selection of parasites. *Diseases of Aquatic Organisms,* **28:** 201-209.

Burton, D., Burton, M. P. & Idler, D. R. (1984). Epidermal condition in post-spawned winter flounder, *Pseudopleuronectes americanus* (Walbaum), maintained in the laboratory and after exposure to crude petroleum. *Journal of Fish Biology*, **25:** 593-606.

Bychowsky, B. E. (1957). *Monogenetic Trematodes, their Classification and Phylogeny.* Academy of Sciences, USSR, Moscow and Leningrad. (In Russian). English translation by W.J. Hargis and P.C. Oustinoff (1961). American Institute of Biological Sciences, Washington.

Chan, B. & Wu, B. (1984). Studies on the pathogenicity, biology and treatment of *Pseudodactylogyrus* for the eels in fish-farms. *Acta Zoologica Sinica,* **30:** 173-180.

Chintala, M. M., Ford, S. E., Fisher, W. S. & Ashton-Alcox, K. A. (1994). Oyster serum agglutinins and resistance to protozoan parasites. *Journal of Shellfish Research,* **13:** 115-121.

Chu, Fu-Lin-E., Burreson, E. M., Zhang, F. & Chew, K. K. (1996). An unidentified haplosporidian parasite of bay scallop *Argopecten irradians* cultured in the Shandong and Liaoning provinces of China. *Diseases of Aquatic Orgarnisms*, **25:** 155-158.

Chubb, J. C. (1977). Seasonal occurrence of helminths in freshwater fishes part I. Monogenea. *Advances in Parasitology*, **15:**133-192.

Combes, C. (1972). Ecologie des Polystomatidae (Monogenea): facteurs influencant le volume et le rythme de la ponte. *International Journal for Parasitology*, **2:** 233-238.

Cone, D. K. (1979). Hatching of *Urocleidus adspectus* Mueller, 1936 (Monogenea: Ancyrocephalinae). *Canadian Journal of Zoology*, **57:** 833-837.

Cone, D. K. & Cusack, R. (1988). A study of *Gyrodactylus colemanensis* Mizelle & Kritsky, 1967 and *Gyrodactylus salmonis* (Yin & Sproston, 1948) (Monogenea) parasitising captive salmonids in Nova Scotia. *Canadian Journal of Zoology*, **66:** 409-415.

Cone, D. K. & Odense, P. H. (1984). Pathology of five species of *Gyrodactylus* Nordmann, 1832 (Monogenea). *Canadian Journal of Zoology*, **62:** 1084-1088.

Cusack, R. (1995). Sea lice and possible interactions with wild fishes. *Bulletin Aquaculturist Association of Canada*, **95:** 26-27.

Cusack, R. & Cone, D. K. (1986). *Gyrodactylus salmonis* (Yin & Sproston, 1948) parasitizing fry of *Salvelinus fontinalis* (Mitchill). *Journal of Wildlife Diseases*, **22:** 209-213.

Dillion,W. A. & Hargis, W. J. (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.

Dimitrov, V., McCarthy, A.M. & Kanev, I.(1991). *Strigea falconispalumbi* (Trematoda:Strigeidae): argentophillic structures of the miracidium. *Angewandte Parasitologie*, **32:**173-175.

Dogiel, V.A. (1964). *General Parasitology*. English translation. Oliver and Boyd. Edinburgh and London.

Engelbrecht, H. (1963). Der Einfluss der Umwelt auf die Entwicklung parasitarer Wurmer. *Ĉsllca Parasitology*, **10:** 73-110. (Translation Fisheries Research Board of Canada No. 456).

Erasmus, D. A. (1972). *The Biology of the Trematodes*. Edward Arnold Ltd. London.

Ernst, I. & Whittington, I.D. (1996). Hatching rhythms in the capsalid monogeneans *Benedenia lutjani* from the skin and *B. rohdei* from the gills of *Lutjanus carponotatus* at Heron Island, Queensland, Australia. *International Journal for Parasitology*, **26:** 1191-1204.

Euzet, L. & Wahl, E. (1970). Biologie de *Rhinecotyle crepitacula* Euzet et Trilles, 1960 (Monogenea) parasite de *Sphyraena piscatorum* Cadenat, 1964 (Teleostei) dans la langune Ebrié (Côte d'I voire). *Revue Suisse de Zoologie*, **77:** 687-703.

Finn, R. N., Fyhn, H. J. & Evjen, M. S. (1991). Respiration and nitrogen metabolism of Atlantic halibut egg (*Hippoglossus hippoglossus*). Marine Biology, **108:** 11-19.

Finlayson, J. E. (1982). The alledged alternation of sexual phases in *Kuhnia scombri*, a monogenean of *Scomber scombrus*. *Parasitology*, **84:** 303-311.

Fischer, S. A. & Kelso, W. E. (1990). Parasite fauna development in juvenile bluegills and largemouth bass. *Transactions of the American Fisheries Society*, **119:** 877-884.

Frankland, H. M. T. (1955). The life history and bionomics of *Diclidophora denticulata* (Trematoda: Monogenea). *Parasitology*, **45:** 313-351.

Fuentes, J. L. & Nasir, P. (1990). Description and ecology of *Ligophorus mugilinus* (Hargis, 1955) Euzet y Suriano, 1477. (Monogenea: Ancyrocephalinae) in *Mugil curema* (Val., 1936) of Margarita Island, Venezuela. *Scientia Marina*, **54:** 187-193.

