An experimental study of the biology of *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoda: Pseudophyllidea).

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy.

by

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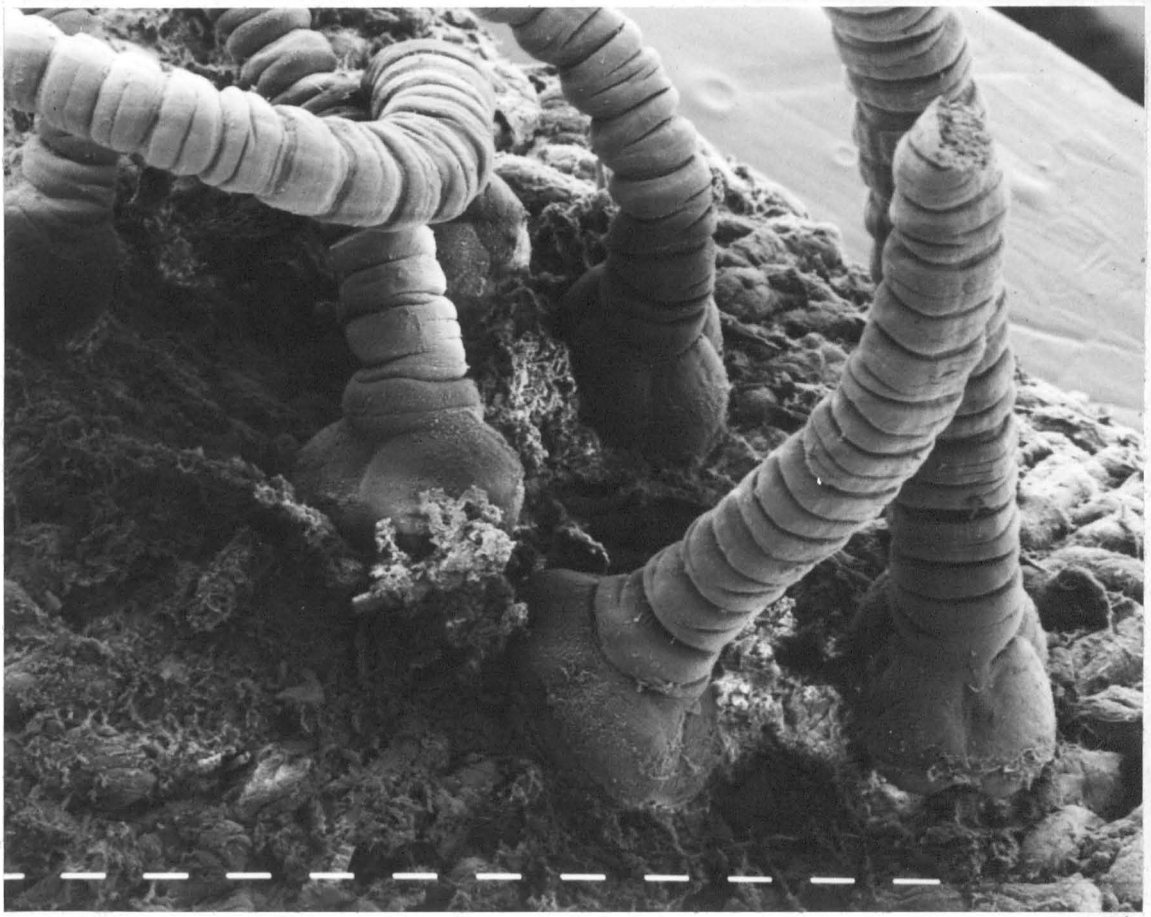

Bothriocephalus acheilognathi in a Cyprinus carpio intestine.

Marker = 100um.

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Acknowledgements.

I would like to thank Dr J. C. Chubb for supervising this research, and for much help and advice; Dr R. G. Pearson, in whose department the work was carried out; and the National Environment Research Council for the provision of a grant.

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Abstract.

Bothriocephalus specimens from a single 0+ Ctenopharyngodon idella were fixed using three different techniques, and the scolex morphology was examined using scanning electron microscopy. The scolices were compared with the five species of Bothriocephalus and one species of Schyzocotyle previousley recorded from cyprinid fishes. The taxonomic history of the Bothriocephalus species parasitising cyprinid fishes was reviewed. It was concluded that the six species were synonymous, and that priority should be given to the name B. acheilognathi Yamaguti, 1934; with B. opsariichthydis Yamaguti, 1934, B. fluviatilis Yamaguti, 1952, B. gowkongensis Yeh, 1955, B. phoxini Molnár, 1968 and Schyzocotyle fluviatilis as synonyms.

A critical examination of the three species of Bothriocephalus reported from cyprinid fishes in Africa is presented. The characters examined did not enable the species to be differentiated, therefore it is suggested that B. aegyptiacus Ryšavý and Moravec, 1975 and B. kivuensis Baer and Fain, 1958 are synonyms of B. acheilognathi.

Laboratory experiments revealed that establishment, development and mortality of each stage of the life cycle of B. acheilognathi was temperature dependant. In addition the infectivity of the coracidium and procercoid was age dependant. Development of the procercoid was influenced by the age of the coracidium when ingested by the copepod intermediate host, and the infectivity of the procercoid was inversely related to the density of the procercoids within the intermediate host.

Estimation of the time taken for the completion of the life

cycle at temperatures ranging from 18-30 ^{o}C showed it to be inversely related to temperature. The size of the Cyprinus carpio host influenced the ability of the plerocercoids to establish, with no establishment occuring in C. carpio greater than 63 mm fork length.

The strobila of B. acheilognathi was observed to undergo a cyclical pattern of contraction and relaxation within the host intestine. The frequency of this behaviour was associated with the feeding behaviour of the C. carpio host. No significant variation in scolex position was observed. Egg release by B. acheilognathi also followed a cyclical pattern, with peak egg production occurring shortly after the entry of food into the intestine.

Praziquantel (Droncit, Bayer) was found to be a suitable anthelmintic for the control of B. acheilognathi. In vitro studies indicated that praziquantel concentrations of 0.1ug per ml 0.9% saline and above, caused muscular contraction and severe tegumental damage. Exposure to drug concentrations of 100 ug praziquantel per ml saline for 24 hours was not lethal to the worms. Praziquantel had no ovicidal activity.

Praziquantel was used to eliminate B. acheilognathi from a batch of 30,000 newly imported Ctenopharyngodon idella. Dose rates of 105 and 125 mg praziquantel kg bodyweight^{-1} administered as a medicated feed over a three day period were used. The fish were raised to 24^{o}C in an indoor recirculating unit prior to treatment. The ponds containing infected fish were drained and limed to kill B. acheilognathi ova and copepods.

General Introduction.

The rapid increase in angling, aquaculture and ornamental fish keeping in recent years has resulted in considerable interest being focussed upon the diseases and parasites of fishes. Much of this research has centred around the ecology and pathology of these parasites, with a view to establishing which species are potentially pathogenic, so that effective control measures can be developed and the introduction of these species into new habitats prevented.

Fish parasites or disease organisms may affect their hosts in a number of ways, causing alterations to their behaviour and blood chemistry, histopathological effects, reduced rates of growth and fecundity, and death (Bauer et al., 1969; Dogiel et al., 1958; Reichenbach-Klinke and Elkan, 1965; Andrews, 1985). Relatively few of the large number of fish diseases and parasites which have been described, are reported to be seriously pathogenic to their fish hosts (see reviews by Meyer, 1966; Roberts, 1978; Sarig, 1971, for examples). One such parasite is the pseudophyllidean cestode Bothriocephalus acheilognathi Yamaguti, 1934, a parasite of fishes of the family Cyprinidae.

B. acheilognathi is a particular threat to underyearling cyprinids under culture conditions, where up to 100% of the fish can be infected , with as many as 467 worms per fish (eg Liao and Shih, 1956; Korting, 1974). At infestation levels of over 15 worms per fish pathological effects become apparent resulting, in extreme cases, in death (Par, 1978).

Geographical distribution.

Bothriocephalus acheilognathi was first described by Yamaguti in 1934 from the cyprinid fish Acheilognathus rhombea Temmink and Schlegal, from Lake Ogura, Japan. It is endemic to China, Japan and the River Amur. As a result of the exportation of Cyprinus carpio L. for food and sport, and Ctenopharyngodon idella Val. for weed control, B. acheilognathi spread rapidly to Austria (Otte et al., 1972), Bulgaria (Petkov, 1972), Ceylon (Fernando and Furtardo, 1963), Czechoslovakia (Par and Parova, 1976), Hungary (Molnár, 1968), New Zealand (Edwards and Hine, 1974), Poland (Panczyk and Zelazny, 1974), Rumania (Radulescu and Georgescu, 1962), Ukraine (Malevitskaya, 1958)U.S.A. (Hoffman, 1976), U.S.S.R. (Bauer and Hoffman, 1976), West Germany (Körting, 1974) and Yugoslavia (Kezeik et al., 1975) (see Fig. 1).

In the late 1960's and early 1970's B. acheilognathi was introduced into the British Isles in a small number of C. idella, imported for experimental work by the Ministry of Agriculture, Fisheries and Food (Stott, 1977). Since that time B. acheilognathi has been imported on a number of occasions (eg Andrews et al., 1981; Pool et al., 1984). In each instance a restriction on the movement of the infected fish, imposed by the relevant Water Authority under section 30 of the 1975 Salmon and Freshwater Fisheries Act, and/or the administration of suitable anthelmintics, has prevented the parasite becoming established in the British Isles.

It is not clear whether B. acheilognathi can persist in natural conditions in the British Isles. In at least one incident where infected Cyprinus carpio were released into a natural water, and anthelmintic treatment was not feasible, the parasite population

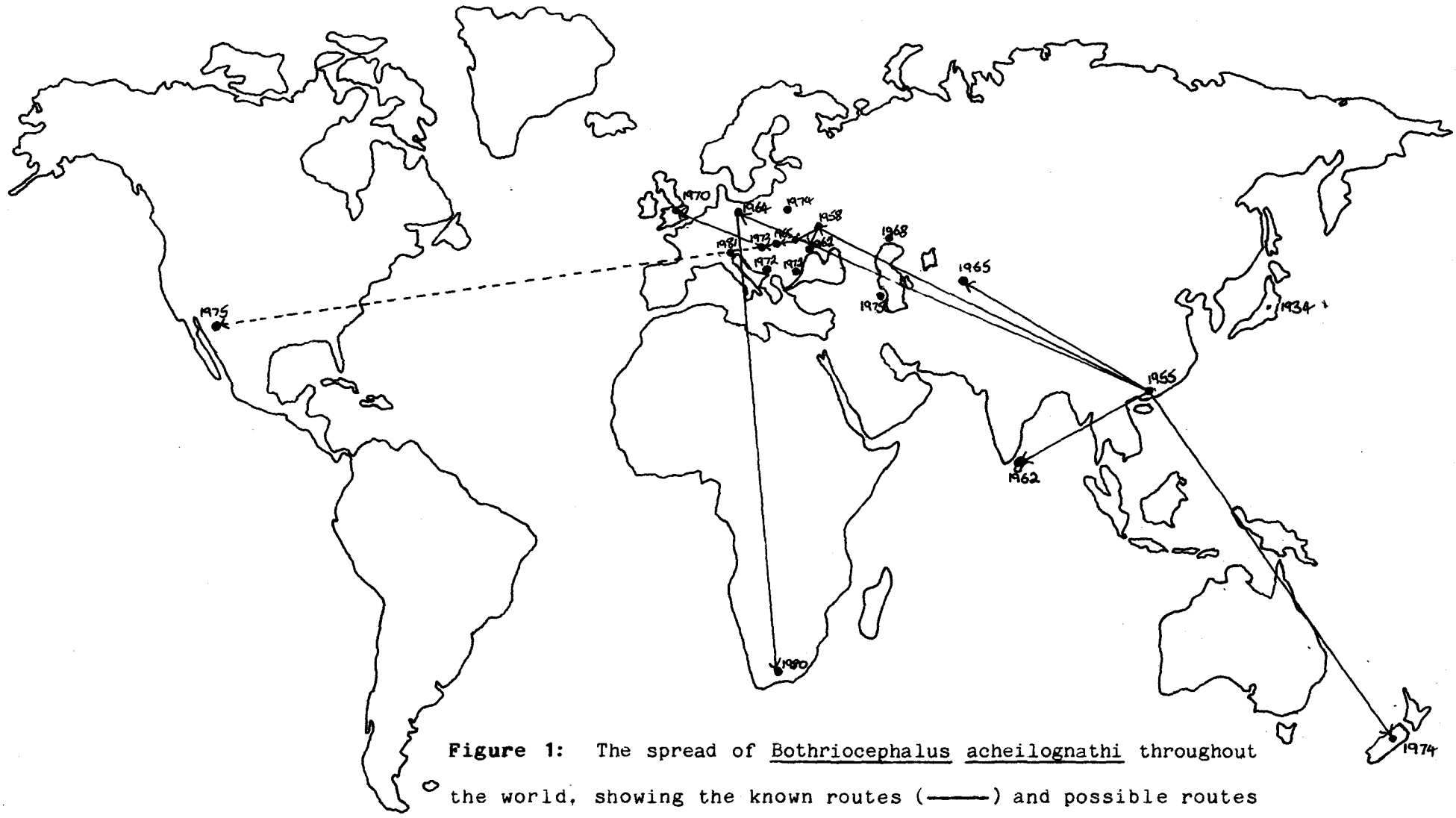


Figure 1: The spread of Bothriocephalus acheilognathi throughout the world, showing the known routes (——) and possible routes (- - - -) of transport.

became extinct over a period of two years. Whereas in other incidents, mainly in the south of England, large numbers of C. carpiofry raised in the British Isles have become infected with B. acheilognathi, probably through contact with previously infected fishes.

The research presented in this thesis falls conveniently into three parts. In part I the taxonomic position of Bothriocephalus acheilognathi was examined in relation to the Bothriocephalus species described from Europe, U.S.S.R., and South East Asia (Chapter 1), and the species described from Africa (Chapter 2).

Part II describes the experimental work. One of the aims of this study was to establish if B. acheilognathi could survive and successfully complete it s' life cycle in the British Isles. The data collected is presented and discussed in chapter 3. While collecting B. acheilognathi for this study it was noted that the position occupied within the intestine varied depending on the period of time between feeding the fish host and dissection. In chapter 4 this was examined in greater detail in order to find the cause of this behaviour.

A large number of anthelmintics have been used to control B. acheilognathi, although few are completely successful at eliminating the parasite. In part III, in vitro experiments (Chapter 5) and large scale field trials (Chapter 6) are described using praziquantel (Droncit, Bayer).

The life cycle of Bothriocephalus acheilognathi.

The developmental cycle of Bothriocephalus acheilognathi is typical of most of the Bothriocephalidae, and is shown diagramatically in Fig. 2. It was first described by Yeh (1955) who noted that unidentified copepods acted as an intermediate host. In the following year Liao and Shih (1956) published an extensive study on the life cycle and biology. Since that time a number of studies have concentrated on all or certain aspects of the life cycle (eg Hoffman, 1976; Korting, 1975; Mitchell and Hoffman, 1980; Nakajima and Egusa, 1976; Pimenova, 1971, 1973; Urazbaev and Allaniyazova, 1977).

More extensive and detailed studies have been made on closely related pseudopyllidean cestodes: eg Clarke (1954) (Schistocephalus solidus); Dubinina (1966) (Ligulidae) and Kuperman (1973) (Triaenophorus species). Much of this work can be related to the development of B. acheilognathi.

I. **The Egg.**

The operculate eggs of B. acheilognathi (Fig. 3a and b) are released from the mature proglottides of the adult worm, through the ventrally placed uterine pore (Fig. 12) into the host intestine. They pass into the water during evacuation of the host faeces and sink to the bottom.

The embryonic development of B. acheilognathi within the egg has been briefly described by Liao and Shih (1956) and Körting (1975). A more detailed description has been provided for other pseudophyllidean cestodes, eg Dibothriocephalus latus (= Diphyllobothrium latum) (Wardle and McLeod, 1952), Triaenophorus

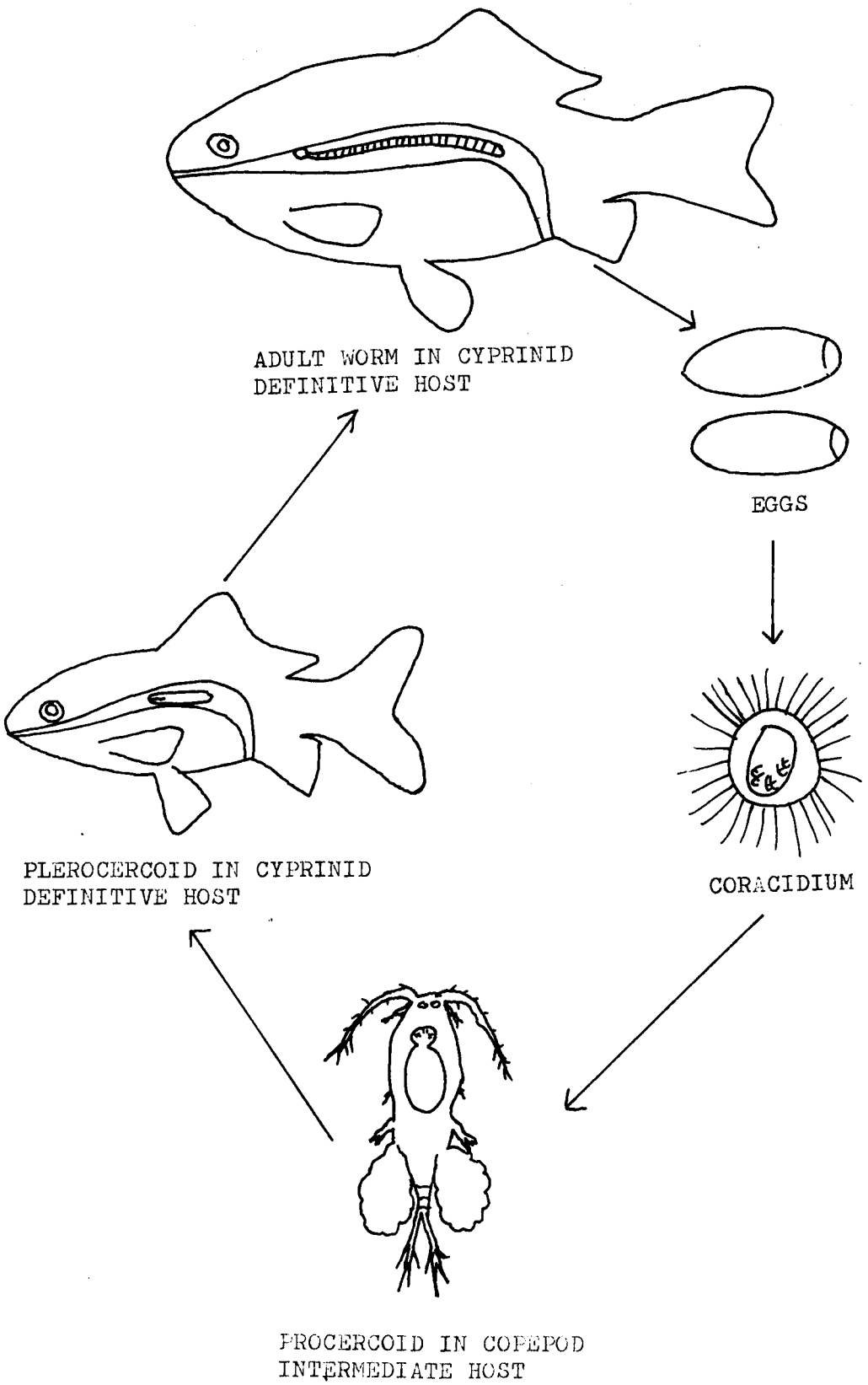
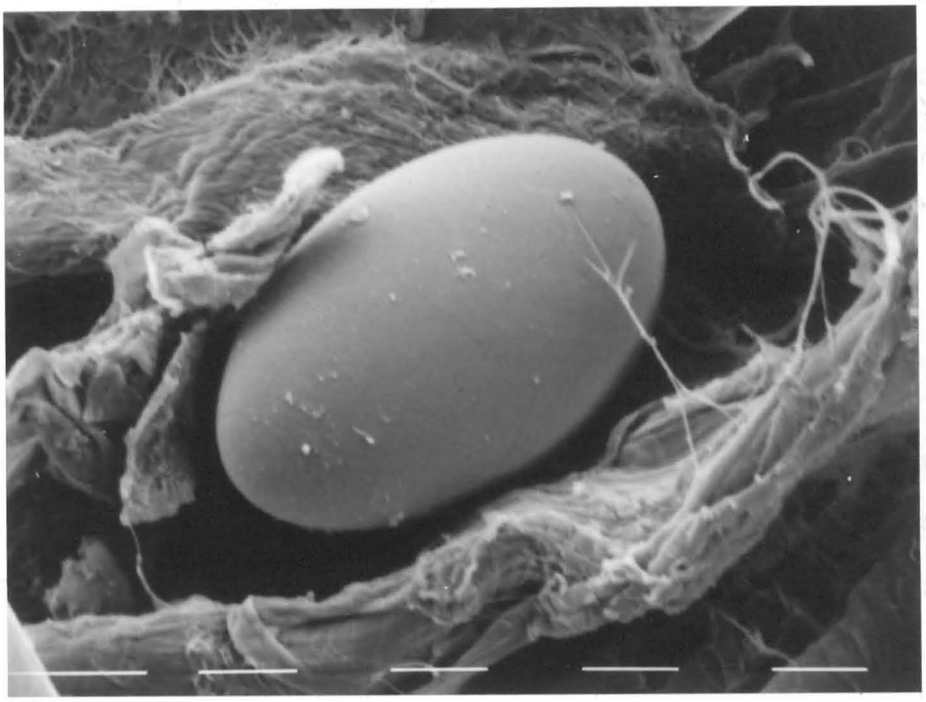


Figure 2: The life cycle of Bothriocephalus acheilognathi.



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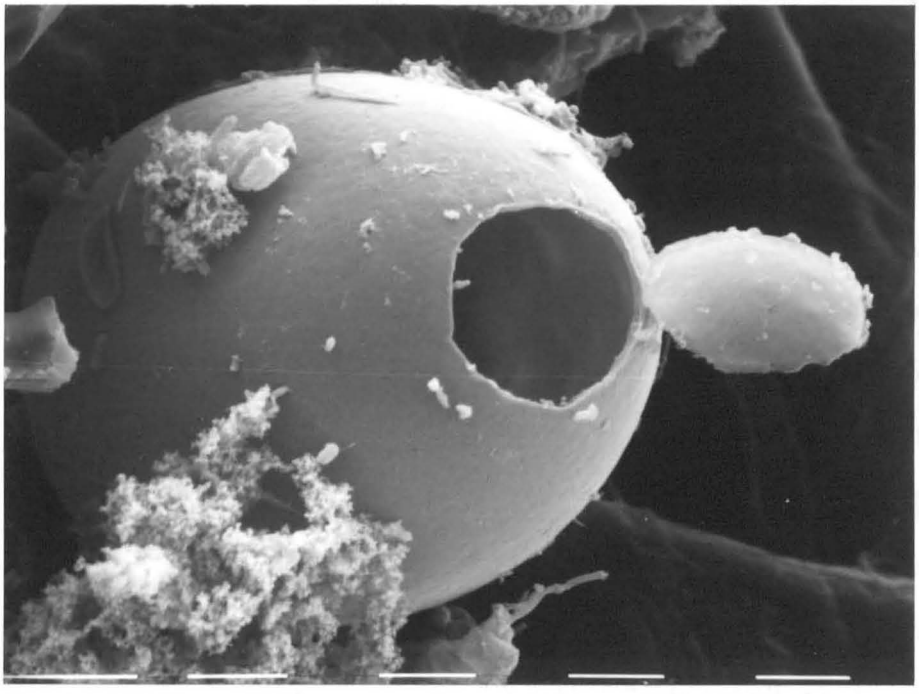


b

Figure 3: The operculate egg of B. acheilognathi.

a. Unhatched. Marker = 10 μm. b. Hatched. Marker = 5.6 μm.

nodulosus (Kuperman, 1973) and Ligulidae (Dubinina, 1966). It is probable that embryonic development is similar in all Pseudophyllidea (Schauinsland, 1885, cited Wardle and McLeod, 1952).

II. **Hatching of the egg.**

As the onc osphere develops within the egg it begins to move. Shortly before hatching these movements are quite vigorous and appear to cause the operculum to open, allowing the coracidium to emerge (Fig. 4). In the opinion of Vogel (1929) these movements together with an increased pressure within the egg caused the operculum of Diphyllobothrium latum eggs to open.

As the eggs of the pseudophyllidean cestodes and the digenean trematodes are very similar (Smyth and Clegg, 1959) the hatching process might be expected to be similar. In Fasciola hepatica the miracidium releases an enzyme which digests the substance binding the operculum to the shell. (Rowan, 1956). Production of this enzyme was stimulated by light. A number of authors have noted that light is required for the hatching of certain members of the Pseudo phyllidea: eg D. latum (Guttowa, 1961; Grabiec et al., 1963) and D. norvegicum (Vik, 1957) (= D. dendriticum). Dubinina (1966) reported that all members of the Pseudophyllidea required light to enable the egg to hatch. However experiments performed with B. acheilognathi indicated that egg hatching occurs normally in the absence of light (Nakajima and Egusa, 1976; personal observations).

III. **Coracidium.**

Immediately after emerging from the egg the coracidium is an

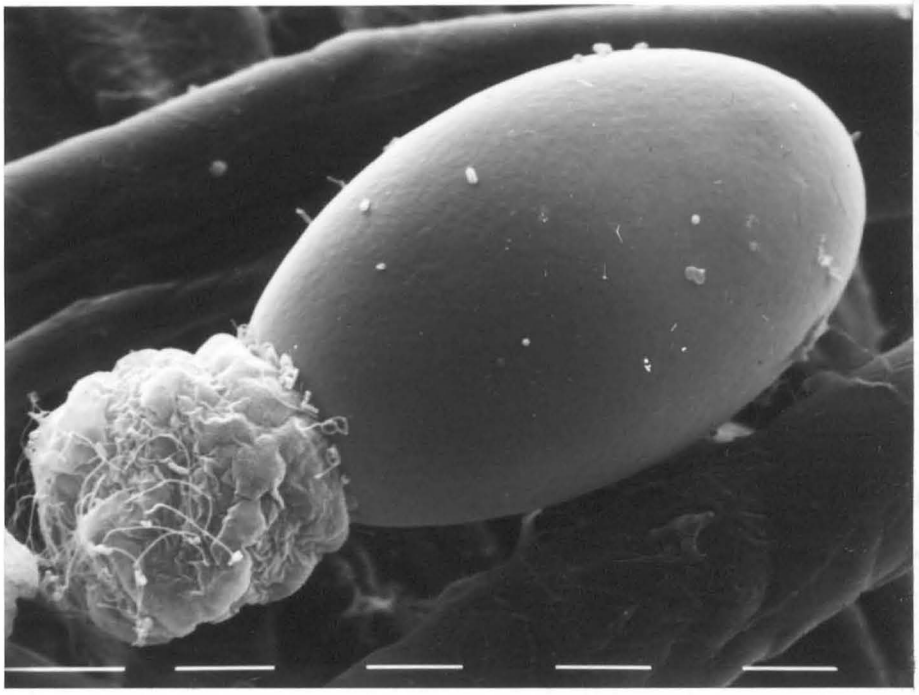


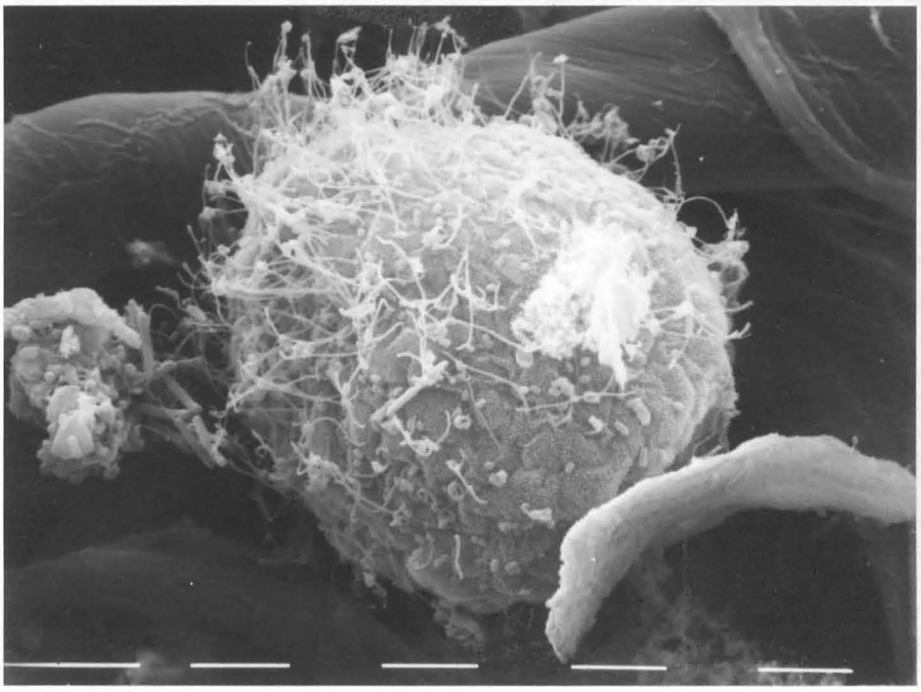
Figure 4: Emergence of the coracidium through the operculate opening of the egg. Marker = 7.14um.

oval shape, with movement being in the form of a flexing of the body. After only a few seconds it becomes a spherical shape, and the cilia begin beating with a definite rhythm (Fig. 5a and b). At this stage the coracidium measures 0.045 - 0.050 mm in diameter. The surface has a regular pattern of protruberences which have not been reported on the coracidia of other pseudophyllidean cestodes. The function of these structures is not known.

As a result of water absorption due to osmosis, the coracidium begins to swell up to a diameter of 0.08 mm (Körting, 1975) (Fig. 6a). Associated with this swelling is the curling and fragmenting of the cilia (Fig. 6b). Thomas (1937) noted that in B. rarus the ciliary action was affected by the salinity of the surrounding medium, and that it could be 'stopped and started at will' by altering the salt concentration of the medium.

When first hatched the onchosphere is located in the centre of the coracidium, being surrounded by a layer of bubble-like chambers filled with a transparent solution. These chambers swell greatly in the water, displacing the onchosphere to one of the poles. Contained in these chambers are numerous phospholipid and polysaccharide granules which act as an energy scource for the beating of the cilia (Kuperman, 1973; Grabiec et al., 1962,1965).

The action of the cilia propels the coracidium forwards, and also causes it to rotate about it's longitudinal axis. Initially it moves towards the surface of the water where it is more likely to come into contact with the copepod intermediate host (Kuperman, 1973). In other pseudophyllidean species this has been shown to be a positive phototaxic response (Dubinina, 1966; Kuperman, 1973; Vogel, 1929). As the coracidium ages, the jerkyswimming action gives way to an oscillatory movement. Eventually this rocking stops



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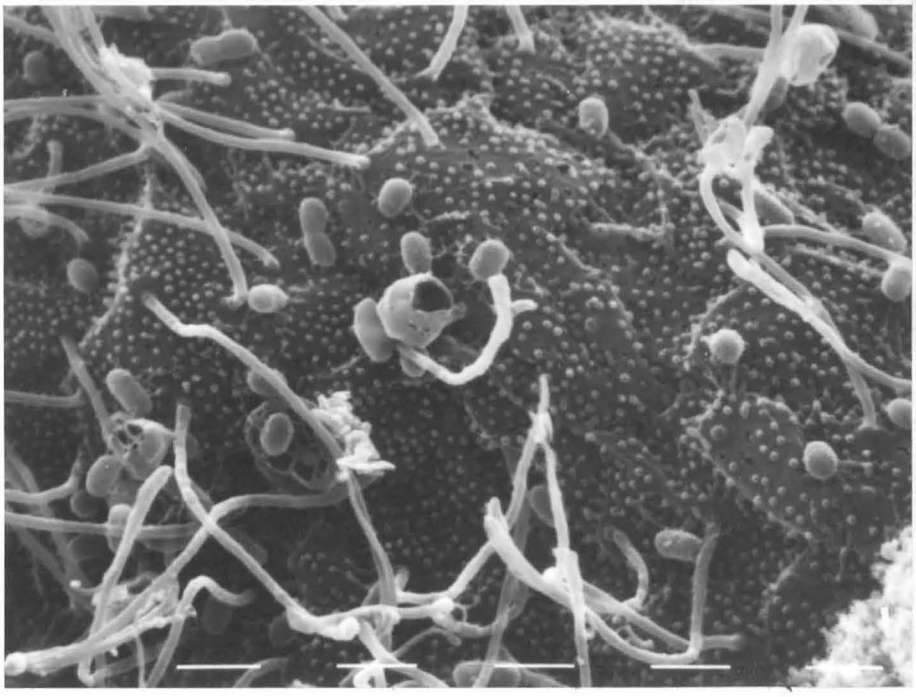

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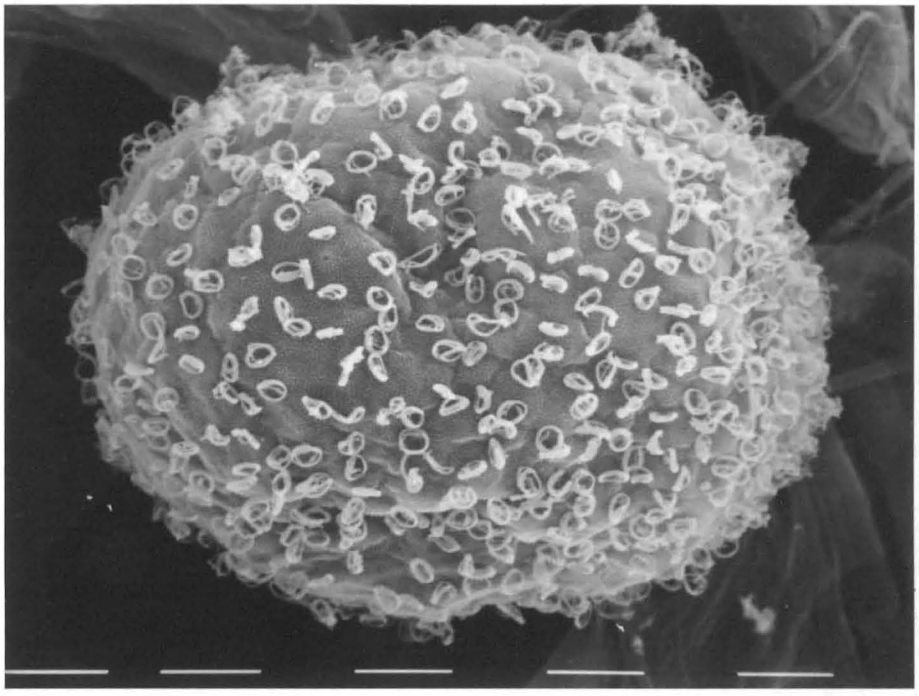
Figure 5: Coracidium soon after emerging from the egg. Note uncoiled cilia and surface protruberances.

a. Marker = 4.9um. b. Marker = 1um.



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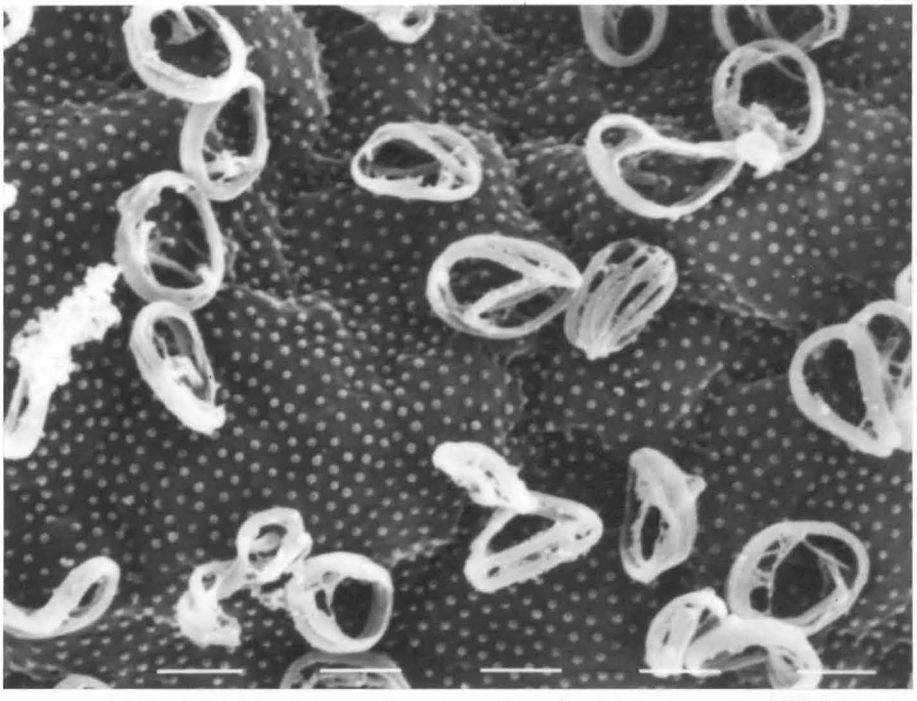

b

Figure 6: Swollen coracidium 5 hours after hatching. Note coiled and fragmented cilia.

a. Marker = 4.9um. b. Marker = 1um.

and the coracidium sinks to the bottom, where the outer envelope splits, releasing the onchosphere, which rapidly dies.

IV. **Procercoid.** (Fig 7).

A wide range of cyclopoid copepods have been recorded as intermediate hosts of B. acheilognathi. Cyclopoid copepods are predatory (Fryer, 1957). They catch the coracidia when they collide with them (most species) or they actively seek out and attack them.

The sequence of events from the consumption of the coracidium to it's establishment in the copepod haemocoel have not been observed for B. acheilognathi. However, it is likely to be similar to that described for the other Pseudophyllidea. Dubinina (1966) observed that the onchosphere of members of the Ligulidae was separated from the outer envelope of the coracidium by the action of the chitonous structures in the oral cavity of the copepod, or by the muscular action of the pharynx.

Despite the penetration by up to 25 onchospheres the copepod intestine wall remains intact. In the onchospheres of representatives of the order Cyclophyllidea (Reid, 1946, 1948; Silverman and Maneely, 1955) and the cercariae of the Trematoda (Levine et al., 1948; Evans, 1953) the rapid penetration of the intestinal wall occurs using hooks (Fig. 8) and the secretion of a cytolitic enzyme (possibly hyaluronidase) which temporarily breaks the intracellular bonds of the walls of the host intestine. Although there is no direct evidence for the secretion of a similar enzyme in members of the Pseudophyllidea, flame cells and excretory organs have been observed in the onchospheres of a number of species, eg B. rarus (Thomas, 1937); D. decipens and D. erinacei (Li, 1929); D. latum (Thomas, 1937) and members of the Ligulidae

Figure 7: Procercoid with well developed cercomer.

Marker = 6.25um.

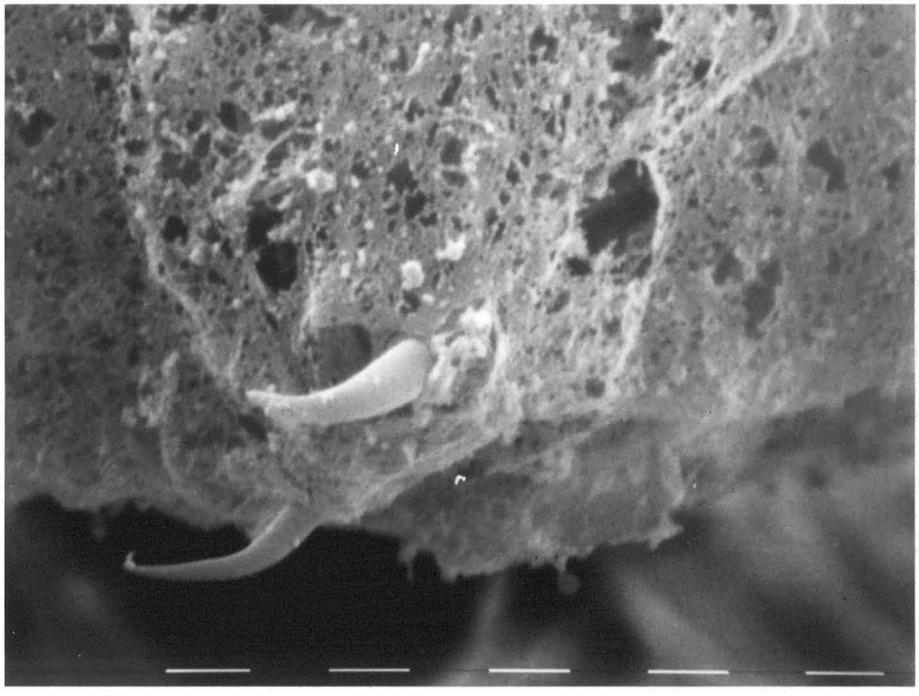

Figure 8: Embryonic hooks on procercoid cercomer. Marker = 1um.

(Dubinina, 1966).

The process of development of the onchosphere into the procercoid has been followed in detail for B. acheilognathi (as B. gowkongensis) by Liao and Shih (1956). This development has also been extensively studied in other pseudophyllidean species, eg D. latum (Vogel, 1930; Guttowa, 1956, 1961; Morozova, 1955); T,nodulosus (Kuperman, 1973); S. solidus (Clarke, 1954; Dubinina, 1966) and members of the Ligulidae (Dubinina, 1966).

Within the copepod haemocoel most of the procercoids developed in the posterior half of the cephalothorax or in the thorax, with the body of the procercoid orientated along the length of the copepod. Soon after penetrating into the body cavity the onchosphere became inactive. As the procercoid, grew occasional contortions and flexing of the body were observed. This activity increased during the formation of the cercoma and the bothria until the consumption of the mature procercoid by a fish host.

In a number of instances procercoids were observed in the antenna of Cyclops agilis (s. str.). These procercoids grew to a maximum size of 1 mm before degenerating and dying. Dubinina (1966) observed this phenomenon in the procercoids of members of the Ligulidae. She believed it to be caused by the onchospheres not loosing their activity when they had penetrated the copepod gut wall.

When five or more procercoids were found in the haemocoel of a single copepod, their development was observed to be uneven; with the size of the procercoids and their establishment in the fish definitive host being influenced by the density of infection. This has also been recorded in a number of pseudophyllidean cestodes, for example in D. latum (Guttowa, 1956, 1963 ; Halvorsen, 1966); T.

nodulosus (Michajlow, 1953); B. acheilognathi (Körting, 1975) and members of the Ligulidae (Dubinina, 1966). These authors believed that the availability of nutrients and the physical space were responsible for the uneven development. Liao and Shih (1956) working with B. gowkongensis concluded that the density of infection in the copepod was not an important influence on the growth and maturation of the procercoids.