Gelnar, M. (1987). Experimental vertification of the effect of physical conditions of *Gobio gobio* (L.) on the growth rate of micropopulations of *Gyrodactylus gobiensis* Glaser, 1974 (Monogenea). *Folia Parasitologia*, **30:** 15-26.

Godø, O. R. & Haug, T. (1988). Tagging and recaptures of Atlantic halibut (*Hippoglossus Hippoglossus* L.) on the continental shelves off eastern Canada and off weatern and eastern Greenland. *Journal of Northwest Atlantic Fisheries Science*, **8:** 25-31.

Goff, G. P. & Lall, S. P. (1989). An initial examination of the nutrition and growth of Atlantic halibut (*Hippoglossus hippoglossus*) fed whole herring with a vitamin supplement. *Proceedings of the Annual Meeting 1989. Aqauaculture Association of Canada Symposium. Bulletin of the Aquaculture Association of Canada*, **89:** 53-55.

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.

Hanek, G. & Fernando, C. H. (1978a). Seasonal dynamics and spatial distribution of *Urocleidus ferox* Mueller 1934, a gill parasite of *Lepomis gibbosus* (L.). *Canadian Journal of Zoology*, **56:** 1241-1243.

Hanek, G. & Fernando, C. H. (1978b). Seasonal dynamics and spatial distribution of *Cleidodiscus stentor* Mueller, 1937 and *Ergasilus centrarchidarum* Wright, 1882, gill parasites of *Ambloplites rupestris* (Raf.). *Canadian Journal of Zoology*, **56:** 1244-1246.

Hara, T. J., Macdonald, S., Evans, R. E., Marui, T. & Akai, S. (1984). Morpholine, bile acids and skin mucus as possible chemical cues in salmonids homing: electrophysiological re-evaluation. In: *Mechanisms of Migration in Fishes* (Eds. McCleave, J.D., Arnold, G.P., Dodson, J.D. & Neill, W.H.). Plenum. New York.

Harboe, T., Huse, I. & Öie, G. (1994). Effects of egg disinfection on yolk sac and first feeding stages of halibut (*Hippoglossus hippoglossus*) larvae. *Aquaculture*, **119:** 157-165.

Harris, P. D. (1988). Changes in the site specificity of *G. turnbulli* Harris, 1980 (Monogenea) during infestation of individual guppies (*Poecilia reticulata*) Peters, 1859. *Canadian Journal of Zoology*, **66:** 2854-2857.

Haug, T. (1990). Biology of the Atlantic halibut, *Hippoglossus hippoglossus* (L., 1758). *Advanced Marine Biology*. **26:** 1-70.

Haug, T. & Fevolden, S. E.(1986). Morphology and biochemical genetics of Atlantic halibut, *Hippoglossus hippoglossus* (L.), from various spawning grounds. *Journal of Fish Biology*, **28:** 367-378.

Haug, T. & Gulliksen, B. (1988). Fecundity and egg sizes in ovaries of female Atlantic halibut, *Hippoglossus hippoglossus* (L.). *Sarsia,* **73:** 259-261.

Haug, T., Kjørsvik, E. & Solemdal, P. (1984). Vertical distribution of Atlantic halibut (*Hippoglossus hippoglossus*) eggs. *Canadian Journal of Fisheries and Aquatic Sciences*, **41:** 798- 804.

Haug, T. & Sundby, S. (1987). A preliminary report on the natural occurrence and ecology of Atlantic halibut, *Hippoglossus hippoglossus*, postlarvae and young immature stages. *Council Meeting of the International Council for the Exploration of the Sea*, **38:** 1-29.

Haug, T. & Tjemsland, J. (1986). Changes in size-and age-distributions and age at sexual maturity in Atlantic halibut, *Hippoglossus hippoglossus*, caught in North Norwegian waters. *Fisheries Research*, **4:** 145-155.

Heuch, P.A., Parsons, A. & Boxaspen, K. (1995). Diel vertical migration: A possible host-finding mechanism in salmon louse (*Lepeophtheirus salmonis*) copepodids?. *Canadian Journal of Fisheries Aquaculture Science*, **52:** 681-689.

Hines, R.S. & Spira, D.T. (1974). Ichthyophthiriasis in the mirror carp *Cyprinus carpio* (L.) III. Pathology. *Journal of Fish Biology*, **6:** 189-196.

Holmyard, N. (1996). UK halibut farms move to commercial stage. *Fish Farming International*, EMAP business publications, October 1996.

Houlihan, D. F. & Macdonald, S. (1979). *Diclidophora merlangi* and *Entobdella soleae*: egg production and oxygen consumption at different oxygen partial pressures. *Experimental Parasitology*, **48:** 109-117.

Jackson, H. (1982). *Protopolystoma xenopodis*: success of reproduction in single and multiple infestations. *Parasitology*, **85:** v.

Jackson, H. C. & Tinsley, R. C. (1988). Environmental influences on egg production by the monogenean *Protopolystoma xenopodis. Parasitology*, **97:** 115-128.

Jahn, T. L. & Kuhn, L. R. (1932). The life history of *Epibdella melleni* MacCallum, 1927 a monogenetic trematode parasitic on marine fishes. *Biological Bulletin*, **62:** 89-111.