The behaviour of heavily infected copepods was observed to be different. Their movements were sluggish and they were always located on the bottom of the tanks. This behaviour, which is caused by the pressure of the procercoids on the brain and eye, may make the copepods more succeptible to predation (Dubinina, 1966).

V. Plerocercoid.

When a copepod containing an infective procercoid (with bothria) is consumed by a suitable definitive host (Table 1) the parasite can develop into the plerocercoid stage (Fig. 9).

The procercoid is released from the copepod by the mechanical action of the pharangeal teeth (in cyprinids) and by the action of the intestinal digestive secre tions (Dubinina, 1966). Once inside the intestine, the procercoid attaches to the villi of the intestine wall by means of the bothria. B. acheilognathi has a very low specificity and has been recorded from over 50 species of freshwater fishes (table 1), although in several it does not develop to maturity, eg Platichthys flesus and Tilapia zilli.

It is probable that the procercoids become attached to the intestine throughout it s' length depending on their individual ability to attach to the intestine wall. Within one day however,

Table 1: The fish species recorded as definitive hosts for *Bothriocephalus acheilognathi*.

Species	**Authority**
Abramis brama	Bachinskii, 1965
Acheilognathus rhombea	Yamaguti, 1934
Alburnoides bipunctatus eichwaldi	Danijarov, 1975
Aristichthys nobilis	Laptev, 1980
Aspiolucius esocinus	Babaev, 1965
Aspius aspius	Babaev, 1965
Barbus altianilis altianilis	Baer and Fain, 1968
B. barbus	Buza *et al.*, 1970
B. brachycephalus	Babaev, 1965
B. bynni	Rysavy and Moravec, 1975
B. capito canocephalus	Babaev, 1965
B. kimberleyensis	Brandt *et al.*, 1981
B. mattozi	Brandt *et al.*, 1981
B. trimaculatus	Van As *et al.*, 1981
Cirrhina chinensis	Liao and Shih, 1956
Cobitis aurata bulgarica	Kakacheva-Avramova, 1977
Crassius auratus	Zitnan, 1983
C. crassius	Zitnan, 1983
Ctenopharyngodon idella	Yeh, 1955
Cyprinus carpio	Bachinskii, 1965
Elopichthys bambusa	Liao and Shih, 1956
Esox lucius	Zitnan, 1983
Gambusia affinis	Babaev, 1965
Gnathopogon elongatus suwae	Yamaguti, 1934

Gobio gobio	Bauer et al., 1969
Gymnocephalus schraester	Kakacheva-Avramova, 1977
Hypophthalmichthys molitrix	Liao and Shih, 1956
H. nobilis	Buza et al., 1970
Ictalurus punctatus	Granath and Esch, 1983
Leuciscus idus	Bachinskii, 1965
Luciobrama macrocephalus	Liao and Shih, 1956
Lucioperca lucioperca	Astakova et al., 1968
Mylopharyngodon aethiops	Liao and Shih, 1956
Nemachilus labiatus	Iksanov et al., 1976
N. stauchi	Iksanov et al., 1976
Netemigonus crysoleucas	Hoffman, 1976
Opsariichthys uncirostris	Yamaguti, 1934
Pelecus cuttratus	Babaev, 1965
Perca fluviatilis	Osmanov, 1971
Phoxinus phoxinus	Molnar, 1968
Pimphales promelas	Hoffman, 1976
Platichthys flesus	Personal observations
Pseudaspius leptocephalus	Akhmerov, 1960
Ptychocheilus lucius	Hoffman, 1976
Punutis sarana	Fernando and Fertardo, 1963
Rutilus rutilus	Babaev, 1965
Scardinius erythrophthalmus	Buza et al., 1970
Siluris glanis	Babaev, 1965
Tilapia zilli	Personal observations
Varichorinus capoeta	Babaev, 1965
Vimba vimba	Bauer et al., 1969

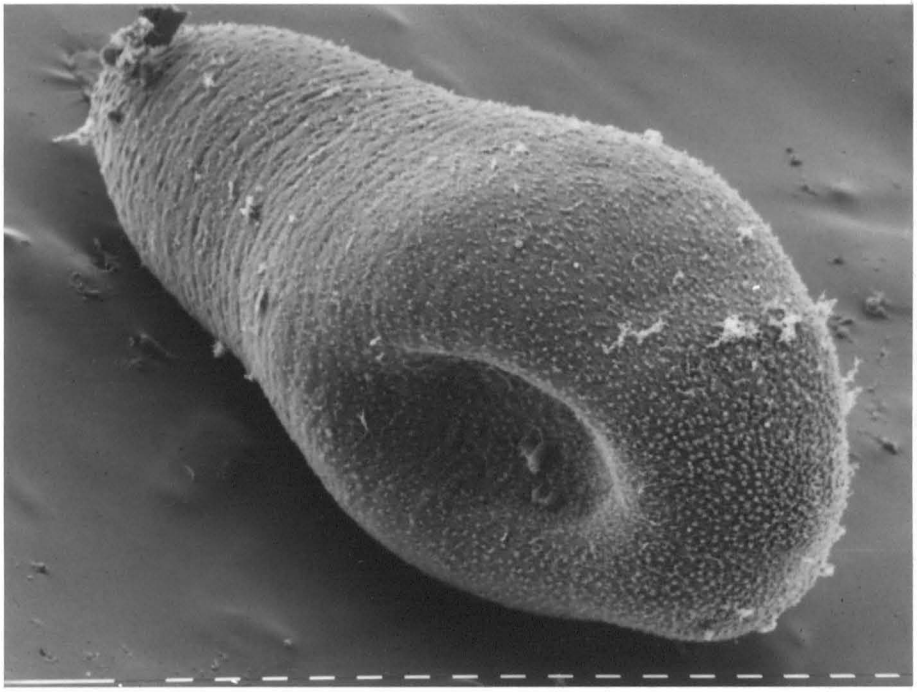

Figure 9: Plerocercoid soon after entering definitive host. Marker = 7.7um.

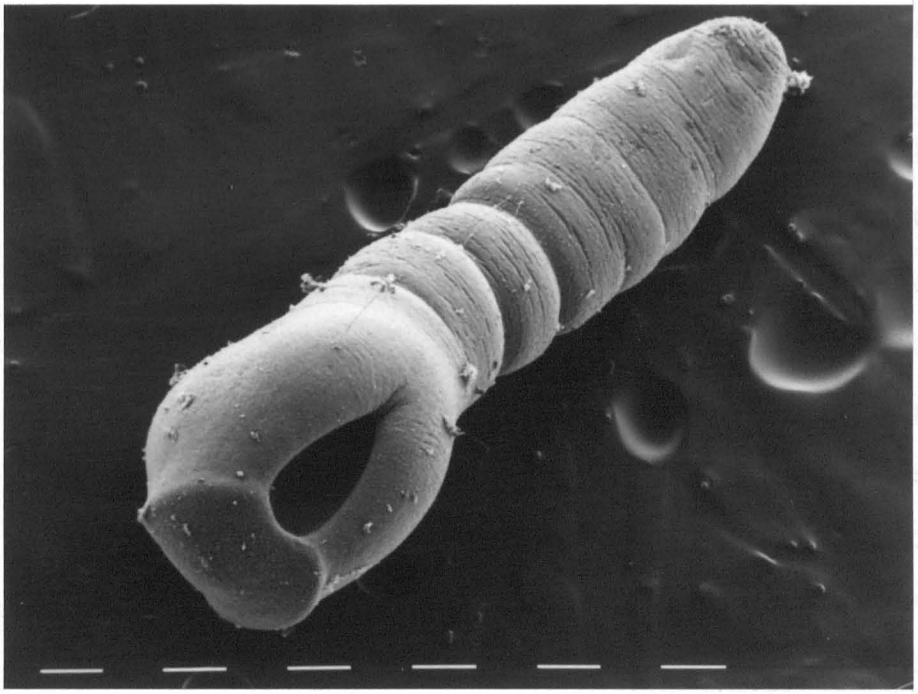

Figure 10: Early adult worm, 30 days after entering definitive host (at 18 ^{o}C). Marker = 5.9um.

all of the plerocercoids were located in the region 10 to 20 percent of the way along the intestine (0% = mouth, 100% = anus). This behaviour has been extensively studied in the hymenolepid cestodes, eg Hymenolepis diminuta (Chandler, 1939; Chappel et al., 1971) and H. microstoma (Cooreman and DeRyke, 1972).

The plerocercoid stage of the life cycle lasts for a very short time, with the parasite being described as an ad ult worm upon the formation of the first proglottid (Fig. 10).

VI **Adult.**

B. acheilognathi is typical of most cestodes in that it is hermaphroditic, with each mature proglottid containing both male and female genitalia (see Yamaguti, 1934; Yeh, 1955; Molnár and Murai, 1973 for a detailed description).

The male and female copulatory organs are located in the dorsally situated genital atrium (Fig. 11). The process of copulation and fertilisation has not been studied in B. acheilognathi. Viable eggs are released from single worm infections indicating that self-insemination must occur. However, it is probable that cross insemination is also important. (as described for H. diminuta (Nollen, 1975)). There is a sparcity of information regarding the copulatory methods of cestodes. Copulation involving the insertion of the cirrus of one worm into the vagina of another has been recorded for H. serula (Cox et al., 1956) and Acanthobothrium quadripartium (Williams and McVicar, 1968). It has also been suggested that the cirrus emits semen into the fluid environment, allowing it to migrate into the vagina (Wardle and McLeod, 1952).

When fertilised, the eggs collect within the uterus of each

proglottide before being expelled through the ventrally situated uterine pore (Fig. 12).

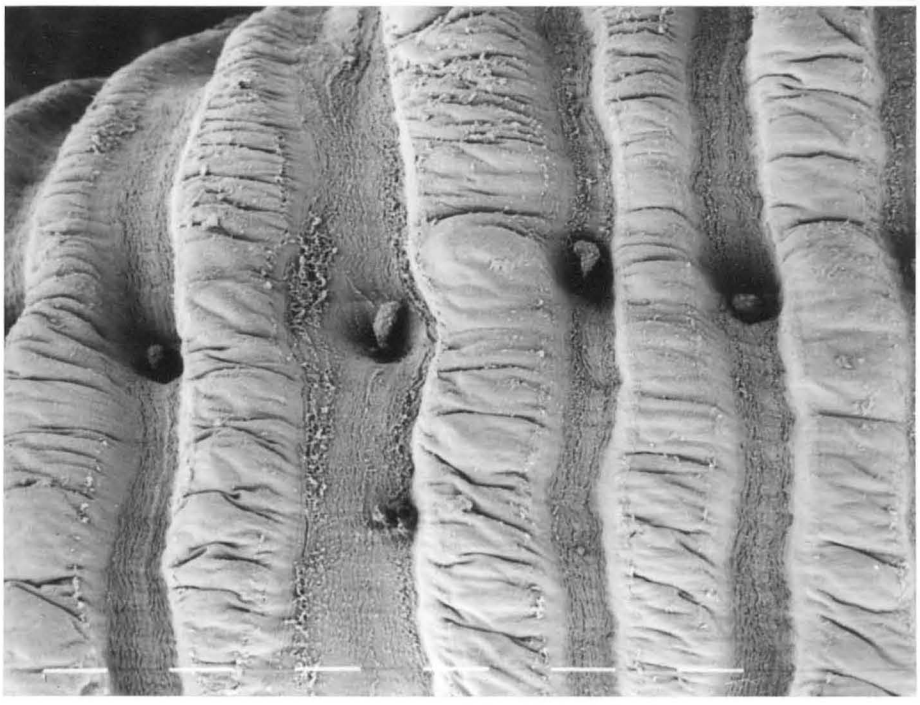

Figure 11: Genital atrium on dorsal surface of mature proglottides showing everted cirrus. Marker = 100um.

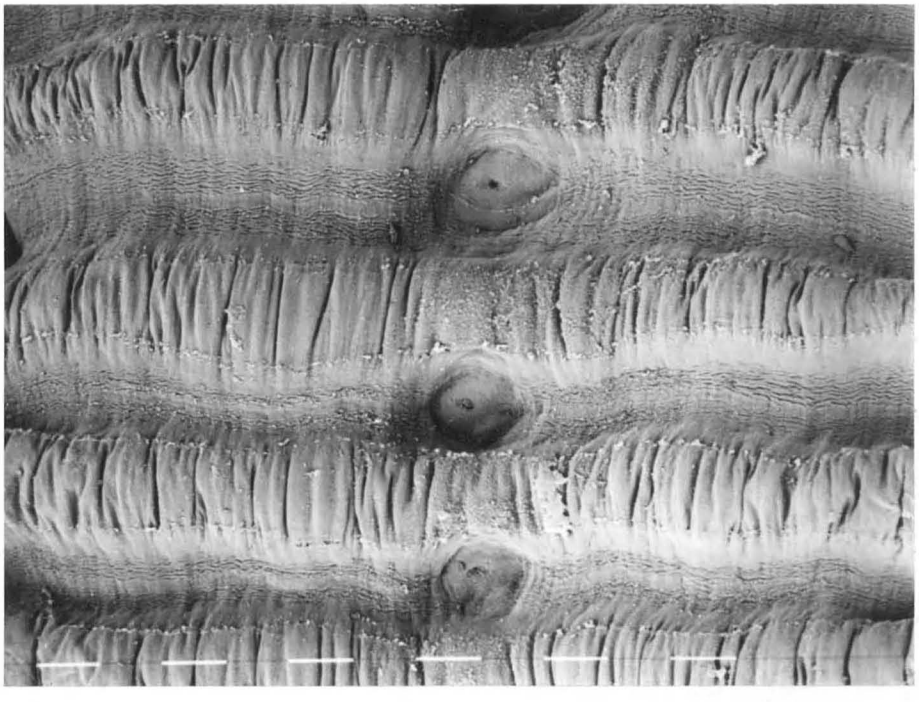

Figure 12: Uterine pore on ventral surface of mature proglottides. Marker = 100um.

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Part I

The taxonomic status of the *Bothriocephalus* species parasitising cyprinid fishes

Introduction

The zoological classification of the tapeworms has been subject to numerous alterations and additions since the first larval fish tapeworms were described and figured by Redi in 1684 (Wardle and Macleod, 1952). Foundations for the present systematics were laid down by Carus (1863) based largely on the morphological characteristics of the scolex. Attempts to formulate a definitive classification based on this have resulted in considerable disagreement and confusion between leading authorities (Wardle and Mcleod, 1952; Stunkard, 1983).

The generally accepted classification adopted by Wardle and Mcleod (1952) allows the five to six thousand known tapeworm species to be divided into two classes, the Cestoda and Cestodaria, which together are usually subdivided into 13 orders. Using this scheme the genus Bothriocephalus can be classified as follows:

Phylum	Platyhelminthes	Carus, 1863
Class	Cestoda	Monticelli, 1892
Order	Pseudophyllidea	Carus, 1863
Family	Bothriocephalidae	Blanchard, 1849
Genus	Bothriocephalus	Rudolphi, 1808

Ten species of Bothriocephalus have been described from cyprinid fishes, namely: B. granularis Rudolphi, 1810; B. capillicollis Megnin, 1883; B. acheilognathi Yamaguti, 1934; B. opsariichthydis Yamaguti, 1934; B. fluviatilis Yamaguti, 1952; B. gowkongensis Yeh, 1955; B. kivuensis Baer and Fain, 1958;

Schyzocotyle (=Bothriocephalus) fluviatilis Akhmerov, 1960; B. phoxini Molnár, 1968; and B. aegyptiacus Rysavy and Moravec, 1975.

There are considerable doubts surrounding the validity of these species, resulting largely from the dubious characteristics used for their specific identity, in particular host specificity and the external morphological features.

Recently Dubinina (1982) has drawn attention to the confusion concerning the taxonomic status of the species of the genus Bothriocephalus parasitising cyprinid fishes. She concluded that in the U.S.S.R. (and in the rest of Europe) there were two species: the widely distributed B. opsariichthydis (synonyms B. gowkongensis and B. phoxini) and the less prevalent B. acheilognathi (synonym S. fluviatilis) which could be distinguished by their differing scolex morphology. This opposed the generally accepted view of Molnár (1977) that the four species of Bothriocephalus described from European cyprinid fishes were in fact one and, in agreement with Körting (1975) that priority should be given to the name B. acheilognathi with B. opsariichthydis, B. gowkongensis and B. phoxini as synonyms.

Wardle and Mcleod (1952) stated that for the cestodes in general "...no two specimens when fixed will be identical in shape and size of holdfast and body even if they were approximately so when alive". The aim of chapter 1 is to examine the consequences of this comment on the proposals made by Dubinina (1982) and to critically review the taxonomic history of the genus Bothriocephalus parasitising cyprinid fishes.

Four species of Bothriocephalus have ben recorded from Africa. Two of these, B. aegyptiacus and B. kivuensis are specific to African freshwater fishes. In chapter 2 the biology and morphology of these species are critically examined in order to investigate their taxonomic status.

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Chapter 1

A critical scanning electron microscope study of the scolex of *Bothriocephalus acheilognathi* Yamaguti, 1934, with a review of the taxonomic history of the genus *Bothriocephalus* parasitising cyprinid fishes

Introduction

The specific identity of Bothriocephalus species imported into fish farms in the British Isles from Europe has been a matter of conjecture since their first reported introduction (Andrews et al., 1981). This was a consequence of the extreme variation in the size of the living worms. Adult Bothriocephalus have no hard structures, and are active worms, capable of considerable mobility of the body, especially the scolex. Therefore, depending on the fixation process, a considerable variation in morphology can be induced. The species described from cyprinids are very similar in morphology, so much so that it is a matter of conjecture whether the differences are specific, intraspecific or artefacts of fixation.

It is the purpose of this chapter to examine the suggestion of Dubinina (1982) that, for the identification of species of this genus, special attention should be paid to the structure of the scolex; and to clarify the taxonomic status of some members of the genus.

Materials and methods

In order to demonstrate constancy or variation in scolex morphology in one species of Bothriocephalus, all the worms were obtained from a single 0+ Ctenopharyngodon idella (Valenciennes) (Cyprinidae). Ten worms of various sizes were fixed using each of the following procedures: 1. in situ in the host intestine using a 10% formaldehyde solution; 2. removed from the intestine and immediately placed in absolute alcohol at 25°C; and 3. removed from the intestine, relaxed in distilled water at 10°C for 5 minutes followed by immersion in a 10% formaldehyde solution. After fixation, specimens in formaldehyde were washed twice in distilled water and dehydrated using an ethanol series. All scolices were treated in a Polaron E3000 critical point drier, followed by coating with 60% gold-palladium using a Poloron E5100 sputter coater and viewed using a Philips 501B scanning electron microscope.

Bothriocephalus plerocercoid and early adult stages were reared in the laboratory using the methods detailed by Pool (1984). The plerocercoids and early adults were allowed to develop within the fish intestine for 16 and 40 days respectively at 20°C before removal. To facilitate handling, owing to their small size, these specimens were placed in a cone of filter paper for fixation using method 3 (above). After drying, the contents of the cone were emptied onto double-sided adhesive tape for attachment to the viewing stub, or the apex of the cone was mounted directly on the stub. The specimens were sputter-coated and viewed as indicated above.

Results

The results are presented as a series of photographs of representative scolices obtained using each of the three fixation procedures.

Figs. 1-4 show scolices fixed *in situ* in the intestine of the host. They were rounded, the bothria were short and orientated towards the apex of the scolex (anterior) and the apical disc was very apparent, with, in some individuals (Figs. 2-4) four distinct lobes. There was little variation in the size or form of the scolices fixed *in situ*.

Figs. 5-7 demonstrate scolices fixed immediately after removal from the intestine. In this instance the scolices were not completely relaxed, the bothria were open and the apical discs were not obvious. The shape of the scolex was a result of the state of contraction or extension of the musculature at the moment of fixation. Accordingly, considerable variation in the form of the scolex resulted from extended (Figs. 5 and 6) to contracted (Fig. 7).

Figs. 8-12 illustrate scolices relaxed in distilled water prior to fixation. These scolices were all an inverted heart-shape with a characteristic apical disc (Fig. 12). In most of these relaxed specimens the bothria were closed and were approximately two-thirds the length of the scolex. The variation between the two bothria of one individual scolex is shown in Figs. 10 and 11. One bothrium (Fig. 10) is contracted around a piece of intestinal tissue, while

the other (Fig. 11) is relaxed. The scolices which were relaxed prior to fixation showed very little variation in morphology.

The apical disc shown on adult Bothriocephalus fixed in situ in the intestine (Figs. 1-4) and in the relaxed individuals (Figs. 8-12), was also visible on late plerocercoid (Fig. 13) and early adult stages (Fig. 14) of the life cycle.

Figures 1-4 *B. acheilognathi* scolices from *C. idella* fixed *in situ* in the host intestine using 10% formaldehyde solution.

1. Viewed at 7.2 kv. Bar micron markers are 100 um each.

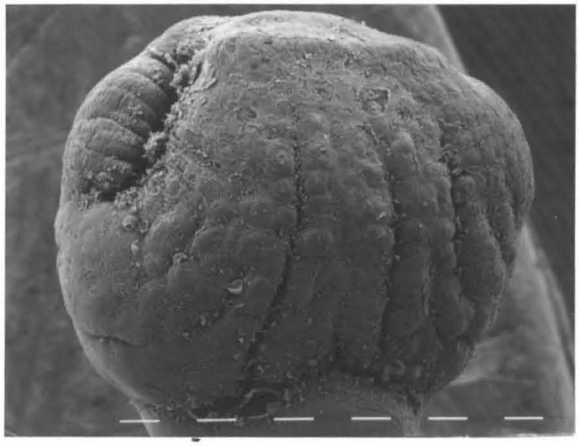
1a. *Schyzocotyle fluviatilis*. From Akhmerov, 1960: Fig. 4,1. p. 20.