Jakupsstovu, S. H. I. & Haug, T. (1988). Growth, sexual maturation and spawning season of Atlantic halibut, *Hippoglossus hippoglossus*, in Faroese waters. *Fisheries Research*, **6:** 201-215.

Jakupsstovu, S. H. I. & Haug, T. (1989). Spawning of Atlantic halibut *Hippoglossus hippoglossus* in deep waters on the continental slope south west of the Faroe Islands. *Frödskaparrit*, **34/35:** 79-80.

Jansen, P. A. & Bakke, T. A. (1991). Temperature-dependent reproduction and survival of *Gyrodactylus salaris* Malmberg, 1957 (Platyhelminthes: Monogenea) on Atlantic salmon (*Salmo salar* L.). *Parasitology*, **102:** 105-112.

Jensen, A. J. & Johnsen, B. O. (1991). Site specificity of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea) on Atlantic salmon (*Salmo salar* L.) in the River Lakselva, northern Norway. *Canadian Journal of Zoology*, **70:** 264-267.

Johnson, S. C., Blaylock, R. B., Elphick, J. & Hyatt, K. D. (1996). Disease induced by the sea louse (*Lepeophtheirus salmonis*) (Copepoda: Caligidae) in wild sockeye salmon (*Oncorhynchus nerka*) stocks of Alberni Inlet, British Columbia. *Canadian Journal of Fisheries Aquatic Science*, **53:** 2888-2897.

Kamiso, H. N. & Olson, R. E. (1986). Host-parasite relationships between*Gyrodactylus stellatus* (Monogenea: Gyrodactylidae) and *Parophrys vetulus* (Pleuronectidae – English sole) from coastal waters of Oregon. *Journal of Parasitology*, **72:**125-129.

Kearn, G. C. (1963a). The life cycle of the monogenean *Entobdella soleae*, a skin parasite of the common sole. *Parasitology*, **53:** 253-263.

Kearn, G. C. (1963b). The egg, oncomiracidium and larval development of *Entobdella soleae*, a monogenean skin parasite of the common sole. *Parasitology*, **53:** 435-447.

Kearn, G. C. (1963c). The oncomiracidium of *Capsala martinieri*, a monogenean parasite of the sunfish (*Mola mola*). *Parasitology*, **53:** 449-453.

Kearn, G. C. (1963d). Feeding in some monogenean skin parasites: *Entobdella soleae* on *Solea solea* and *Acanthocotyle* sp. on *Raja clavata*. *Journal of the Marine Biological Association of the United Kingdom*, **43:** 749-766.

Kearn, G. C. (1964). The attachment of the monogenean *Entobdella soleae* to the skin of the common sole. *Parasitology*, **54:** 327-335.

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

Kearn, G. C. (1967b). Experiments on host-finding and host-specificity in the monogenean skin parasite *Entobdella soleae*. *Parasitology*, **57:** 585-605.

Kearn, G. C. (1968). The development of the adhesive organs of some diplectanid, tetraonchid and dactylogyrid gill parasites (Monogenea). *Parasitology*, **58:** 149-163.

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

Kearn, G. C. (1973). An endogenous circadian hatching rhythm in the monogenean skin parasite *Entobdella soleae* and its relationship to the activity rhythm of the host *Solea solea*. *Parasitology*, **66:** 101-122.

Kearn, G. C. (1974a). Nocturnal hatching in the monogenean skin parasite *Entobdella hippoglossi* from the halibut, *Hippoglossus hippoglossus*. *Parasitology*, **68:** 161-172.

Kearn, G. C. (1974b). A comparative study of the glandular and excretory systems of the oncomiracidia of the monogenean skin parasites *Entobdella hippoglossi*, *E. diadema and E. soleae*. *Parasitology*, **69:** 257-269.

Kearn, G. C. (1974c). The effect of fish skin mucus on hatching in the monogenean parasite *Entobdella soleae* from the skin of the common sole, *Solea solea*. *Parasitology*, **68:** 173-188.

Kearn, G. C. (1975). The mode of hatching of the monogenean *Entobdella soleae*, a skin parasite of the common sole (*Solea solea*). *Parasitology*. **71:** 419-431.

Kearn, G. C. (1976). Body surface of fishes. In: *Ecological Aspects of Parasitology*. North Holland Publishing Company, Amsterdam/Oxford.

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. (1980). Light and gravity responses of the oncomiracidium of *Entobdella soleae* and their role in host location. *Parasitology*, **81:** 71-89.

Kearn, G. C. (1982). Rapid hatching induced by light intensity reduction in the monogenean *Entobdella diadema*. *Journal of Parasitology*, **14:** 63-69.

Kearn G.C. (1984). The migration of the monogenean *Entobdella soleae* on the surface of its host, *Solea solea*. *International Journal for Parasitology*, **14:** 63-69.

Kearn, G. C. (1985). Observation on egg production in the monogenean *Entobdella soleae*. *International Journal for Parasitology*, **15:** 187-194.

Kearn, G. C. (1986). The Eggs of Monogeneans. *Advances in Parasitology*, **25:** 175-262.

Kearn, G. C. (1988). Orientation and locomotion in the monogenean parasite *Entobdella soleae* on the skin of its host (*Solea solea*). *International Journal for Parasitology*, **18:** 753-759.

Kearn G. C. (1992). Mating in the capsalid monogenean *Benedenia seriolae*, a skin parasite of the yellowtail, *Seriola quinqueradiata*, in Japan. *Publications of the Seto Marine Biology Laboratory*, **35:** 351-362.

Kearn, G. C. & Baker, N. O. (1973). Ultrastructural and histochemical observations on the pigmented eyes of the oncomiracidium of *Entobdella soleae*, a monogenean skin parasite of the common sole, *Solea solea*. *Zeitschrift fur Parasitenkunde*, **41:** 239-254.

Kearn, G. C. & Macdonald, S. (1976). The chemical nature of host hatching factors in the monogenean skin parasites *Entobdella soleae* and *Acanthocotyle lobianchi*. *International Journal for Parasitology*, **6:** 457-466.

Kearn, G. C., Ogawa, K. & Maeno, Y. (1992a). Egg production, the oncomiracidium and larval development of *Benedenia seriolae*, a skin parasite of yellowtail, *Seriola quinqueradiata*, in Japan. *Publications of the Seto Marine Biology Laboratory*, **35:** 351-362.

Kearn G. C., Ogawa K. & Maeno Y.(1992b). The oncomiracidium of *Heteraxine heterocerca*, a monogenean gill parasite of the yellowtail *Seriola quinqueradiata*. *Publications of the Seto Marine Biology Laboratory*, **35:** 347-350.

Khan, R.A. & Kiceniuk, J. (1988). Histopathological effects of crude oil on Atlantic cod following chronic exposure. *Canadian Journal of Zoology*, **62:** 2038-2043.

Khan, R. A. & Lee, E. M. (1989). Influence of *Lernaeocera branchialis* (Crustacea: Copepoda) on growth rate of Atlantic cod, *Gadus morhua*. *Journal of Parasitology*, **75:** 449-454.

Kingston, N., Dillon, W.A. & Hargis, W.J.Jnr. (1969). Studies on larval Monogenea of fishes from the Chesapeake Bay area. *Journal of Parasitology*, **55:** 544-558.

Kjørsvik, E., Haug, T. & Tjemsland, J. (1987). Spawning season of the Atlantic halibut (*Hippoglossus hippoglossus*) in northern Norway. *Journal du Conseil Internationale pour Exploration de la Mer*, **43:** 285-293.

Kjørsvik, E. & Holmefjord, I. (1995). Atlantic halibut (*Hippoglossus hippoglossus*) and cod (*Gadus morhua*). In: *Broodstock Management and Egg and Larval Quality*. (Eds. Bromage and Roberts). Blackwell Science, Oxford.

Kohler, A.C. (1964). Movements of halibut on the Nova Scotian and Grand Banks. *Journal of the Fisheries Research Board of Canada*, **21:** 837-840.

Kollmann, A. (1970). *Dactylogyrus vastator*, Nybelin, 1924 (Trematoda: Monogenoidea) as a pathogen on the gills of common carp (*Cyprinus carpio*) ((*Dactylogyrus vastator*, Nybelin, 1924 (Trematoda: Monogenoidea) als Krankheitserreger auf den Kiemen des Karpfens (*Cyprinus carpio* L.)). Part 1. *Zeitschrift fur fisherei und deren Hilfswissenschaften, Berlin.* **18:**129-150.

Kollmann, A. (1972). *Dactylogyrus vastator*, Nybelin, 1924 (Trematoda: Monogenoidea) as a pathogen on the gills of common carp (*Cyprinus carpio*) ((*Dactylogyrus vastator*, Nybelin, 1924 (Trematoda: Monogenoidea) als Krankheitserreger auf den Kiemen des Karpfens (*Cyprinus carpio* L.)). Part 3. Teil. *Zeitschrift fur Wissenschafliche Zoologie, Leipzig.* **185:** 1-54.

Körting, W. (1975). Das Wirt-Parasit-Verhältnis aus der Sicht des Fischereibiologen. *Fisch und Umwelt*, **1:** 3-11.

Ktari, M. H. (1969). Recherches sur l'anatomie et la biologie de *Microcotyle salpae* Parona et Perugia, 1890 parasite de *Box salpa* L. (Téléostéen). *Annales de Parasitologie Humaine et Comparée*, **44:** 425-440.

Ktari, M. H. (1971). Recherches sur la reproduction et le developpement de quelques (Polyopisthocotylea) parasites de poissons marins. *Universite Sciences Naturella et Technique, Languedoc, Montpellier,* pp.327.

Lambert, A. (1977). Recherches Sur les affinités phylétiques des Polystomatidae (Monogenea). *Compté Rendus de l Académie des Sciences, Paris.* **Series D:**1243-1246.

Lambert, A. (1980). Oncomiracidiums et phylogenèse des Monogenea (Platyhelminthes). Deuxième partie: Structures argyrophiles des oncomiracidiums et phylogenèse des Monogenea. *Annales de Parasitologie Humaine et Comparéé*, **55:** 281-325.

Lambert, A. (1981). Sensors and effectors in the behaviour of oncomiracidia: ciliated epidermis and sensilla. (Third European Multicolloquium of Parasitology. Cambridge, Sept. 7-13, 1980 workshop proceedings). *Parasitology*, **82:** 59-60.

Lambert, A & Maillard, C. L. (1975). Repartition branchiale de deux monogenes: *Diplectanum aequans* (Wagener, 1857) Diesing 1858 et *Diplectanum lauberi* Lambert et Maillard, 1974 (Monogenean: Monopisthocotylea) parasites simultanes de *Dicentrachus labrax* (Teleosteen). *Annales de Parasitologie Humaine et Comparéé,* **50:** 691-699.