2. Viewed at 30 kv using back-scattered electrons. Markers 76.92 um each.

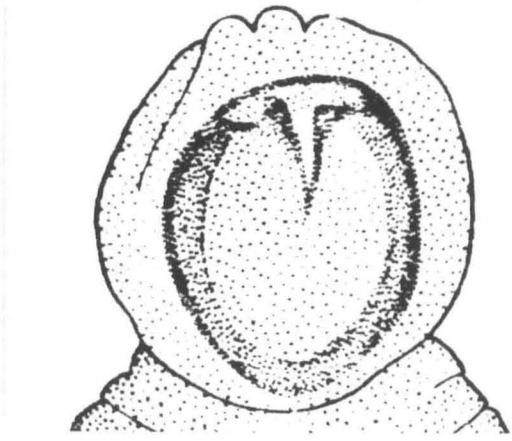
3. Viewed at 3.6 kv. Markers 100 um each.

3a. *B. opsariichthydis*. From Dubinina, 1971: Fig. 10, p. 90.

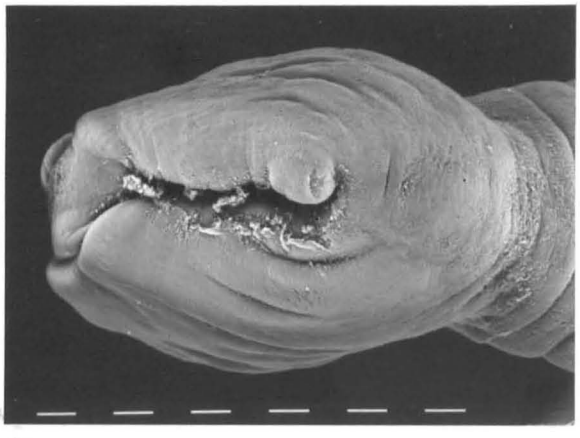
3b. *B. gowkongensis* or *B. opsalichthydis* (=*opsariichthydis*). From Korting, 1974: Fig. 2 p. 168.



1



1a



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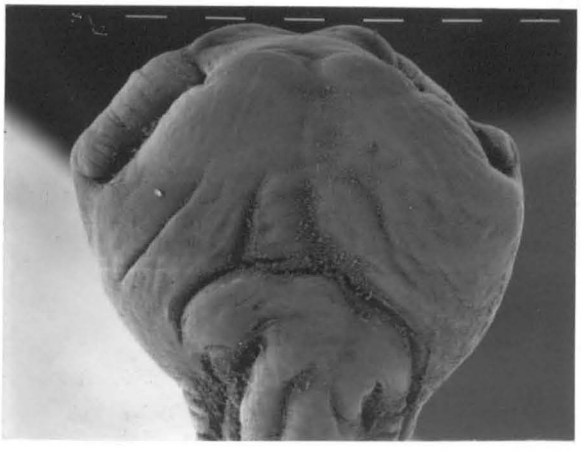

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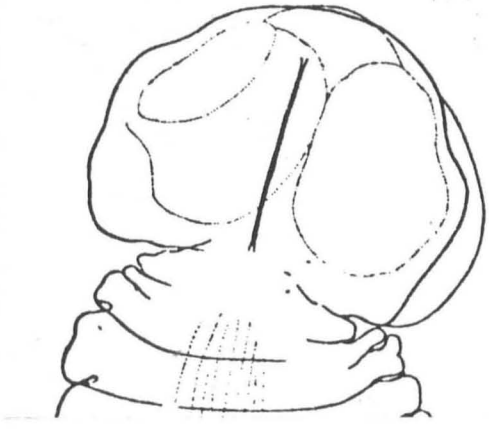

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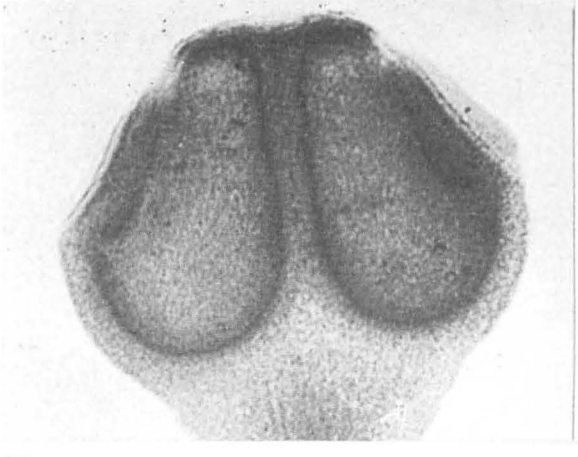


3b

4. Viewed at 7.2 kv. Markers 88.33 um each.

4a. Schyzocotyle fluviatilis. From Akhmerov, 1960: Fig. 3,1, p. 20.

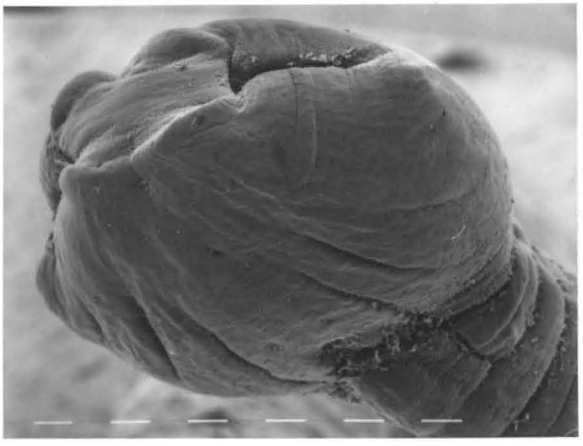
Figures 5-7 B. acheilognathi scolices from C. idella fixed immediately after removal from intestine by immersion in absolute alcohol at 25°C.

5. Viewed at 7.2 kv. Markers 71.43 um each.

5a. B. gowkongensis. From Yeh, 1955: Fig. 2 between pp.73-74. Bar marker = 100 um.

5b. B. gowkongensis or B. opsalichthydis (= opsariichthydis). From Körting, 1974: Fig. 1, p. 168.

5c. B. gowkongensis or B. opsalichthydis (= opsariichthydis). From Korting, 1974: Fig. 3, p. 169.



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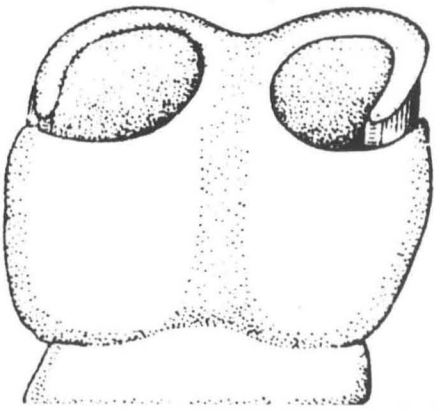
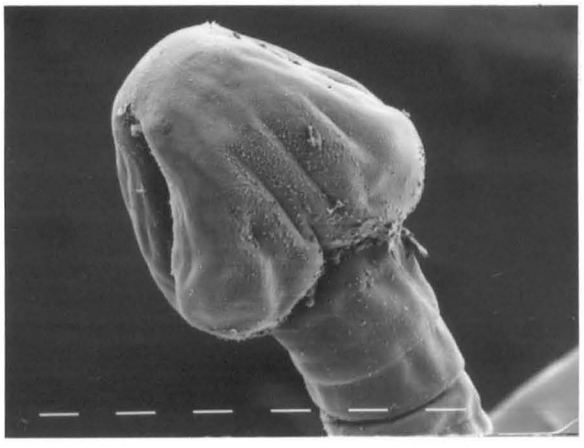


4 a



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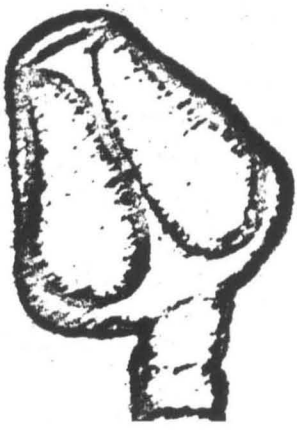

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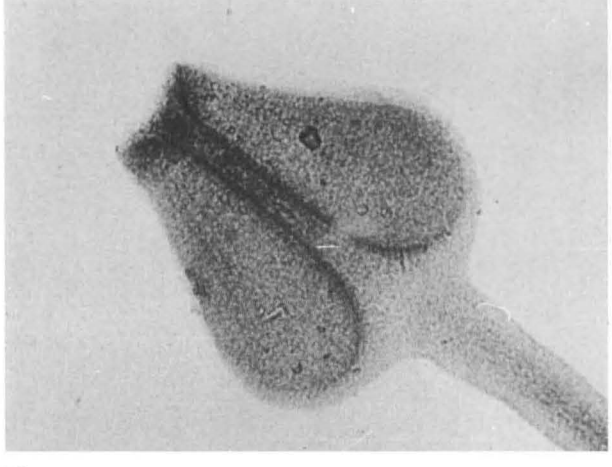

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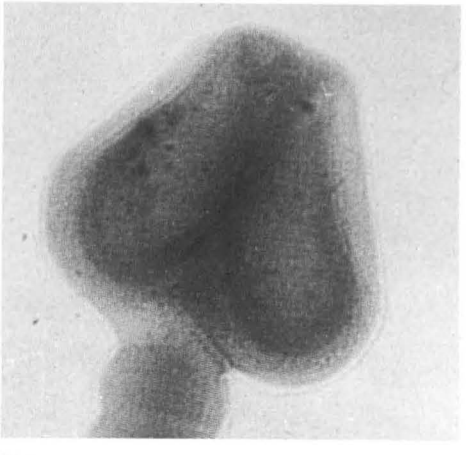


5c

6. Viewed at 3.6 kv. Markers 76.92 um each.

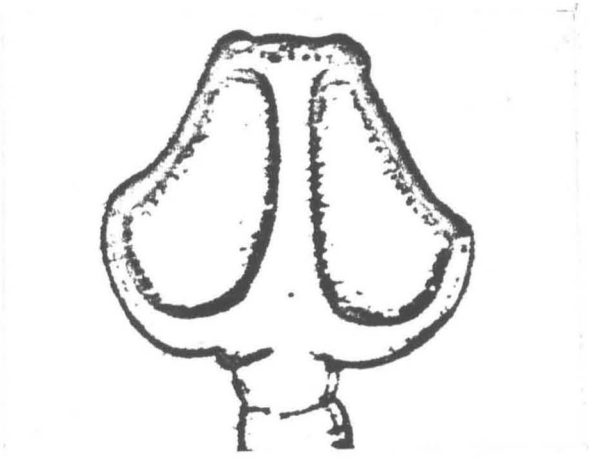
6a. B. gowkongensis. From Yeh, 1955: Fig. 1 between pp. 73-74. Bar marker = 100 um.

6b. B. opsariichthydis. From Yamaguti, 1934: Fig. 23, p.17.

7. Viewed at 7.2 kv. Markers 71.43 um each.

7a. B. gowkongensis. From Yeh 1955: Fig. 3 between pp. 73-74.

6



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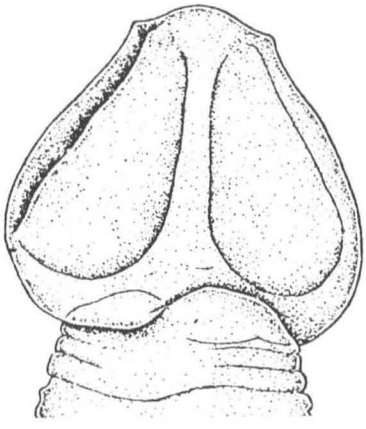
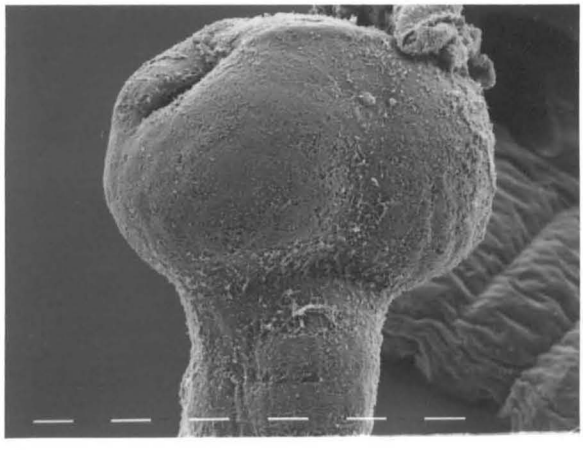


6b



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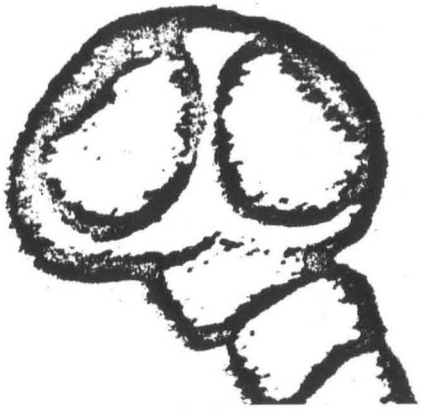


7a

Figures 8-12 B. acheilognathi scolices from C. idella, relaxed in distilled water at 10°C for 5 minutes prior to fixation in 10% formaldehyde solution.

8. Viewed at 7.2 kv. Markers 100 um each.

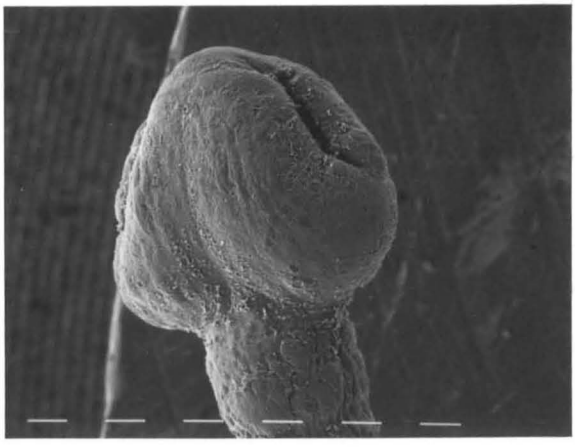
8a. B. acheilognathi. From Yamaguti, 1934: Fig. 20, p.15.

8b. B. gowkongensis. From Molnár & Murai, 1973: Fig. 2 opposite p. 102. Bar marker = 100 um.

8c. B. phoxini. From Molnár & Murai, 1973: Fig. 7 opposite p. 103. Bar marker = 50 um.

9. Viewed at 7.2 kv. Markers 76.92 um each.

9a. B. gowkongensis. From Musselius, 1973: Fig. 8a p. 28.



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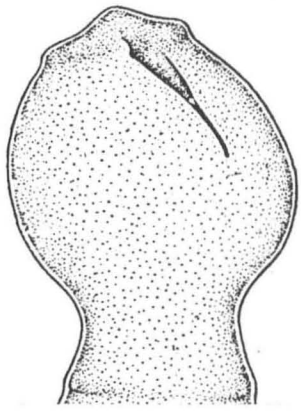
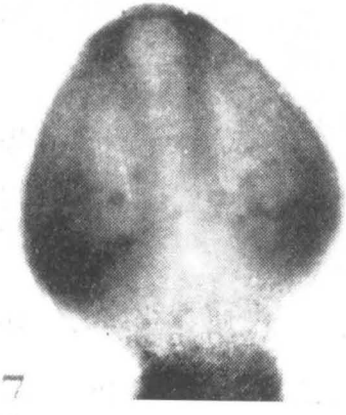
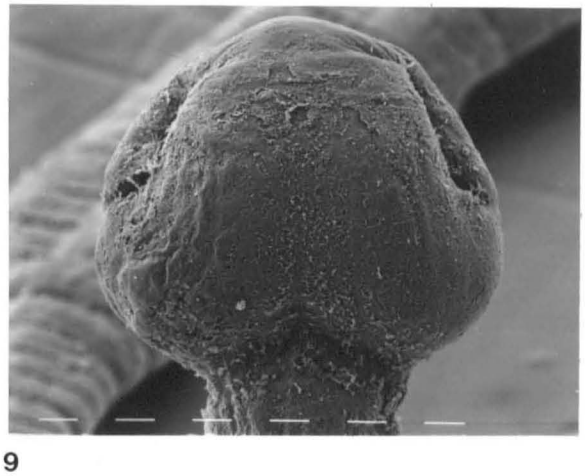


8a

8b



8c



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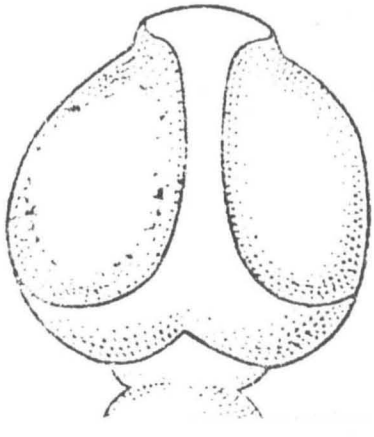


9a

9b. B. gowkongensis. From Molnár & Murai, 1973: Fig. 1 opposite p. 102. Bar marker = 100 um.

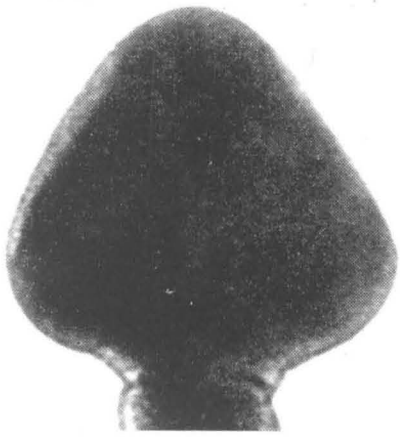
9c. B. phoxini. From Molnár, 1968: Fig. 1D p. 184.

10. Viewed at 7.2 kv. Markers 71.43 um each. Same scolex as Fig. 11. Bothrium contracted around intestinal material.

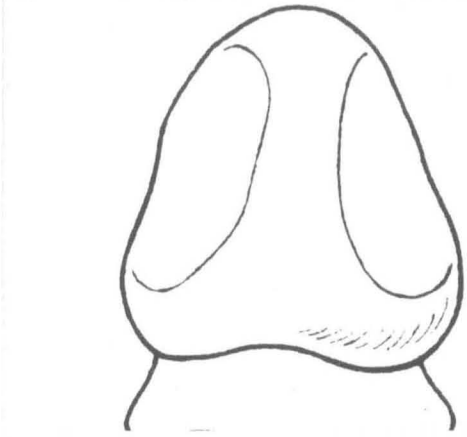
11. Viewed at 7.2 kv. Markers 71.43 um each. Same scolex as Fig. 10. Other bothrium relaxed.

11a. B. gowkongensis. From Protasova, 1977: Fig. 14a p. 88. Bar marker = 200 um.

11b. B. phoxini. From Molnár & Murai, 1973: Fig. 8 opposite p. 103. Bar marker = 50 um.



9 b



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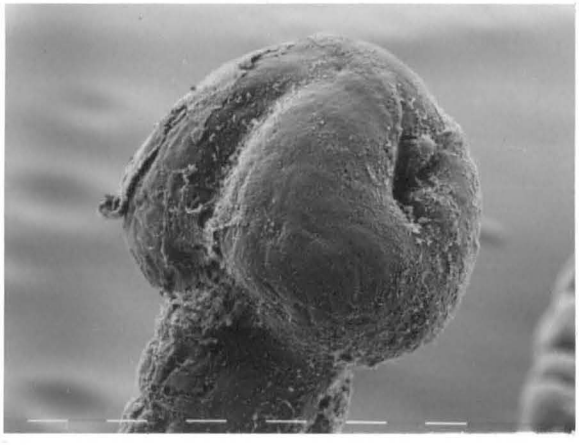

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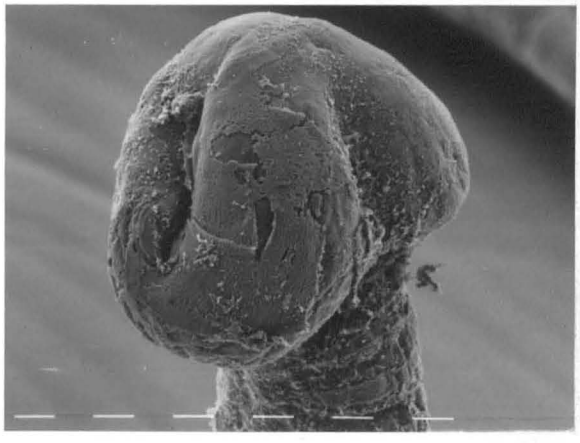

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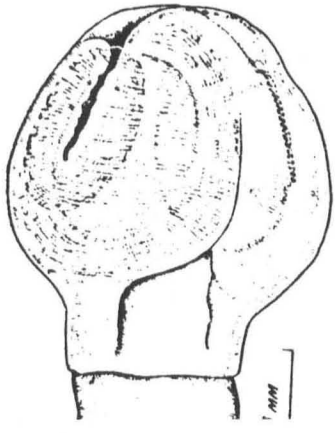
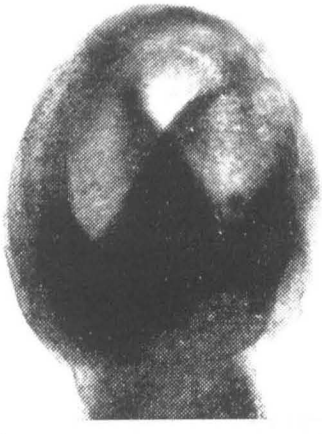


11a



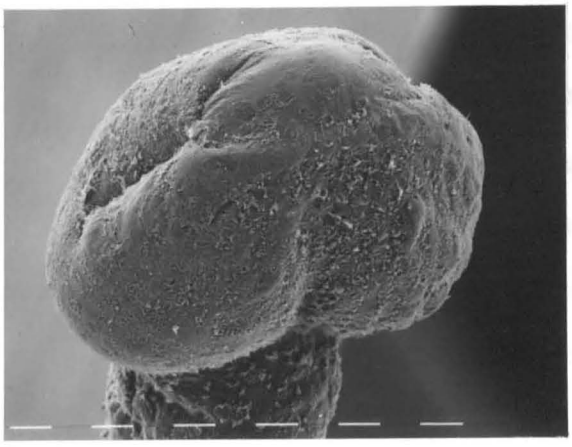
11b

12. Viewed at 7.2 kv. Markers 71.43 um each.

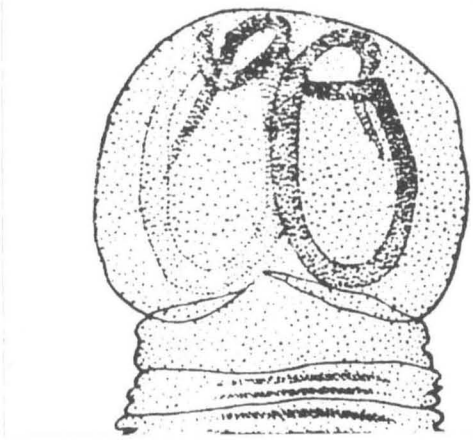
12a. S. fluviatilis. From Akhmerov, 1960: Fig. 4 p. 20.