Langdon, J.S., Thorne, T. & Fletcher, W.J. (1992). Reservoir hosts and new clupeoid host records for the myoliquefactive myxosporean parasite *Kudoa thyrsites* (Gilchrist). *Journal of Fish Diseases*, **15:** 459-471.

Lein, I. (1996). Environmental aspects of the yolk sac stage and early feeding of Atlantic halibut larvae. Ph.D thesis. University of Bergen, Norway.

Lester, R. J. G. (1972). Attachment of *Gyrodactylus* to *Gasterosteus* and host response. *Journal of Parasitology*, **58:** 717-722.

Lester, R. J. G. & Adams, J. R. (1974). *Gyrodactylus alexanderi*: reproduction, mortality and effect on the host. *Canadian Journal of Zoology*, **52:** 827-833.

Llewellyn, J. (1956). The host-specificity, micro-ecology, adhesive attitudes, and comparative morphology of some trematode gill parasites. *Journal of the Marine Biological Association of the United Kingdom* **35:** 113-127.

Llewellyn, J. (1957). The larvae of some monogenetic trematode parasites of Plymouth fishes. *Journal of the Marine Biological Association of the United Kingdom*, **36:** 243-259.

Llewellyn, J. (1962). The life cycle histories and population dynamics of monogenean gill parasites of *Trachurus trachurus* (L.). *Journal of the Marine Biological Association of the United Kingdom*, **42:** 587-600.

Llewellyn, J. (1972). Behaviour of monogeneans. In: *Behavioural Aspects of Parasite Transmission. Zoological Journal of the Linnean Society* (Supplement No.1),. **51:** 19-30.

Llewellyn, J. & Macdonald, S. (1980). Host-specificity and speciation in diclidophoran (Monogenea) gill parasites of Trisopteran (gadoid) fishes at Plymouth. *Journal of the Marine Biological Association of the United Kingdom*, **60:** 73-79.

Logan, F. H. & Odense, P. H. (1974). The integument of the ocean sunfish (*Mola mola* L.) (Plectognathi) with observations on the lesions from two ectoparasites, *Capsala martinierei* (Trematoda) and *Philorthagoriscus serratus* (Copepoda). *Canadian Journal of Zoology*, **52:** 1039-1045.

Lønning, S., Kjørsvik, E., Haug, T.& Gulliksen, B. (1982). The early development of the halibut, *Hippoglossus hippoglossus* (L.), compared with other marine teleosts. *Sarsia*, **67:** 85-91.

Lyons, K.M. (1972). Sense organs of monogeneans. In: *Behavioural Aspects of Parasite Transmission. Zoological Journal of the Linnean Society* (Supplement No.1). **51:** 19-30.

Macdonald, S. (1974). *Aspects of the biology of some monogenean parasites*. Ph.D thesis. University of East Anglia, Norwich.

Macdonald, S. (1975). Hatching rhythms in three species of *Diclidophora* (Monogenea) with observations on host behaviour. *Parasitology*, **71:** 211-228.

Macdonald, S. (1977). A comparative study of the structure of the oncomiracidia of the monogenean gill parasites *Diclidophora merlangi, D. luscae and D. denticulata. International Journal for Parasitology*, **7:** 113-118.

Macdonald, S. & Combes, C. (1978). The hatching rhythm of *Polystoma integerrimum*, a monogenean from the frog *Rana temporaria. Chronobiologia,* **5:** 277-285.

Macdonald, S. & Jones, A. (1978). Egg-laying and hatching rhythms in the monogenean *Diplozoon homoion gracile* from the southern barbel (*Barbus meridionalis*). *Journal of Helminthology*, **52:** 23-28.

Molnár, K. (1971). Studies on gill parasitosis of the grass-carp (*Ctenopharyngodon idella*) caused by *Dactylogyrus lamellatus* Achmerow, 1952. I. Morphology and biology of *Dactylogyrus lamellatus. Acta Veterinaria Academiae Scientiarum Hungaricae,* **21:** 267-289.

Musselius, V. A. & Ptashuk, S.V. (1970). On the development and specificity of *Dactylogyrus lamellatus* (Monogenoidea, Dactylogyridae). *Parazitologiya*, **4:**125-132.

Nagasawa, K. & Maruyama, S. (1987). Occurrence and effects of *Haemobaphes diceraus* (Copepoda: Pennellidae) on brown sole *Limanda herzensteini* off the Okhotsk coast of Hokkaido. *Nippon Suisan Gakkaishi*, **53:** 991-994.

Nickerson, J. T .R. (1978). The Atlantic halibut and its utilization. *Marine Fisheries Review*, **40:** 21-25.

Nilakarawasam, N. (1993). *Ecological studies on the parasites of Etroplus suratensis (Bloch) (Pisces:Cichilidae) with special reference to Enterogyrus spp (Monogenea: Ancyrocephlinae).* Ph.D Thesis, University of Stirling.

Noakes, D.L.G. (1973). Parental behaviour and some histological features of scales in *Cichlasoma citrinellum* (Pisces, Cichlidae). *Canadian Journal of Zoology*, **51:** 619-622.

Ogawa, K. (1986). A monogenean parasite *Gyrodactylus masu* sp. n. (Monogenea: Gyrodactylidae) of salmonid fish in Japan. *Nippon Suisan Gakkaishi*, **52:** 947-950.

Ogawa, K. (1988). Development of *Bivagina tai* (Monogenea: Microcotylidae). *Nippon Suisan Gakkaishi,* **54:** 61-64.

Ogawa, K., Bondad-Reantaso, M. G. & Wakabayashi, H. (1995a). Redescription of *Benedenia epinepheli* (Yamaguti, 1937) Meserve, 1938 (Monogenea : Capsalidae) from cultured and aquarium marine fishes of Japan. *Canadian Journal of Fisheries and Aquatic Science*, **52:** 62-70.

Ogawa, K., Bondad-Reantaso, M.G. Fukudome, M. & Wakabayashi, H. (1995b). *Neobenedenia girellae* (Hargis, 1955) Yamaguti, 1963 (Monogenea : Capsalidae) from cultured marine fishes of Japan. *Journal of Parasitology*, **81:** 223-227.

Oliver, G. (1977). Effet pathogene de la fixation de *Diplectanum aequans* (Wagener, 1857) Diesing, 1858 (Monogenea, Monopisthocotylea, Diplectanidae) sur les branchies de *Dicentrarchus labrax* (Linnaeus, 1758), (Pisces, Serranidae). *Zeitschrift fur Parasitenkunde*, **53:** 7-11.

Oliver, L.M., Fisher, W.S., Burreson, E.M., Ragone-Calvo, L.M, Ford, S.E. & Gandy, J. (1996). *Perkinsus marinus* tissue distribution and seasonal variation in oysters (*Crassostrea virginica*) from Florida, Virginia and New York. *Journal of Shellfish Research*, **15:** 497.

Owen, I. L. (1970). The oncomiracidium of the monogenean *Discocotyle sagittata*. *Parasitology*, **61:** 279-292.

Paling J. E. (1965). The population dynamics of the monogenean gill parasite *Discocotyle sagittata* Leuckart on Windermere trout, *Salmo trutta*, L. *Para*sitology, **55:** 667-694.

Paling J. E. (1969). The manner of infection of trout gills by the monogenean parasite *Discocotyle sagittata*. *Journal of Zoology*, **159:** 293-309.

Paperna, I. (1963a). Some observations on the biology and ecology of *Dactylogyrus vastator* in Israel. *Bamidgeh,* **15:** 8-28.

Paperna, I. (1963b). Dynamics of *Dactylogyrus vastator* Nybelin (Monogenea) populations on the gills of carp fry in fish ponds. *Bamidgeh*, **15:** 31-50.

Paperna, I. (1964). Host reaction to infestation of carp with *Dactylogyrus vastator* Nybelin, 1924 (Monogenea). *Bamidgeh*, **16:** 129-141.

Paperna, I. (1980). *Caligus minimus* (Caligadae: Copepoda) infections of the sea bass *Dicentrachus labrax* in the Bardwill Lagoon, Israel. *Annales Parasitologie Humaire et Comparée*, **55:** 687-706.

Peters, L. E. (1966). Epidermal cell patterns in the miracidium of digenetic trematodes. *Papers of the Michigan Academy of Science, Arts and Letters*, **51:**109-117.

Petrushevski, G. K. & Shulmann, S. S. (1961). The parasitic diseases of fishes in the natural waters of the USSR. In: *Parasitology of fishes* (Eds Dogiel, Petrushevski, Polyanski & Yu). English translation, Oliva and Boyd, Edinburgh and London.

Pichelin, S. (1995a). The taxonomy and biology of the Polystomatidae (Monogenea) in Australian freshwater turtles (*Chelidae pleurodira*). *Journal of Natural History.* **29:** 1345-1381.

Pichelin, S. (1995b). *Parapolystoma johnstoni* n. sp. from *Litoria nyakalensis* (Amphibia) in Australia and taxonomic considerations on the Diplorchiinae (Monogenea). *Journal of Parasitology*, **81:** 261-265.

Pickering, A. D.(1974). The distribution of mucous cells in the epidermis of the brown trout *Salmo trutta* (L.) and the char *Salvelinus alpinus* (L.). *Journal of Fish Biology*, **6:** 111-118.

Pickering, A. D. (1977). Seasonal changes in the epidermis of the brown trout *Salmo trutta* (L.). *Journal of Fish Biology*, **10:** 561-566.

Pickering, A. D. & Macey, D. J. (1977). Structure, histochemistry and the effect of handling stress on the mucous cells of the epidermis of the char *Salvelinus alpinus* (L.). *Journal of Fish Biology*, **10:** 505-512.

Pilcher, M. W., Whitfield, P. J. & Riley, J. D. (1989). Seasonal and regional infection characteristics of three ectoparasites of whiting , *Merlangius merlangus* L., in the North Sea. *Journal of Fish Biology*, **35:** 97-110.

Pittman, K., Berg, L. & Naas, K. (1987). Morphological development of halibut (*Hippoglossus hippoglossus*) larvae with special reference to mouth development and metamorphosis. *Council Meeting of the International Council for the Exploration of the Sea*, **18:** 1-22.

Pottinger, T.G., Pickering, A. D. & Blackstock, N. (1984). Pathogenesis and autoradiographic studies of the epidermis of salmonids infected with *Ichthyobodo necator* (Henneguy, 1883). *Journal of Fish Diseases*, **4:** 113-125.