Figure 13. B. acheilognathi late plerocercoid relaxed in distilled water at 10°C for 5 minutes, prior to fixation in 10% formaldehyde. Viewed at 7.2 kv. Markers 7.7 um each.

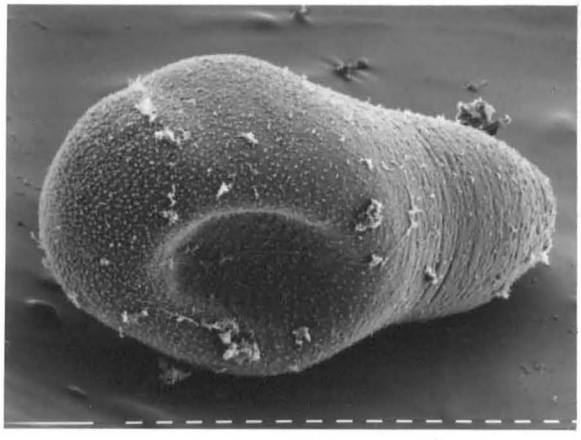
Figure 14. B. acheilognathi early adult relaxed in distilled water at 10°C for 5 minutes, prior to fixation in 10% formaldehyde. Viewed at7.2 kv. Markers 5.9 um each.



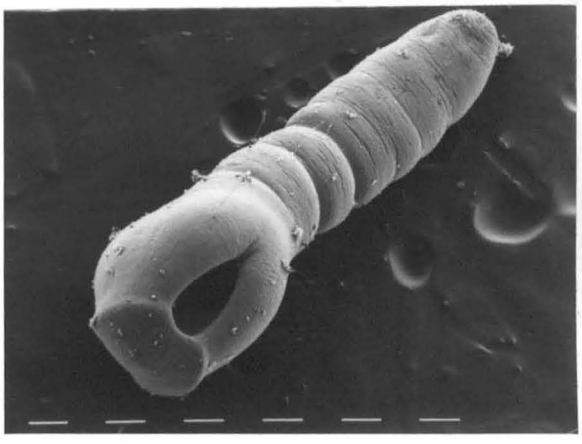
12



12a



13



14

Discussion

The results illustrated in this paper clearly demonstrate the well-known fact that the shape of soft-bodied helminths will vary according to their treatment during autopsy and fixation. To provide a controlled situation, gravid Bothriocephalus from a single species-infection in one individual fish were used, and, therefore, any variation was owing to intraspecific variation or artefacts of fixation. In fact, specified conditions and methods of fixation appeared more significant in affecting scolex morphology than intraspecific variation, which should not surprise anyone who has worked with living pseudophyllidean cestodes, as in many species the ability of the scolex to change shape can be clearly seen in vitro (Brandt et al., 1981).

How does this conclusion influence the proposals of Dubinina (1982) and others concerning the species found in cyprinid fishes? It is convenient to review the literature briefly and where possible to relate the scolex characteristics as given in the literature to the three conditions used in this study.

Yamaguti (1934) described B. acheilognathi from a single worm 80 mm long found in the intestine of Acheilognathus rhombea (Temminck & Schlegel) (Cyprinidae) from Lake Ogura, Japan. Although he thought the worm had a somewhat atypical scolex, the internal anatomy justified its inclusion in the genus Bothriocephalus. His illustration is shown alongside Fig. 8. It appears to represent the scolex typical of a worm fixed after relaxation. In the same publication Yamaguti (1934) described a second species of

Bothriocephalus from a cyprinid fish Opsariichthys uncirostris (Temminck & Schlegel) from Lake Biwa and the River Yodo, Japan. Yamaguti misspelt the generic name of the host. It should have been Opsariichthys, hence the cestode was incorrectly named B. opsalichthydis, although later Yamaguti (1959) emended the spelling to opsariichthydis. B. opsariichthydis was a common worm, up to more than 100 mm long with an inverted heart-shaped scolex with a prominent terminal disc and deep bothridial grooves. His illustration of the scolex is reproduced alongside Fig. 6. It represents a worm fixed immediately after removal from the host intestine. Yamaguti (1934) noted in B. opsariichthydis that the eggs (collected in July) contained embryos which were segmented but not yet fully embryonated. This point is important later in connection with B. gowkongensis. Yamaguti (1952) subsequently described a third species, B. fluviatilis, from a Japanese cyprinid fish Hymenophysa curta (Temminck & Schlegel) also from the River Yodo, Japan. B. fluviatilis was a small worm 22.5 mm long, presumably not gravid, as no details of egg dimensions were provided. A mature proglottis was figured (Yamaguti, 1952, Plate I, Fig. 1, opposite p. 76), but not the scolex.

Yeh (1955) described Bothriocephalus gowkongensis from Ctenopharyngodon idella from Gowkong, near Canton, South China. The worms were 35-80 mm long, with a large inverted heart-shaped scolex. Yeh figured three scolices which are reproduced as Figs. 5a, 6a and 7a. They correspond to worms fixed immediately after removal from the fish intestine, and show a range of variation characteristic of this situation. In B. gowkongensis the eggs were fully embryonated

when laid, according to Yeh the first Bothriocephalus species recognised as showing this phenomenon. Yeh found variation in a large number of specimens of B. gowkongensis and as a consequence suggested that B. opsariichthydis was a synonym of B. acheilognathi. It should be noted that Yeh (1955) was the first to correct the spelling of B. opsalichthydis to B. opsariichthydis.

In 1956 Liao & Shih demonstrated that the degree of embryonation of the eggs of B. gowkongensis varied with season and water temperature. Eighty-nine percent of the eggs were fully-embryonated on release from April to October (24-29°C), whereas only 2% were from November until February (14.8-21.3°C).

Baer & Fain (1958) also suggested that B. opsalichthydis (=opsariichthydis) was a synonym of B. acheilognathi because they could not separate the two species on morphological features. Yamaguti (1959) accepted that B. opsariichthydis and B. acheilognathi were synonyms as well asammending the orignal spelling of the specific epithet opsalichthydis as indicated earlier.

It is interesting to note that Baer & Fain (1958) considered Clestobothrium crassiceps (Rudolphi, 1819) to be in the same genus as B. acheilognathi and B. kivuensis Baer & Fain, 1958 on the basis of their similar scolex morphology. They reduced Clestobothrium to a subgenus and renamed the three species B. (C.) crassiceps, B. (C.) acheilognathi and B. (C.) kivuensis. This view was supported by Tadros (1967), who transferred B. acheilognathi and B. kivuensis to the genus Clestobothrium and amended the diagnosis of the genus.

An examination of the descriptions of the genera Clestobothrium

and Bothriocephalus provided by Wardle and McLeod (1952) indicates that they differ most notably in the presence of operculate eggs in Bothriocephalus and their absence in Clestobothrium. Therefore, despite the similarity in scolex morphology, C. crassiceps should not be included in the same genus as B. acheilognathi.

Bothriocephalus phoxini Molnár, 1968 was described from Phoxinus phoxinus(L.) (Cyprinidae) in a tarn near Lake Balaton, Hungary. In this host the worms had a maximum length of 45 mm and an inverted heart-shaped scolex. A terminal disc and two bothria were readily visible in relaxed specimens. Such a description corresponds to our findings (Figs. 8-12).

According to Molnár (1968) the range of the host P. phoxinus, the endemic nature of the infection he discovered and the small size of individuals of B. phoxini, readily served to differentiate them from all known European bothriocephalids and also from B. opsariichthydis from the Far East. The finding of B. gowkongensis in Hungary resulted in a comparison of the morphology of B. gowkongensis and B. phoxini by Molnár & Murai (1973) which, according to them substantiated the separation of the two species.

Molnár & Murai (1973) fixed their materials using mostly hot 5% formalin. Molnár (1977) by means of cross infection experiments using eggs from tapeworms found in Cyprinus carpio and Phoxinus phoxinus, demonstrated that typical B. gowkongensis worms were recovered from C. carpio and typical B. phoxini specimens were recovered from P. phoxinus.

Otte et al. (1972) considered the Bothriocephalus species found in Cyprinus carpio imported into Austria from Hungary to be B.

acheilognathi, with B. opsariichthydis as a synonym. Körting (1974) commented about "B. gowkongensis or B. opsalichthydis", he stated "the name of the species is not yet finally established". By 1975 Körting observed that, in the event of the three species B. acheilognathi, B. opsariichthydis and B. gowkongensis being accepted as synonymous, the priority should be with B. acheilognathi. The Bothriocephalus species he studied from Cyprinus carpio in southern Bavaria appeared to be B. opsariichthydis as no fully embryonated eggs were detected when they were expelled from the uterus. However, in the light of Liao and Shih (1956) establishing that the maturity of the eggs of B. gowkongensis was dependent upon the season of the year and that the tapeworm could deliver both fully embryonated and half-embryonated eggs, together with the fact that B. opsariichthydis and B. gowkongensis could not be differentiated by morphological features, Körting (1975) favoured the opinion that the two species were identical. As a consequence of the cross-infection experiments performed by Molnár (1977) he proposed that the names B. opsariichthydis Yamaguti, 1934, B. gowkongensis Yeh, 1955 and B. phoxini Molnár, 1968 were synonyms of a single species which, by virtue of priority, should be called B. acheilognathi Yamaguti, 1934.

In Japan, Nakajima & Egusa (1974) examined Bothriocephalus in Cyprinus carpio from farm ponds in Nagano, Yamagata and Akita prefectures. They decided that the criteria for identification were shape and structure of the body. In these characters their species resembled B. acheilognathi, B. opsariichthydis, B. fluviatilis and

B. gowkongensis, all of which had been found in Japan or China. Their species differed from B. gowkongensis however, in that the egg was never embryonated when laid. The body length of their worms (average of 10 of the largest mature individuals, 173 mm) was close to B. opsariichthydis (more than 100 mm long according to Yamaguti, 1934), but differed from B. acheilognathi (about 80 mm) and B. fluviatilis (22 mm). They proposed that B. acheilognathi and B. fluviatilis were young stages of B. opsariichthydis. Their Fig. 2 A-D clearly revealed how the form of the scolex was changed according to the state of contraction and angle of view.

To summarise at this point, the opinion held by most authorities was that there was one species in cyprinids (Nakajima & Egusa, 1974; Körting, 1975; Molnár, 1977). This view was adopted by Andrews et al. (1981) and Chubb (1981) when Bothriocephalus was found in three fish farms in the British Isles during 1979.

However, Dubinina (1982) proposed an alternative view, based largely on the hypothesis that for the identification of the species of the genus Bothriocephalus special attention should be given to the structure of the scolex. On the basis of scolex morphology she suggested that B. opsariichthydis and B. acheilognathi were distinct species. She further suggested that B. gowkongensis and B. phoxini were synonyms of B. opsariichthydis, and that Schyzocotyle fluviatilis Akhmerov, 1960 was a synonym of B. acheilognathi.

The figures of the scolex of Schyzocotyle fluviatilis provided by Akhmerov (1960) clearly show it to be a bothriocephalid, as proposed by Dubinina (1982). These are given here as Figs. 1a, 4a and 12a. The sketch of the scolex in Fig. 4a is perplexing,

although it may correspond to my Fig. 4 or may be a damaged scolex. The details of the proglottis confirm the worm as a Bothriocephalus.

Dubinina (1982) suggested that Yamaguti (1959) confirmed the independence of the two species B. acheilognathi Yamaguti, 1934 and B. opsariichthydis Yamaguti, 1934. Yamaguti (1959) has two entries: p. 44 "B. acheilognathi Yamaguti, 1934, in Acheilognathus rhombea; Japan. Also in Gnathopogon elongatus suwae, Japan." and p. 46 "B. opsariichthydis Yamaguti, 1934,[1]) syn. of B. acheilognathi - Yeh, 1955, in Opsariichthys uncirostris; Japan." The footnote on p. 46 reads: "[1]) Orignal spelling opsalichthydis emended to opsariichthydis". In my opinion the dash between the two sections of the entry for B. opsariichthydis on p. 46 leaves no doubt that Yamaguti accepted the proposal of Yeh (1955) that B. opsariichthydis was a synonym of B. acheilognathi. In this respect Körting (1975) and Protasova (1977) were correct. Nakajima & Egusa (1974) also agreed that the two species were synonyms, however they opted for the alternative view, of B. acheilognathi Yamaguti, 1934 as a synonym of B. opsariichthydis Yamaguti, 1934.

A number of other authors have figured a scolex of Bothriocephalus from cyprinid fishes, including Dubinina (1971), Musselius (1973) a nd Protasova (1977). The Dubinina (1971) scolex (named as B. opsariichthydis) corresponds broadly to my Fig. 3, a specimen fixed in situ in 10% formalin; that of Musselius (1973, as B. gowkongensis) approaches my Fig. 9, an individual relaxed in cold water and fixed in 10% formaldehyde, and that of Protasova (1977, as B. gowkongensis) resembles my Fig. 11, a specimen also fixed after

relaxation.

Some other Bothriocephalus species need a mention. B. capillicollis Megnin, 1883 was found in a 'carpe de mer' from the coast of Norwegian Lapland. According to Gozmany (1979) the 'carpe de mer' is Labrus bergylta Ascanius, 1767 (Labridae) and is therefore not relevant here. Linstow (1889) listed B. capillicollis from Idus melanotus and Zschokke (1903) from Leuciscus idus, however they provide no further information, therefore I agree with Protasova (1977) that this form should be treated as a species inquirenda. Rudolphi (1810) described B. granularis in 'Cyprinus' observed by Zeder in 1788. The description given by Rudolphi provides too little information for precise evaluation of the worm, so that again I agree with Protasova (1977) that it should be treated as a species inquirenda.

B. kivuensis, from Barbus altianalis altianalis Blgr. in Lake Kivu, has a scolex resembling that of B. acheilognathi. However, its great length, 700 mm to 1 m, more than twice as long as the largest B. acheilognathi specimen I have examined, and the presence of a vaginal sphincter suggest that it is a distinct species. B. aegyptiacus Ryšavý & Moravec, 1975 was recorded from Barbus bynni in the River Nile. The scolex is similar to that of 'B. opsalichthydis' but it has a larger number of testes (100 - 200 as compared with 60 - 100) and is somewhat larger (502 - 611 x 3.8 - 4.3 mm compared to 100 x 1.25 mm). A critical investigation into their taxonomic status is presented in chapter 2.

Prigli (1974, 1975) noted the role of aquatic birds in the spread of B. acheilognathi (as B. gowkongensis) in Hungary.

Borgarenko (1981) observed this phenomenon in Tadzhikstan and figured a scolex from the intestine of the little bittern Ixobrychus mintus L. Shinde and Jadhav (1977) recorded a single specimen of B. opsariichthydis in a marine fish Histophorus gladius at Veraval on the west coast of India. Although they described and figured this worm I doubt their identification and suspect it was a marine species of Bothriocephalus.

Conclusions

The conclusion of Dubinina (1982) that B. opsariichthydis Yamaguti, 1934 and B. acheilognathi Yamaguti, 1934 are separate species, is not supported by my results as shown in Figs. 1-14. The scolex characters utilized by her in this instance need represent no more than variation of form produced by different methods of fixation. Thus, as indicated earlier, the Yamaguti (1934) specimen of B. acheilognathi has a scolex form consistent with a worm fixed after relaxation but viewed from a semi-dorsoventral angle (as Fig. 8). The Yamaguti (1934) specimen of B. opsariichthydis (as B. opsalichthydis) has a scolex corresponding with a worm fixed immediately on removal from the host intestine (as Fig. 6). The results confirm the view of Dubinina (1982) that Schyzocotyle fluviatilus Akhmerov, 1960 is a species of Bothriocephalus, it having a scolex corresponding to Fig. 1, a worm fixed in situ in the host intestine.

I propose the continued use of the name B. acheilognathi Yamaguti, 1934 in preference to that of B. opsariichthydis Yamaguti, 1934, as emended by Yeh (1955) and Yamaguti (1959). Although Yamaguti reserved the specific diagnosis of B. acheilognathi until additional specimens came to hand, he did describe a gravid individual, and there is no difficulty with the spelling of the specific epithet as exists for B. opsariichthydis. In addition the name B. acheilognathi has been accepted into common usage, and no rule of priority is contravened by its continued use. I agree with the other authorities noted earlier in this section that B.

opsariichthydis Yamaguti, 1934, B. fluviatilis Yamaguti, 1952, B. gowkongensis Yeh, 1955, B. phoxini Molnár, 1968 and Schyzocotyle fluviatilis Akhmerov, 1960 are synonyms of B. acheilognathi Yamaguti, 1934.

In conclusion, this experiment with cestodes having highly mobile scolices without any hard structures has clearly shown the importance of adopting a standarized fixation procedure. It has also shown how if a standardized procedure is not used considerable taxonomic confusion can result. I agree with Dubinina (1982) that scolex characteristics play an important role in the identification of species of the genus Bothriocephalus, and also other cestodes, but only if the exact conditions of fixation are clearly stated.

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Chapter 2

The taxonomic status of *Bothriocephalus aegyptiacus* Ryšavý and Moravec, 1975 and *B. kivuensis* Baer and Fain, 1958.

Introduction

The exportation of carp for food and sport, and grass carp for weed control has resulted in the rapid spread of Bothriocephalus acheilognathi throughout the world. The parasite was first recorded in South Africa (as B. gowkongensis) from Barbus kimberleyensis in 1978 (Brandt et al., 1981) and has subsequently been recorded in Cyprinus carpio, Barbus mattozzi, and B. trimaculatus (Brandt et al. 1981; Van As et al., 1981). The route of entry of B. acheilognathi into South Africa is reported to be via infected Ctenopharyngodon idella imported into that country from West Germany in 1975 (Brandt et al., 1981).

In addition, two species of Bothriocephalus have been described only from African cyprinid freshwater fishes: B. kivuensis Baer and Fain, 1958 in Barbus altianilis altianilis from Lake Kivu and B. aegyptiacus Ryšavý and Moravec, 1975 in Barbus bynni from the River Nile. B. aegyptiacus is described as having a scolex resembling that of B. opsariichthydis, but could be distinguished from it by the larger number of testes per proglottid (140-200 as compared with 60-100), the larger strobila (502-611 x 3.8-4.3mm as compared with 100 x 1.25mm) and the shape and location of the ovaries. The anatomy of the reproductive organs and scolex morphology is, however, similar to that of B. phoxini. It differs in having a larger strobila (502-611 x 3.8-4.3mm) as compared with 45 x 1.4mm), a greater number of testes (140-200 as compared with 60-70) and the larger diameter of the cirrus sac (0.13-0.156mm as compared with 0.08mm). As B. opsariichthydis and B. phoxini are synonyms of B. acheilognathi (see Chapter 1), B. aegyptiacus differs from B. acheilognathi only in the size of the strobila and the number of testes per proglottid.

B. kivuensis also has a scolex resembling that of B. achielognathi; however it differs in having a considerably larger strobila (7000-1000 x 25.4-4.0mm as compared with 80-100 x 1.25mm) and in possessing a vaginal sphincter muscle (Baer and Fain, 1958).

Molnár (1977) noted that there was considerable morphological variability in B. acheilognathi depending on the size and species of the fish definitive host. It is therefore possible that the three Bothriocephalus species recorded from cyprinid fishes in Africa are in fact the same species, with the observed morphological variation being a result of them infecting three different hosts. The purpose of this chapter is to investigate this suggestion by examining the morphological variability of B. aegyptiacus and B. kivuensis and comparing it with that of B. acheilognathi.

Method

Stained and mounted syntype and topotype specimens of *B. aegyptiacus* and syntype specimens of *B. kivuensis* were obtained from Dr. F. Moravec and Professor O.M. Amin, and from the Museum of Natural History of Geneva respectively. These specimens were examined using light microscopy, and the variation in the number and size of the internal organs was noted. Paratype material of *B. acheilognathi* (as *B. gowkongensis* Yeh, 1955), obtained from the British Museum for Natural History, and specimens collected from *Ctenopharyngodon idella* and *Cyprinus carpio*, and stained with Erlichs Haematoxylm, were examined in the same way for comparison.