Poulin, R., Curtis, M. A. & Rau, M. E. (1990). Responses of the fish ectoparasites *Salmincola edwardsii* (Copepoda) to stimulation, and their implication for host-finding. *Parasitology*, **100:** 417-421.

Price, E. W. (1939). North American monogenetic trematodes III. The family Capsalidae (Capsaloidea). *Journal of The Washington Academy of Science,* **29:** 63- 92.

Prost, M. (1959). Badania and Wplywem Zasolenia Wody na faune Monogenoidea ryb. *Acta Parasitologica*, **7:** 615-630.

Prost, M. (1963). Investigations on the development and pathogenicity of *Dactylogyrus anchoratus* (Duj.1845) and *D. extensus* Mueller et v. Cleave, 1932 for breeding carps. *Acta Parasitologica*, **11:** 17-47.

Reda, E. S. A. (1988). An analysis of parasite fauna of bream, *Abramis brama* L. in Vistula near Warszawa in relation to the character of fish habit. II. Seasonal dynamics of infestation. *Acta Parasitologica*, **33:** 35-58.

Remley, L. W. (1942). Morphology and life history studies of *Microcotyle spinicirrus* McCallum 1918, a monogenetic trematode parasitic on the gills of *Aplodinotus grunniens*. *Transactions of the American Microscopical Society*, **61:** 141-155.

Riis-Vestergaard, J. (1982). Water and salt balance of halibut eggs and larvae (*Hippoglossus hippoglossus*). *Marine Biology*, **70:** 135-139.

Roff, D. E. (1982). Reproductive strategies in flatfish: A first synthesis. *Canadian Journal of Fisheries and Aquatic Sciences*, **39:** 1686-1698.

Rogers, W. P. (1962). *The nature of parasitism.* Academic press, New York.

Rodriguez, L. & George-Nascimento, M. (1996). The metazoan parasite fauna of the Patagonian toothfish *Dissostichus eleginoides* Smitt, 1898 (Pisces: Nototheniidae) of central Chile: Taxonomic, ecological and zoogeographic aspects. *Revista Chilena Historia Natural*, **69:** 21-33.

Rogers, W. A. & Gaines, J. L. (1975). Lesions of protozoan diseases in fish. In : *The Pathology of Fishes*. (Eds. Ribelin & Migaki) University of Wisconsin Press.

Rohde, K. (1976). Marine parasitology in Australia. *Search*, **7:** 477- 482.

Rohde, K. (1978). Latitudinal differences in host-specificity of marine Monogenea and Digenea. *Marine Biology,* **47:** 125-134.

Rohde, K. (1980). Comparative studies on microhabitats utilization by ectoparasites of some marine fishes from the North Sea and Papua New Guinea. *Zoologischer Anzeiger,* **204:** 27-64.

Rohde, K. (1981). Ultrastructure of the buccal organs and associated structures of *Zeuxapta seriolae* (Meserve, 1938) Price, 1962, and *Paramicrocotyloides reticularis* Rohde, 1978 (Monogenea, Polyopisthocotylea). *Zoologischer Anzeiger,* **206:** 279-291.

Roubal, F. R. (1994). Attachment of eggs by *Lamellodiscus acanthopagri* (Monogenea: Diplectanidae) to the gills of *Acanthopagrus australis* (Pisces: Sparidae), with evidence for auto-infection and postsettlement migration. *Canadian Journal of Zoology*, **72:** 87-95.

Roubal, F. R., Bullock, A. M., Robertson, D. A. & Roberts, R. J. (1987). Ultrastructural aspects of infestation by *Ichthyobodo necator* (Henneguy, 1883) on the skin and gills of the salmonids *Salmo salar* L. and *Salmo gairdneri* Richardson. *Journal of Fish Diseases*, **10:**181-192.

Schram, T. A. & Haug, T. (1988). Ectoparasites on the Atlantic halibut, *Hippoglossus hippoglossus* (L.), from northern Norway. Potential pests in halibut aquaculture. *Sarsia*, **73:** 213-227.

Scott, M. E. (1982). Reproductive potential of *Gyrodactylus bullatarudis* (Monogenea) on guppies (*Poecilia reticulata*). *Parasitology*, **85:** 217-236.
Scott, M. E. & Anderson R. M. (1984). The population dynamics of *Gyrodactylus bullatarudis* (Monogenea) within laboratory populations of the fish host *Poecilia reticulata*. *Parasitology*, **89:**159-194.

Scott, M. E. & Nokes, D. J. (1984). Temperature-dependent reproduction and survival of *Gyrodactylus bullatarudis* (Monogenea) on guppies (*Poecilia reticulata*). *Parasitology*, **89:** 221-227.

Shaharom-Harrison, F. (1983). *Monogenetic trematodes (Dactylogyridae: Ancyrocephalinae) on the gills of tilapia (a warm water cultured fish) with special reference to Cichlidogyrus sclerosus Paperna and Thurston 1969.* Ph.D. Thesis, University of Stirling.

Shaharom-Harrison, F. (1986). The reproductive biology of *Dactylogyrus nobilis* (Monogenea: Dactylogyridae) from the gills of big head carp (*Aristichthys nobilis*). In *Proceedings of the First Asian Fisheries Forum, Manila, Philippines* (Maclean, J. L., Dizon, L. B. & Hossillos eds.), pp. 265-268. The First Asian Fisheries Forum, Asian Fisheries Society, Manila, Philppines.

Skinner, R. H. (1982). The interrelation of water quality, gill parasites and gill pathology of some fishes from south Biscayne Bay, Florida. *Fishery Bulletin*, **80:** 269-272.

Stobo, W. T., Neilson, J. D.& Simpson, P.G. (1988). Movements of Atlantic halibut (*Hippoglossus hippoglossus*) in the Canadian North Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences*, **45:** 484-491.

Svendsen, Y. S. & Haug, T. (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.

Thomas, J. D. (1964). Studies on the growth of trout, *Salmo trutta* L. from four contrasting habitats. *Proceedings of the Zoological Society of London*, **142:** 459-509.

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

Tinsley, R. C. (1983). Ovoviviparity in platyhelminth life-cycles. *Parasitology*, **86:**161-196.

Tinsley, R.C. & Owen, R. W. (1975). Studies on the biology of *Protopolystoma xenopodis* (Monogenoidea): the oncomiracidium and life cycle. *Parasitology*, **71:** 445-463.

Urawa, S. & Kusakari, M. (1990). The survivability of the ectoparasitic flagellate *Ichthyobodo necator* on the chum salmon fry (*Oncorhynchus keta*) in seawater and comparison to *Ichthyobodo* sp. on Japanese flounder (*Paralichthys olivaceus*). *Journal of Parasitology*, **76:** 33-40.

Van Oosten, J. (1957). The skin and scales. In: *The Physiology of Fishes* vol. 1 (Ed. Brown) Academic press. New York.

Vedel – Tåning, Å. (1936). On the eggs and young stages of the halibut. *Meddelelser fra Kommisjonen for Danmarks Fiskeri-og Havundersøgelser, Serie Fiskeri*, **10:** 1-23.

Wagner, A. (1961). Papillae on three species of Schistosome cercariae. *Journal of Parasitology*, **47:** 614-618.

Wells, P.R. & Cone, D.K. (1990). Experimental studies on the effects *of Gyrodactylus colemanensis* and *G. salmonis* (Monogenea) on density of mucous cells in the epidermis of fry of *Oncorhynchus mykiss. Journal of Fish Biology*, **37:** 599-603.

Whitaker, D. J. & Kent, M. L. (1991). Myxosporean *Kudoa thyrsites*: A cause of soft flesh in farm-reared Atlantic salmon. *Journal of Aquatic Animal Health*, **3:** 291-294.

Whittington, I. D. (1987). A comparative study of the anatomy of the oncomiracidia of The hexabothriid monogeneans *Rajonchocotyle emarginata* and *Hexabothrium appendiculatum. Journal of the Marine Biological Association of the United Kingdom*, **67:** 757-772.

Whittington, I. D. (1990). The egg bundles of the monogenean *Dionchus remorae* and their attachment to the gills of the remora, *Echeneis naucrates. International Journal for Parasitology,* **20:** 45-49.

Whittington, I. D. & Kearn G. C. (1989). Rapid hatching induced by light intensity reduction in the polyopisthocotylean monogenean *Plectanocotyle gurnardi* from the gills of gurnards (Triglidae), with observations on the anatomy and behaviour of the oncomiracidium. *Journal of the Marine Biological Association of the United Kingdom,* **69:** 609-624.

Whittington, I. D. & Kearn, G. C. (1990). A comparative study of the anatomy and behaviour of the oncomiracidia of the related monogenean gill parasites *Kuhnia scombri, K. sprostonae* and *Grubea cochlear* from mackerel, *Scomber scombrus. Journal of the Marine Biological Association of the United Kingdom,* **70:** 21-32.

Whittington, I. D. & Kearn, G. C. (1993). A new species of skin-parasitic benedenine monogenean with a preference for the pelvic fins of its host, *Lutjanus carponotatus* (Perciformes: Lutjanidae) from the Great Barrier Reef. *Journal of Natural History*, **27:** 1-14.

Whittington, I. D., Kearn, G. C. and Beverly-Burton, M. (1994). *Benedenia rohdei* n. sp.(Monogenea: Capsalidae) from the gills of *Lutjanus carponotatus* (Perciformes: Lutjanidae) from the Great Barrier Reef, Queensland, Australia, with a description of the oncomiracidium. *Systematic Parasitology*, **28:** 5-13.

Wilkins, N.P. & Janscar, S. (1979). Temporal variations in the skin of Atlantic salmon, *Salmo salar* L. *Journal of Fish Biology*, **15:** 299-307.

Williams, I. C., Ellis, C. & Spaull, V. W. (1973). The structure and mode of action of the posterior adhesive organ of *Pseudobenedenia nototheniae* Johnston, 1931 (Monogenea: Capsaloidea). *Parasitology*, **66:** 473-485.

Winch, J. (1983). The biology of *Atrispinum labracis* n. comb. (Monogenea) on the gills of the bass, *Dicentrarchus labrax. Journal of the Marine Biological Association of the United Kingdom*, **63:** 915-927.

Wootten, R., Smith, J. W. & Needham, E. A. (1982). Aspects of the biology of the parasitic copepods *Lepeophtheirus salmonis* and *Caligus elongatus* on farmed salmonids, and their treatment. *Proceedings of the Royal Society of Edinburgh*, **81B:** 185-197.

Yamaguti, S. (1963). *Systema Helminthum*, Volume 4. *Monogenea and Aspidocotylea.* New York & London: Interscience Publishers.