The scolices of 6 immature *B. aegyptiacus* (paratype specimens provided by Dr. F. Moravec) and 2 immature *B. kivuensis* (syntype specimens provided by the Musée Royal de l'Afrique Centrale) were prepared for electron microscopy using the procedure described in Chapter 1, and examined using a Philips 501B scanning electron microscope. A comparison was made with the scolices of *B. acheilognathi* presented in Chapter 1.

Results and Discussion

Considerable variation in the number and size of the anatomical and morphological structures of B. acheilognathi, B. aegyptiacus and B. kivuensis was evident from this study and has been described by various authors. This information is presented in table 1.

Amin (1978) and Ryšavý and Moravec (1975) recorded 140-200 testes per proglottid for B. aegyptiacus. Examination of the material provided by Amin and Moravec revealed that in mature proglottides the testes were obscured by the numerous vitelline cells. However by examining proglottides in which the uterus did not contain eggs, it was possible to distinguish 145-215 vitelline cells (Fig. 1) in the cortical parenchyma around the margins of the proglottides, and 66-88 testes (Fig. 1) in a single lateral layer in the medullary parenchyma. The size of the testes (0.055-0.060mm x 0.080-0.095mm) compares favourably with that described for B. acheilognathi (as B. gowkongensis, Molnár and Murai, 1973). The measurements of the vitelline cells (0.015-0.03 x 0.035-0.045mm in immature proglotties and 0.03-0.05 x 0.04-0.055mm in mature proglottides) coincide with those given for the testes by Ryšavý and Moravec (1975) (0.026-0.048 x 0.040-0.056mm) and Amin (1978) (0.013-0.048 x 0.022-0.070mm, Fig. 2). This together with similarity between the numbers of vitelline cells given here, and of the testes given by Amin, 1978 and Ryšavý and Moravec, 1975, suggests that these authors have confused the testes and vitelline cells.

In the descriptions of B. aegyptiacus by Ryšavý and Moravec (1975) and Amin (1978) and of B. kivuensis by Baer and Fain (1958) these species are distinguished from B. acheilognathi by the length of the strobila. When using the length of a cestode as a specific diagnostic feature it is necessary to consider the size and age of

Table 1: The principal characteristics of the 3 Bothriocephalus species recorded from Africa.

Species	Length mm	Width mm	Number of testes	Cirrus pouch mm	Eggs um	Authority
B. acheilognathi Yamaguti, 1934	80 -40		80-100	0.16 x 0.08	51-54 x 33-37	Yamaguti, 1934
	600					Granath & Esch, 1983
	-440	-2.6	44-86	0.13-0.16 x 0.09	50-54 x 33-36	Körting (pers. comm.) Personal observations
(as B. opsariichthydis)	100	1.25	60-100	0.16 x 0.09	50-54 x 36-40	Yamaguti, 1934
(as B. gowkongensis)	35-80	0.5-1.2	50-90		53-54 x 33-38	Yeh, 1955
(as B. gowkongensis)	100-320	2-2.5	60-70	0.09-0.125	50-52 x 33-37	Molnár & Murai, 1973
(as B. phoxini)	45	1.4	40-60	0.08	49-54 x 35-40	Molnár & Murai, 1973
B. aegyptiacus Ryšavý and Moravec, 1973	502-611	3.8-4.3	140-200	0.13-0.156	66 x 34-46	Ryšavý & Moravec, 1975
	161-611	2.10-4.30	140-280	0.098-0.156	38-55 x 26-46	Amin, 1978
	40-100 (immature)	0.8-1.5	66-88	0.09-0.132	50-55 x 35-42	Personal observations
B. kivuensis Baer and Fain, 1958	700-1000	2.5-4.0	50-75	0.16-0.183 x 0.078-0.091	45-50 x 27.30	Baer and Fain, 1958
	80-240	1.2-2.5	55-63	0.17-0.18 0.079-0.082	45-51 x 27.29	Personal observations

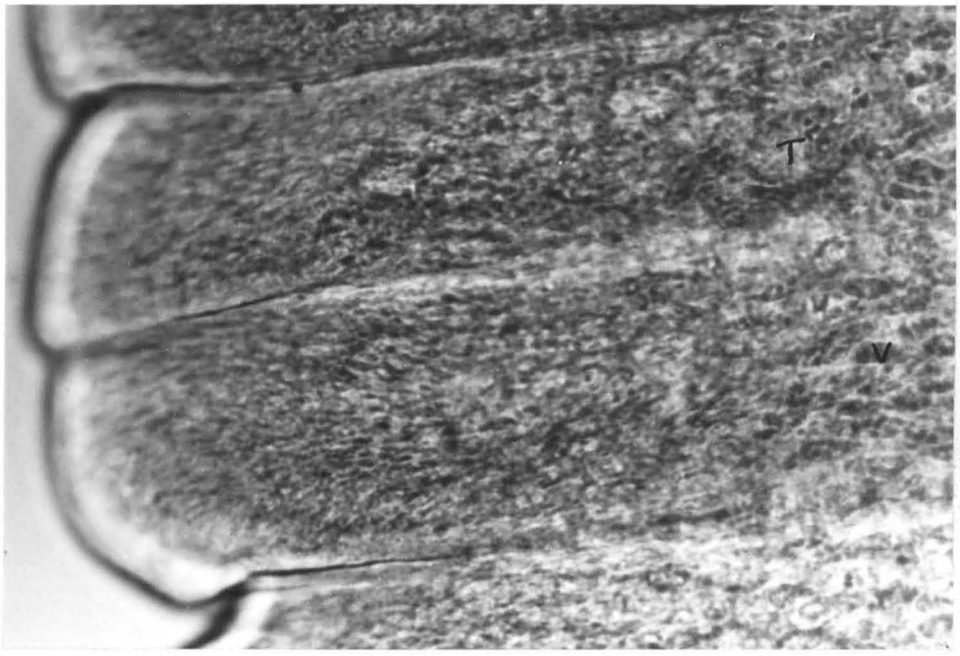


Figure 1. Vitelline cells (V) and testes (T) within the proglottides of B. aegyptiacus.

Marker = 0.1mm

Size of testes, from Amin, 1978

Size of testes, from Ryšavý and Moravec, 1975.

Size of testes, from personal observations

Size of vitelline cells, from Ryšavý and Moravec, 1975

Size of vitelline cells, from personal observations.

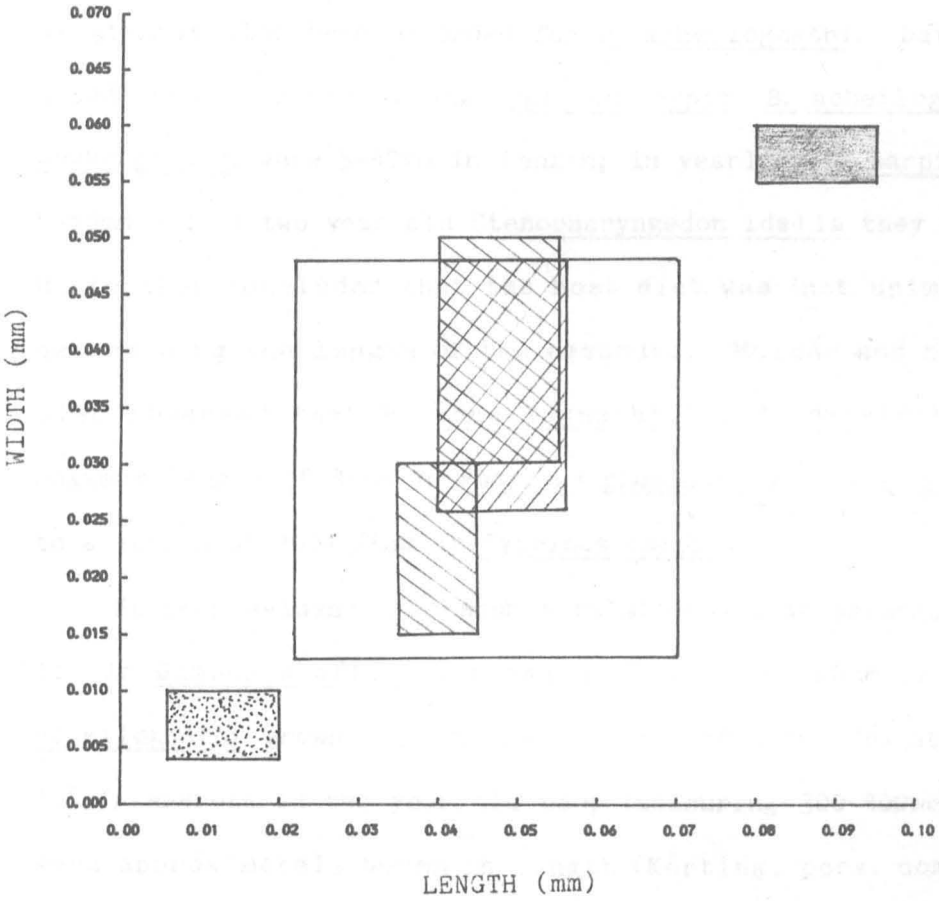


Figure 2. A comparison of the measurements of testes and vitelline cells of B. aegyptiacus as described by Ryšavý and Moravec (1975), Amin (1978) and in this study.

the host (Davydov, 1978), the species of host (Read and Voge, 1954; Freze, 1977), the infection intensity (Pavlovski and Gnezdilov, 1949) and the state of contraction or relaxation of the parasite.

Variation in length related to the host species can be considerable, as illustrated by Diphyllobothrium latum, which grows to a maximum length of 8-11cm in the golden hamster, and 7-14 metres in the bear (Freze, 1977). Read and Voge (1954) suggested that variation such as this was related to the size of the host intestine.

A relationship between host size and species and parasite length has also been recorded for B. acheilognathi. Davydov (1978) noted that in underyearling Cyprinus carpio, B. acheilognathi (as B. gowkongensis) were 5-40mm in length; in yearling C. carpio they were 5-80mm and in two year old Ctenopharyngodon idella they were 5-90mm. He further concluded that the host diet was 'not unimportant' in determining the length of the cestodes. Molnár and Murai (1973) also observed that B. acheilognathi (as B. phoxini) grew to a maximum length of 45mm in Phoxinus phoxinus and (as B. gowkongensis) to a length of 100-320mm in Cyprinus carpio.

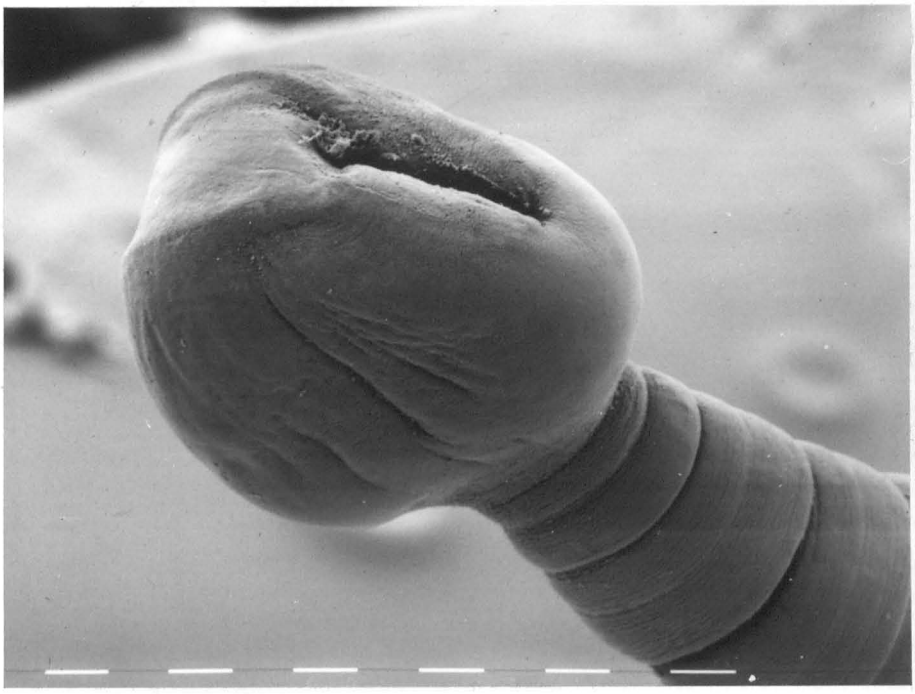
Further evidence for such a relationship is presented in table 1: in Gambusia affinis, a small fish up to 60mm in length, B. acheilognathi grows to a maximum length of 40mm (Granath and Esch, 1983), whereas in two year old carp (measuring 300-400mm) the worms were approximately 600mm in length (Körting, pers. comm.). It is important to note that Körting's specimens were measured immediately after removal from the intestine. Upon relaxation B. acheilognathi specimens have been observed to increase in length by a factor of 1.5-2.0 therefore the specimens may have measured 900-1200mm when

relaxed. The range in length of B. acheilognathi recorded from different hosts (40-1200mm) would encompass that described for B. aegyptiacus (161-611mm when relaxed) and B. kivuensis (700-1000mm when relaxed).

It appears then, that the length of a particular B. acheilognathi specimen is dependent, in part, on the size and species of the fish host. The B. aegyptiacus specimens collected by Ryšavý and Moravec (1975) were from Barbus bynni measuring 320-380mm in length (Moravec, pers. comm.); and the B. kivuensis specimens described by Baer and Fain (1958) were from Barbus altianilis altianilis measuring 250-400mm in length (Fain, pers. comm.). Therefore, in both cases, the large size of the hosts could, in part, account for the large size of the Bothriocephalus species recorded from them.

In the original descriptions, the scolex of B. kivuensis is reported to be "of the same type as B. acheilognathi (Baer and Fain, 1958), while that of B. aegyptiacus resembles B. opsariichthyclis and B. phoxini (Ryšavý and Moravec, 1975). Examination of the scolices using the scanning electron microscope confirmed these statements. The scolex of B. aegyptiacus (Figs. 3a and b) resembles those of B. acheilognathi specimens relaxed in water at 10°C prior to fixation in a 10% formaldehyde solution (Figs. 8 and 12, Chapter 1). The scolex of B. kivuensis (Figs 4a and b) is consistent with B. acheilognathi specimens fixed in situ in the host intestine (Figs. 1 to 4, Chapter 1).

In his redescription of B. aegyptiacus, Amin (1978) describes the eggs as being oval and operculate, with a knob on the opposite side to the operculum. No such structure was apparent on the eggs contained within the uterus of mature proglottides. The eggs



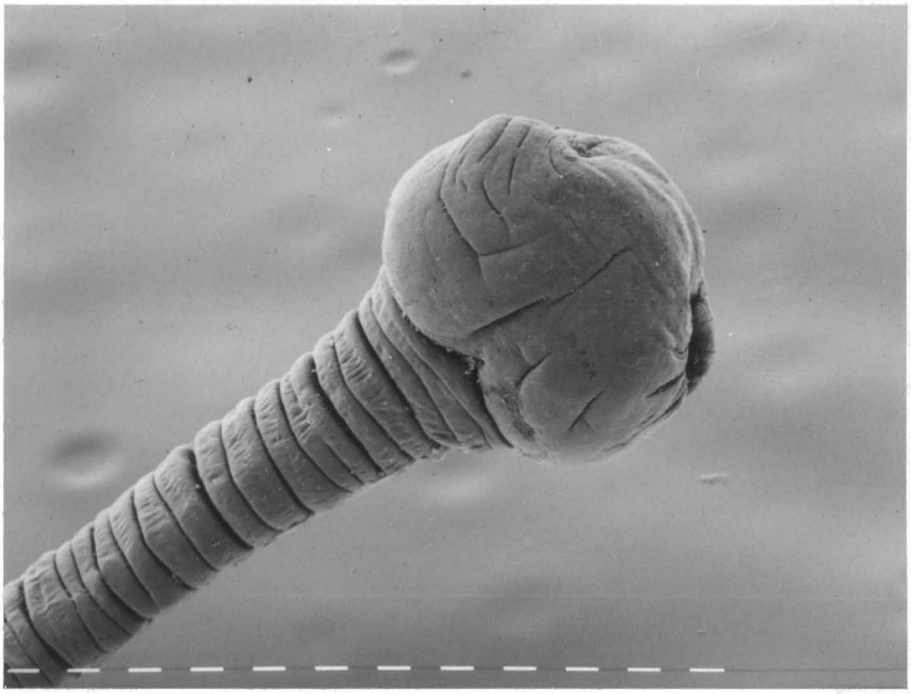
a

b

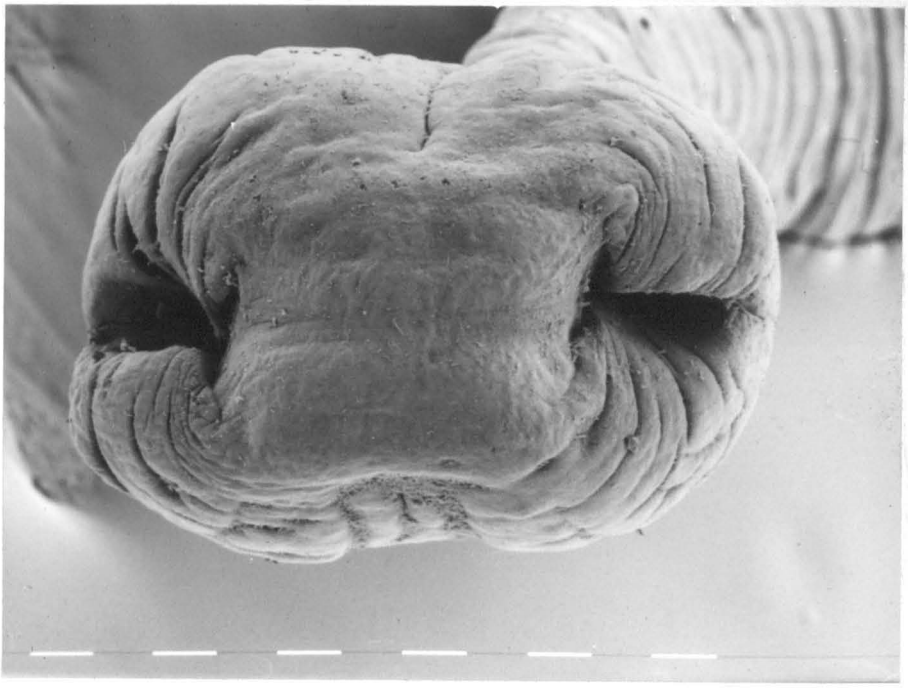
Figure 3. Scanning electron micrographs of the scolices of B. aegyptiacus

a) Markers = 100um

b) Markers = 76.9um



a



b

Figure 4. Scanning electron micrographs of the scolices of B. kivuensis

a) Markers = 71.43um

b) Markers = 100um

examined agreed with the description given by Ryšavý and Moravec (1975). Those described by Amin might have been dessicated during the fixation process, and therefore somewhat atypical.

Baer and Fain (1958) differentiated B. kivuensis from B. acheilognathi by their overall size and the presence in B. kivuensis of a vaginal sphincter muscle. Previous authors have not described this muscle in B. acheilognathi. However, examination of longitudinal and transverse sections of the mature proglottides of B. acheilognathi stained with haematoxylin and eosin enabled the vaginal sphincter muscle to be seen (Fig. 5). The muscle was less obvious than in B. kivuensis on account of the smaller size of the worms examined.

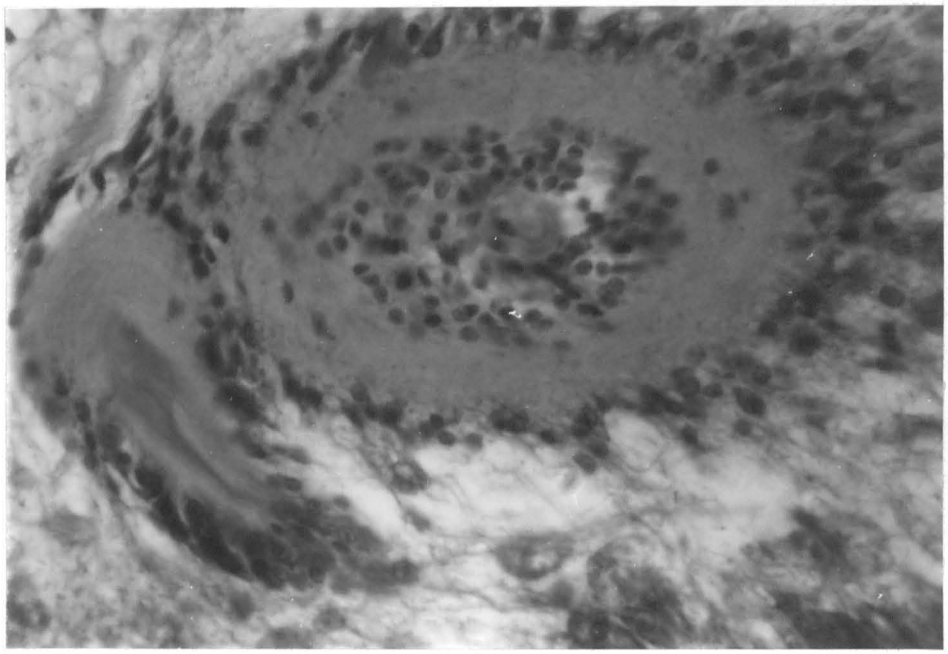


Figure 5. An optical microscope photograph of a transverse section through a mature proglottid of B. acheilognathi showing the cirrus sac and vaginal sphincter muscle.

Marker = 0.1mm

Conclusion

Since the characters examined in this study are not useful in differentiating the three Bothriocephalus species recorded from Africa, I am of the opinion that B. acheilognathi Yamaguti, 1934, B. kivuensis Baer and Fain, 1958 and B. aegyptiacus Ryšavý and Moravec, 1975 are synonymous. The name B. acheilognathi has priority. Further evidence, to clarify this situation could be obtained using isoenzyme analysis, or better, by cross-infection experiments.

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Part II

An experimental study of some aspects of the biology of *Bothriocephalus acheilognathi*

Introduction

The influence of a range of biotic and abiotic factors on the biology of Bothriocephalus acheilognathi have been studied in detail (e.g. Liao and Shih, 1956; Körting, 1975; Nakajima and Egusa, 1976, 1977; Davydov, 1978; Granath and Esch, 1983). Although much of this work has centred around the effect of temperature on the various stages of the life cycle, in many instances the results were contradictory.

At the present time B. acheilognathi is not established in the British Isles, although it has been introduced on a number of occasions (Stott, 1977; Andrews et al., 1981; Pool et al., 1984). Two factors may be of particular importance in explaining this absence. Firstly, owing to the imposed movement restrictions and the careful anthelmintic treatment of infected fishes any B. acheilognathi entering Britain are eliminated; and secondly, the climatic conditions (particularly the temperature) of the British Isles may not enable B. acheilognathi to become established or survive.

In Chapter 3 the influence of temperature on the life cycle of B. acheilognathi is examined. The aim of the study was to assess if B. acheilognathi could survive at the temperatures encountered in the British Isles, and how successfully it could complete its life cycle at these temperatures. The success of the parasite was examined by estimating the transmission dynamics at each stage of the life cycle.

While obtaining B. acheilognathi specimens for this study, and for the taxonomic work (Part I), it was noted that the position of the strobila within the intestine of the definitive host varied depending on the time of the day when the fish was sampled. In

addition it was noted that faecal samples collected at different times of the day contained varying numbers of eggs. In Chapter 4 this is studied in greater detail, and the factors responsible for the two observations are examined.

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Chapter 3

An experimental study of the population dynamics of *Bothriocephalus acheilognathi*

Introduction

The population dynamics of cestodes in natural or experimental conditions are poorly understood. In field investigations many authors have reported the presence of seasonal fluctuations in abundance (reviewed by Chubb 1980, 1982), but few provide quantitative data on rates of transmission, infection or mortality. Those studies that have been conducted indicate that the rate of transmission from one stage of the life cycle to the next is very low, and the natural mortality rates throughout the life cycle are very high (e.g. Jarroll (1980) for Bothriocephalus rarus; Halvorsen and Andersen (1974) for Diphyllobothrium ditremum and Anderson (1974) for Caryophyllaeus laticeps).

Much of the experimental work has concentrated on the infection of the Tribolium intermediate host by the eggs of Hymenolepis diminuta (e.g. Anderson and Lethbridge, 1975; Keymer and Anderson, 1979; Keymer, 1980, 1982) and indicated that the rate of infectivity was age dependent, and proportional to the density of eggs and the feeding behaviour of the host. No studies appear to have been carried out upon the dynamics of transmission between other stages in the cestode life cycle, or at any stage in the life cycle of a pseudophyllidean cestode.

The life cycle of Bothriocephalus acheilognathi is typical of the Bothriocephalidae, and a number of studies have concentrated on all, or certain aspects of it (e.g. Liao and Shih, 1956; Pimenova, 1971, 1973; Körting, 1975; Hoffman, 1976 and Nakajima and Egusa, 1976). None of these studies have examined the population or transmission dynamics.

In the following chapter the population dynamics of B. acheilognathi were studied experimentally at a range of

temperatures. This provided information on a) if B. acheilognathi could survive at the temperatures encountered in the British Isles, and (b) how successful it would be at these temperatures.

1) The fecundity of *B. acheilognathi*

Method

1-2cm *C. carpio* were infected with single specimens of *B. acheilognathi*. Infection was accomplished by placing a single *C. carpio* into a crystalising dish and adding one *Cyclops agilis* containing a single mature procercoid. The infected fish were maintained individually at 18°C in a 16L:8D photoperiod and fed daily on Mainstream Salmon Fry 02 crumb or Omega No. 4 trout pellets, depending on the size of the fish.

At approximately 100 day intervals the faeces from each fish were collected daily for a 5 day period. To facilitate the collection of faeces the aquaria were inclined at an angle of approximately 30° , allowing the faecal material to be removed using a siphon. Each sample was examined in the form of smears under the microscope and all of the eggs were counted. The dry weight of each faecal sample was measured by washing the faeces onto a predried and weighed filter paper and drying for 2-3 days at 60°C.

Results

The data collected are illustrated in figure 1. Egg release was observed after the B. acheilognathi (specimens) had been in the C. carpio intestine for 200 days, and continued until day 600, the last sample before the worms passed out of the intestine. While examining the growth of B. acheilognathi it was noted that egg release first occurred after 130 days (section 7). This information has been included in figure 1.

It is important to note that there was considerable variation in the numbers of eggs released by each worm at each sampling time, as indicated by the large standard error values. This variation can be partly explained by the small number of worms used in the study. Of the 10 C. carpio infected, only 4 contained worms after 200 days, 3 contained worms after 400 days and 2 contained worms after 600 days. The possible reasons for the gradual loss of worms are covered in section 7. Examination of the egg release by a single worm also showed considerable variation. This may be influenced by the activity of the fish, variations in the meal size consumed by the fish etc. Obviously further work to find the causes of variation is necessary.

Comparison of the faeces weight and the number of eggs released showed there to be no significant correlation ($r = 0.1011$, $p > 0.2$) indicating that the quantity of intestinal material passing over the worm did not influence the release of eggs. It was noted however, that a large proportion of the eggs counted, were associated with expelled intestinal mucosa.

Comparison of the daily values of egg release over the 400 day period using Kruskal-Wallis analysis indicated that there was no significant variation between the worms at each sampling time ($p >$

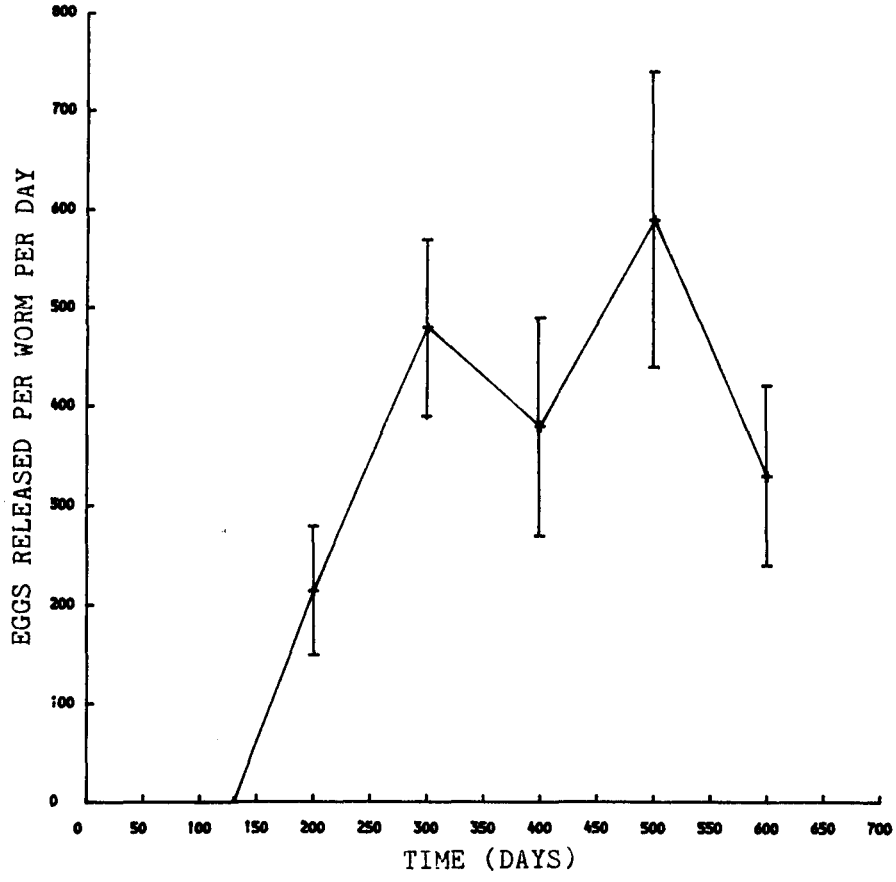


Figure 1: The egg release by B. acheilognathi from single worm infections in C. carpio. ($\bar{x} \pm SE$).

0.5 in each case) or between the samples ($H(c) = 6.157$, $p > 0.5$). Over this period the size of the *B. acheilognathi* showed no significant increase due to the rates of pseudapolysis and of proglottid formation and maturation being approximately equal (section 7). As the size (length or weight) of a cestode is reported to influence the rate of egg production (e.g. Davydov, 1978; Kennedy, 1983) the observed constant rate of egg release might be expected.

Few other studies have examined the pattern of egg release from cestodes. Davydov (1978) examined the rate of egg release by *B. acheilognathi in vitro* over a 12 day period. He found that an 80mm *B. acheilognathi* weighing 240mg and from a single infection produced 60,500 eggs over the 12 day period. Davydov's values are for a limited period of time under *in vitro* conditions, and so give no indication of the total egg output or the variation in egg release of a single worm as observed in this study.

In the present study it can be estimated (from Fig. 1) that a single *B. acheilognathi* from a single worm infection would produce 180,950 eggs (S.E. $\pm$ 45,437) during its life span. This value is considerably less than the values obtained from cestodes with warm blooded hosts. For example *Hymenolepis diminuta* produces 250,000 eggs per day (Keymer, 1980); *Taenia saginata* (*Taeniarhynchus saginatus*) produces 700,000 eggs per day; *T. solium* 300,000 and *T. hydatigena* 60,000 (Moore 1981).

As indicated by Kennedy (1983) the fecundity of a parasite is influenced by many factors (e.g. temperature, host immunity, host diet and genetic differences of the host). Therefore when comparing fecundity estimates it is important to note the precise conditions

in which the worms were maintained. The value for fecundity calculated here is only accurate for the experimental conditons used, and as such may vary considerably from the fecundity of B. acheilognathi in natural conditions. However the value does give an indication of the order of magnitude of egg release, and provides a starting point for the calculation of the population dynamics of B. acheilognathi.

2) The effect of temperature on the hatching of eggs of *B. acheilognathi*

Method

Large numbers of eggs were obtained from 10 randomly selected gravid *B. acheilognathi* by placing the worms into distilled water at 18°C. Bacterial contamination of the egg cultures was reduced by washing the worms in distilled water immediately after removal from the intestine and by removing the worms from the egg cultures after 5 minutes. Approximately 100 eggs together with 5ml of distilled water were placed on a number of watch glasses and 10 watch glasses were incubated at 0, 4, 10, 16, 20, 25, 30, 35 and 40°C in a 16 hours light:8 hours dark photoperiod. The shallow layer of water over the eggs and the absence of bacterial contamination allowed sufficient oxygen to reach the eggs to prevent retardation of development (Dubinina 1966, Kuperman 1973).

The watch glasses were examined at 12 (30, 35 and 40°C), 24 (16, 20 and 25°C) or 48 hourly periods (0, 4 and 10°C), and the percentage of eggs hatching was noted. Each experiment was continued until all of the eggs had hatched, or the remaining eggs were obviously dead (indicated by the lack of organisation within the egg).

Results

The data collected are summarised in figures 2a-d and Table 1, and clearly show that the incubation period and survival of B. acheilognathi eggs are temperature dependent. An increase in temperature (betwee 0 and 30°C) accelerates the rate of development and increases the percentage and rate of development of the coracidia.

The eggs of B. acheilognathi hatched at temperatures ranging from 4-35°C, taking 450-1686 hours (19-70 days) at 4°C, 301-1307 hours (12-54 days) at 10°C, 136-616 hours (6-26 days) at 16°C, 59-434 hours (2.5-18 days) at 20°C, 45-262 hours (2-11 days) at 25°C, 40-144 hours (1.6-6 days) at 30°C and 37-117 hours (1.5-4.9 days) at 35°C. These values agree closely with those obtained by previous authors (e.g. Granath and Esch, 1983b; Körting, 1975; Bauer et al. 1969). The range of temperature at which hatching occurred is however, greater than that recorded previously. Thus Liao and Shih (1956) observed little hatching below 12°C, and none above 37°C, whereas Nakajima and Egusa (1976) found no hatching below 15°C or above 37°C, with rapid death of the embryo from 2-7°C.

At the upper and lower temperature limits the percentage of eggs hatching was greatly reduced (Fig. 2a). At these extremes it was noted that most of the eggs developed to the embryonated stage before dying. In addition at temperatures of 0 and 40°C (at which no hatching occurred), many of the eggs underwent complete development before dying. Similar data at the upper temperature limits was obtained by Granath and Esch (1983b) for B. acheilognathi and at both temperature extremes for members of the Diphyllobothridae, Ligulidae (reviewed Dubinina, 1966) and Triaenophoridae (reviewed Kuperman, 1973). Dubinina (1966) further noted that exposing the eggs of Schistocephalus solidus to a

Table 1. The time taken in hours for the emergence of *B. acheilognathi* coracidia at different water temperatures (x ± SE)

Developmental stages	Temperatures (°C)								
	0	4	10	16	20	25	30	35	40
Emergence of coracidium	-	450 ±15.7	301 ±9.9	136 ±5.9	59.2 ±4.8	45.0 ±5.0	40.0 ±0	36.7 ±2.6	-
Emergence of 50% of coracidia	-	1296.4 ±233.0	633.3 ±31.4	284.5 ±9.7	114.8 ±4.3	77.5 ±7.2	49.4 ±4.2	65.5 ±3.1	-
Emergence of 100% of coracidia	-	1686.2 ±56.3	1307 ±27.7	616 ±31.4	433.8 ±14.8	262 ±9.7	144 ±11.5	117 ±0	-
% of coracidia emerging	-	9.7 ±1.6	24.7 ±4.3	63.9 ±13.1	77.2 ±5.99	92.1 ±4.8	96.2 ±1.3	61.4 ±2.7	-

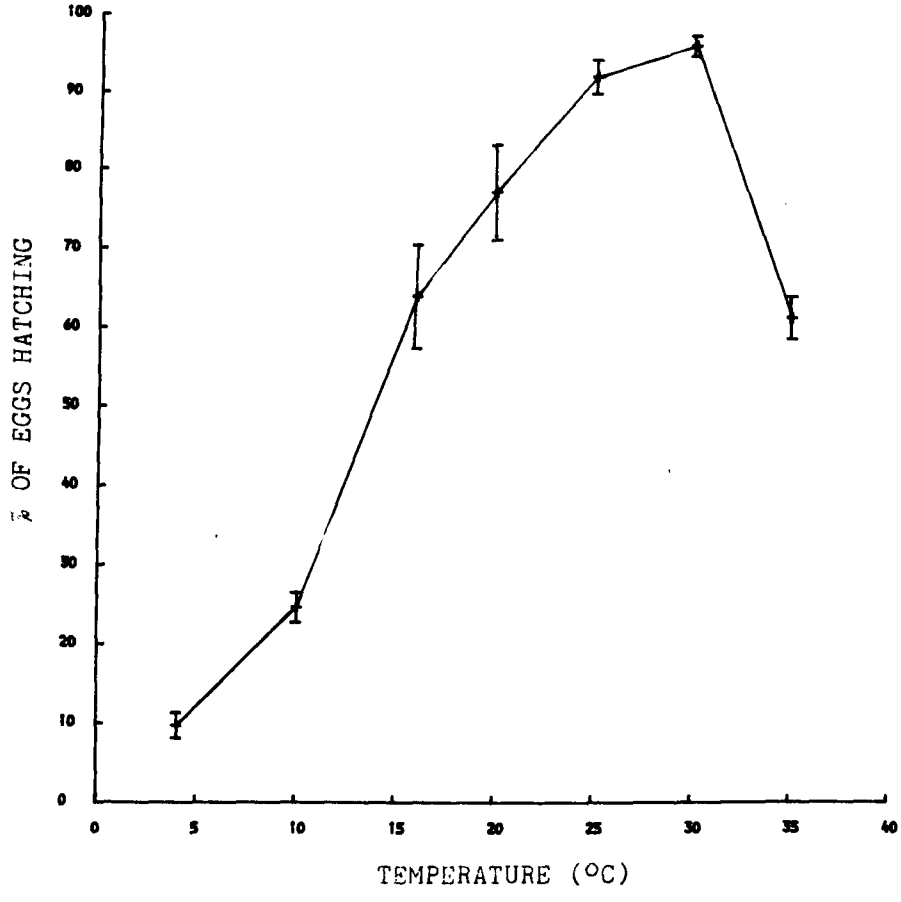


Figure 2a: The effect of temperature on the percentage of *B. acheilognathi* eggs that hatch. ($\bar{x}$ + SE).

temperature of 43°C for up to 4 hours did not reduce their viability when the temperature was reduced, however hatching did not occur at this elevated temperature. Similar Zelazny (1979) found that the eggs of B. acheilognathi could be frozen at -4°C for up to 72 hours before killing the embryos. Therefore it would appear that the final stages of embryonic development or emergence from the egg are the most temperature sensitive, and as such restrict the range of temperatures over which complete development and hatching of the egg can occur.

It is interesting to compare the temperature optima, and temperature limits for normal embryonic development in B. acheilognathi with those for other members of the order Pseudophyllidea from freshwater fishes. The optimum temperature for embryonic development in B. acheilognathi is 30°C. At this temperature the eggs hatch in the minimum time with maximal survival. As indicated above the eggs hatch at temperatures ranging from 4-35°C. Normal development for members of the family Ligulidae takes place between 10 and 32°C, with the optimal temperatures for Ligula intestinalis being 23-25°C and Schistocephalus solidus being 18-20°C (reviewed Dubinina 1966) ; for members of the family Diphyllobothridae at 5-30°C, with the optimum temperature for D. latum being 18-20°C (reviewed Dubinina, 1966); and for the Triaenophoridae at 2-26°C (Watson and Lawler, 1963), with the optimum temperature for Triaenophorus crassus being 17-20°C, T. nodulosusbeing 20°C and T. meridionalis 22-24°C (Kuperman, 1973). Therefore the eggs of B. acheilognathi have a higher optimum temperature and a greater temperature range than other pseudophyllideans.

In addition the embryonic development of B. acheilognathi (Time taken for emergence of coracidia, Table 1) is more rapid than in other members of the order Pseudophyllidea reviewed by Dubinina (1966). This may reflect the adaptation of the parasites to the higher temperatures encountered in its area of origin (S. Asia). The tolerance of a wide range of temperatures may help to explain the success of B. acheilognathi in the temperate conditions of Europe and N. America.

The ability of B. acheilognathi eggs to survive at 0°C and hatch at 4°C suggests that development could occur throughout the winter in the British Isles. In addition preliminary experiments indicated that eggs held at 4°C for long periods (60-70 days) developed normally when raised to 18°C. This suggests that the life cycle of B. acheilognathi could overwinter in the egg phase. Similar retardation of development has been observed for members of the Ligulidae (Dubinina, 1966) and Spirometra mansonoides (Mueller, 1959), and has been used to store eggs for experimental work.

Over the range of temperatures at which normal embryonic development occurs (4-30°C) the time for 50 and 100 percent of the eggs to hatch is exponentially related to temperature (Fig. 2c and d). (Time for 50% of eggs to hatch: $\log_{10}Y = -0.05 x + 3.21$, $r^2 = 0.91$ and Time for 100% of eggs to hatch: $\log_{10}Y = -0.04 x + 3.44$, $r^2 = 0.99$) where Y = time for 50/100% of eggs to hatch (hours) and x = temperature (°C). Previous authors have not described the relationship between egg hatching and temperature for other members of the Pseudophyllidea to enable comparisons to be made.

Examination of the effect of temperature on the time until the first emergence of coracidia (Fig. 2b) shows 2 distinct linear relationships. From 4-20°C an increase in temperature leads to a

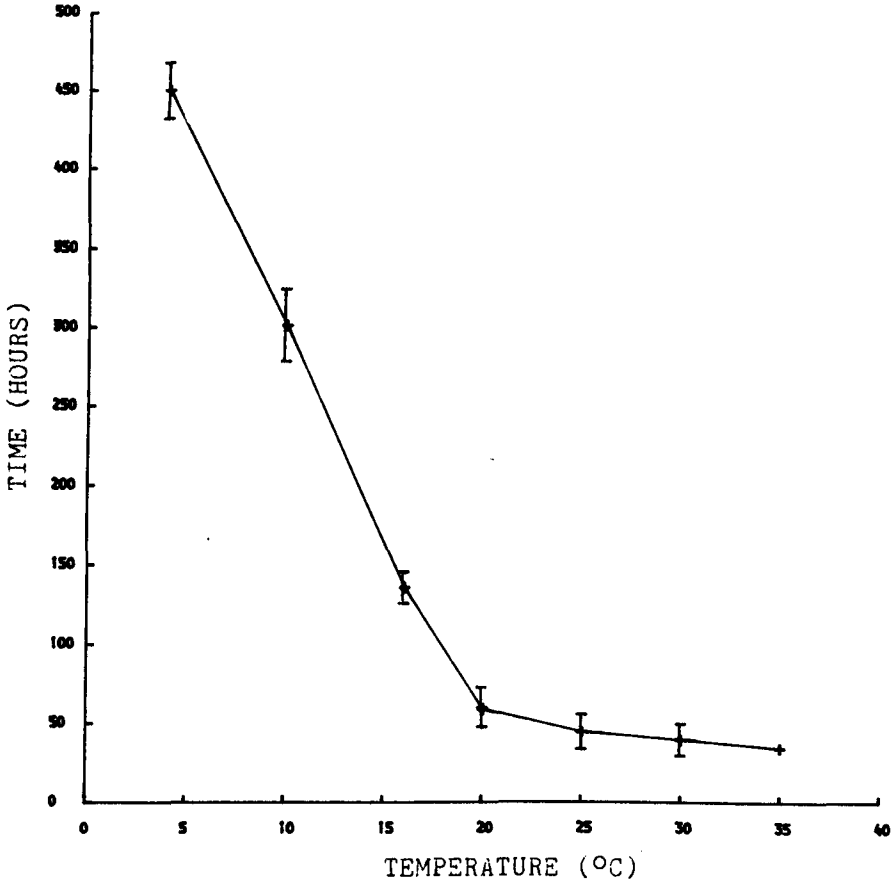


Figure 2b: The effect of temperature on the time for the first B. acheilognathi eggs to hatch. ($\bar{x}$ + SE).

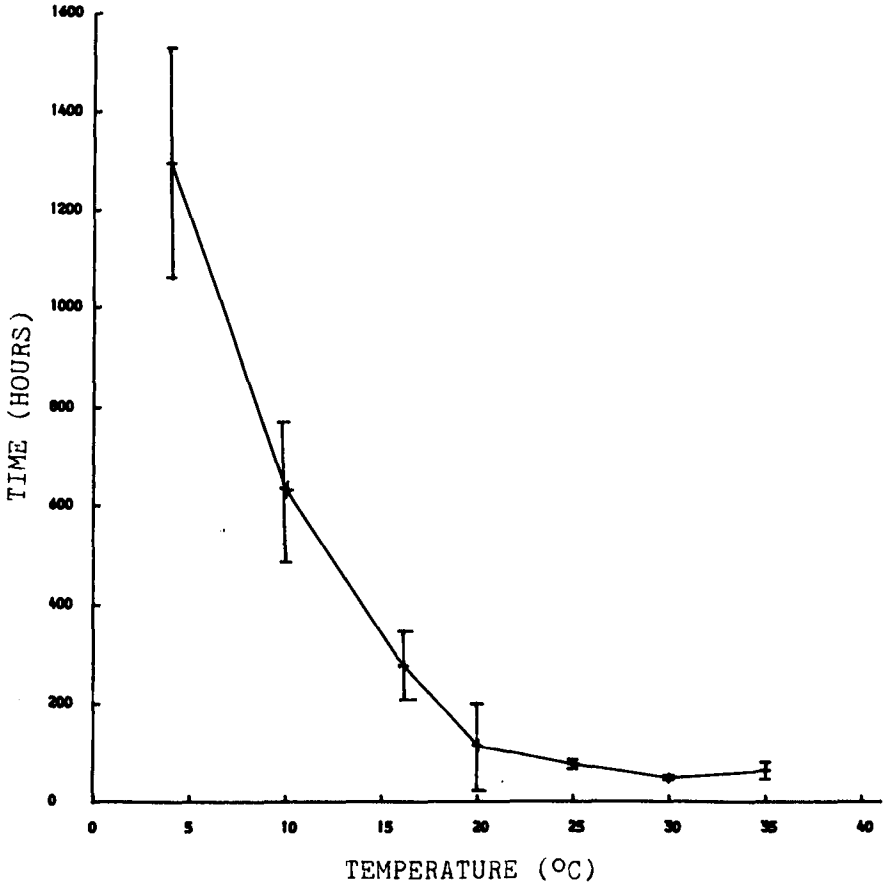


Figure 2c: The effect of temperature on the time taken for 50% of the eggs to hatch. ($\bar{x}$ + SE).

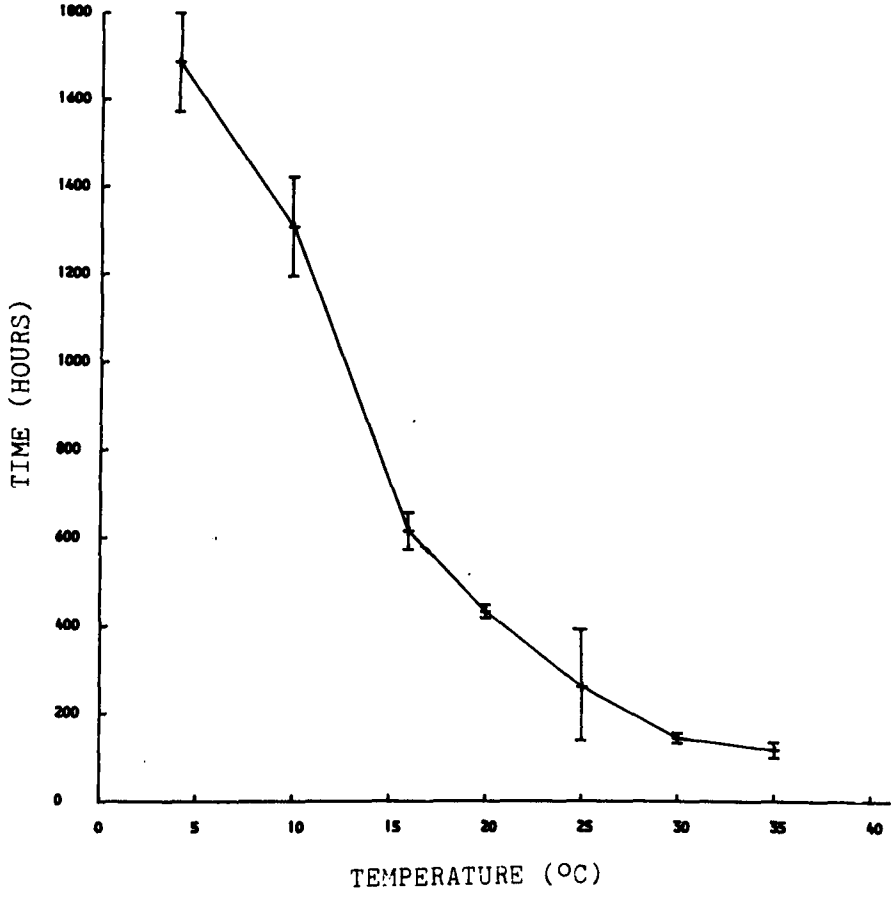


Figure 2d: The effect of temperature on the time taken for 100% of the eggs to hatch. ($\bar{x}$ + SE).

large decrease in the time required for embryonic development (Y = -24.88 x +547.6, where Y = time until first emergence of coracidia, X = temperature between 4 and 20°C). Whereas from 20-35°C an increase in temperature results in a smaller, though still significant decrease in the time taken for the first emergence of coracidia (t between time taken for emergence of first coracidium at 20°C and 35°C = 4.447, 10 df. $0.002 > p < 0.001$. Y = 1.45 x +85.1 where Y = time for emergence of first coracidium, X = temperature between 20 and 25°C). Therefore 20°C appears to be a critical temperature for the influence of temperature on the rate of embryonic development.

Guttowa (1958) observed a similar relationship between temperature and the eggs of Triaenophorus lucii, and concluded that there was a critical temperature (5°C) above which hatching of the eggs occurred normally.

Throughout these experiments very little variation was apparent in the time taken for the eggs to hatch. This is primarily a result of the time interval between observations. However large variation was observed while incubating eggs for other experiments. In one notable example the eggs hatched after only 2 hours at a temperature of 18°C. In this case the worm had been transferred from 10°C to 25°C prior to dissection, suggesting that the conditions encountered by the worm within the host may influence the degree of development of the egg while in the uterus. Kuperman (1973) cites a similar example in which the eggs of T. crassus, T. amurensis and T. orientalis from pike, hatched 3 hours after removal from the worm. Kuperman also observed that this occurred after a rise in temperature. This variation in the time for embryonic development is dependent on the degree of development within the uterus which

is, in turn influenced by the temperature, and was noted by Liao and Shih (1956) who observed that, of the eggs released at 24-29°C, 89% were fully embryonated, whereas at 14-21°C only 2% were embryonated.

3) Effect of temperature and age of the coracidium on coracidium survival and infectivity

Method

B. acheilognathi eggs were incubated at 10, 14, 18, 25 and 30°C until the coracidia started to emerge. The water was then replaced and any coracidia emerging in the following 30 minutes were collected. At each temperature, groups of 10 coracidia were aged for periods ranging from 1 to 14 hours.

Uninfected Cyclops agilis (s.str) aged 1-2 days were used in the infection experiments since these were found to be more susceptible to infection that older specimens and other species.

Ten replicates were conducted at each temperature and for each age group of coracidia. Each experiment involved placing an acclimated C. agilis into 10 drops of dechlorinated tapwater and adding a group of 10 coracidia of the same age for a 60 minute incubation period. The number of coracidia consumed was noted and after 24 hours the number of procercoids present in each copepod was recorded as a percentage of those consumed.

The survival of coracidia at 18°C was recorded at 2 hourly intervals until they were all dead.

Results

The survival of coracidia at 18°C is shown in figure 3a. The coracidia survived for a maximum of 30 hours, although some individuals died immediately after hatching. Anderson and Whitfield (1974) observed a similar pattern of survival for the cercariae of Transversometra patialense and concluded that it was a result of age dependent mortality, caused by the progressive utilisation of the non-replaceable food reserves. Their conclusions may also explain the observed age dependant mortality of B. acheilognathi. The gradual absorbtion of water by the coracidium (Pool, 1984; Dubinina, 1966) may also contribute to the age dependent mortality.

The duration of the life of the coracidia of B. acheilognathi is also temperature dependent (Fig. 3b). The relationship being linear and represented by the equation $Y = -1.45 x + 53.1$ ($r^2 = 0.98$) (where Y = time in hours and x = temperature, °C). Coracidia have no mechanism for absorbing external nutrition, and their energy is obtained from reserves of fat and glycogen stored in the form of granules within the embryonic envelope (Dubinina 1966). The rate of utilisation of these substances will depend on the metabolic rate, which in turn is related to the temperature. Therefore at higher temperatures the energy reserves will be exhausted more rapidly, resulting in the coracidium having a shorter lifespan (Dubinina, 1966; Kuperman, 1973; Grabiec et al., 1962, 1965).

Similar temperature dependent mortality has been described, for example, for the coracidia of Diphyllobothrium latum (Neuymin, 1953) and members of the family Ligulidae (reviewed Dubinina, 1966) and Triaenophoridae (reviewed Kuperman, 1973), together with the miracidia of Schistosomatum douthitti (Farley, 1962) and the

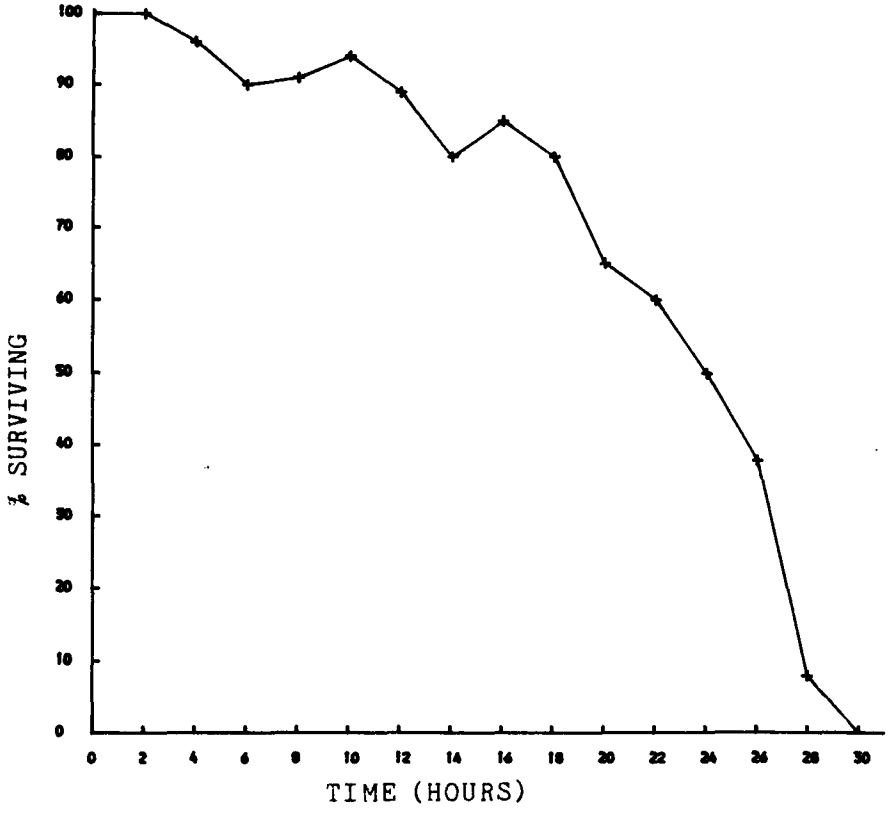


Figure 3a: The survival of the coracidia of B. acheilognathi maintained at 18 °C.

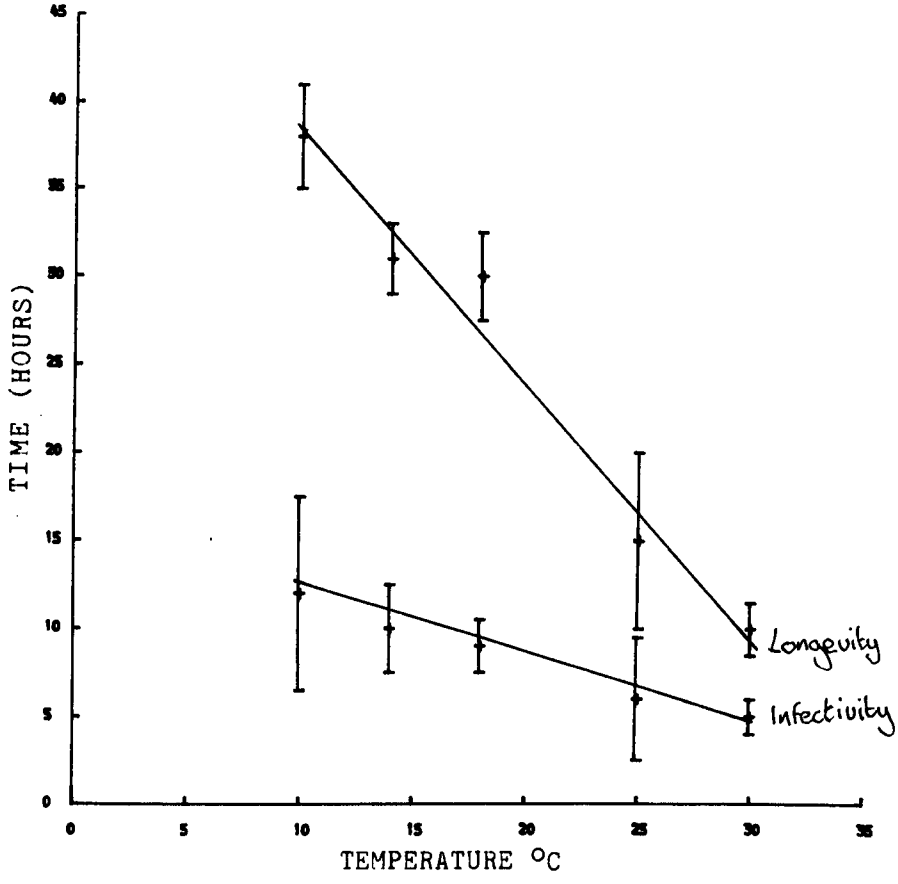


Figure 3b: The effect of temperature on the longevity and time when the coracidia of B. acheilognathi were infective. ($\bar{x} \pm SE$)

Longevity: $Y = -1.45X + 53.1 \quad r^2 = 0.98$

Infectivity: $Y = -0.42X + 17.0 \quad r^2 = 0.93$

cercariae of Transversotrema patialense (Anderson and Whitfield, 1974) and Orientobilharzia datlai (Dutt and Srivasta, 1962).

At a temperature of 4°C and 35°C hatching of the eggs occurred, (see section 2), but the numbers of coracidia emerging and surviving was too low to enable them to be included in the study. The coracidia at 4°C showed very litle activity, but did undergo the same degeneration observed at higher temperatures. They were active for approximately 48 hours. Granath and Esch (1983b) obtained coracidia at 35°C, however they observed that the peak incidence of motile coracidia was lower than at 30, 25 and 20°C.

It is interesting to compare the life span of the coracidia of B. acheilognathi (Fig. 3a) with those of other pseudophyllidean cestodes. Schistocephalus solidus coracidia survive for 96-120 hours at 5-8°C, 48 hrs at 1-18°C and less than 24 hours at 22-25°C; Ligula intestinalis coracidia survive for 24-36 hours at 24-28°C, and less than 4 hours at 35°C (reviewed Dubinina, 1966) whereas the coracidia of Triaenophorus nodulosus survive for 240 hours at 5-7°C, 24-72 hours at 18-20°C and 1 hour at 29°C (Kuperman, 1973). In general therefore, the coracidia of B. acheilognathi survive for a longer period of time at a given temperature than those of other pseudophyllidean cestodes.

The infectivity of the coracidia was found to be age and temperature dependant (Fig. 3b and c). As infection is an active response requiring the production of a glandular secretion and penetration of the copepod intestine (Dubinina, 1966), it will be less efficient in older coracidia due to diminishing energy reserves. The coracidia are not infective throughout their life span, suggesting that the older specimens have not got sufficient

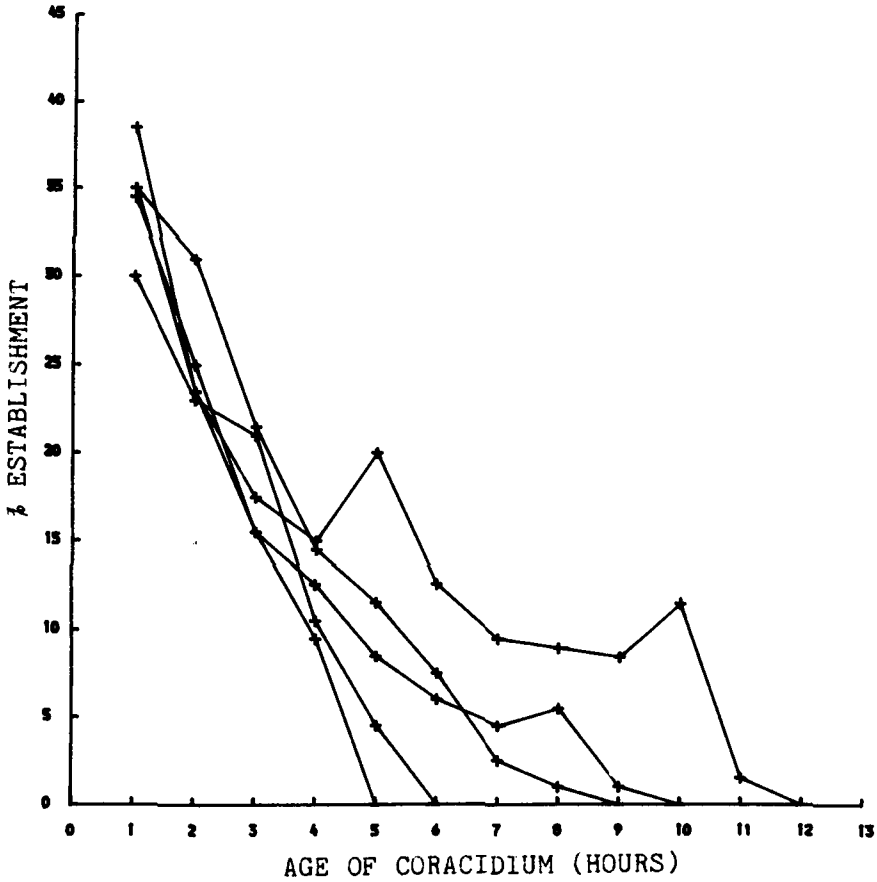


Figure 3c: The effect of temperature and age on the infectivity of the coracidia of B. acheilognathi.

energy reserves to enable infection of the copepod to occur. Observation of the coracidia suggested that they were not infective after they had stopped actively swimming and dropped to the bottom of the dish. A similar relationship was thought to be responsible for the decreased infectivity of older cercaria of Transversotrema patialense (Anderson and Whitfield, 1975).

The time taken for the coracidia to reach this uninfective stage decreased linearly with temperature (Fig. 3b) and was described by the equation $Y = -0.42 \times 17.0$, $r^2 = 0.93$ (where Y = the time when the coracidium is infective in hours and X = temperature (^{o}C)). The decreased infective period at higher temperatures can be explained by the increased role of utilisation of the energy reserves (Anderson and Whitfield, 1975).

Comparison of the infectivity of 0-1 hour old coracidia at the temperatures tested using Kruskal-Wallis analysis showed there to be no significant difference ($H = 3.16$, $p > 0.05$), suggesting that the coracidia emerging at different temperatures had the same energy reserves initially.

It is interesting to note that the number of coracidia consumed by the copepods varied with the age of the coracidia. Young coracidia (0-1 hours old) were eaten less frequently than innactive, old coracidia. This may have been a result of the older coracidia sinking to the bottom of the infection chamber, and so coming into contact with the copepods more frequently. In natural conditions the active swimming and positive phototaxic response of the young coracidia would cause them to rise to the surface of the water body, and into the area frequented by the copepods (Dubinina, 1966).

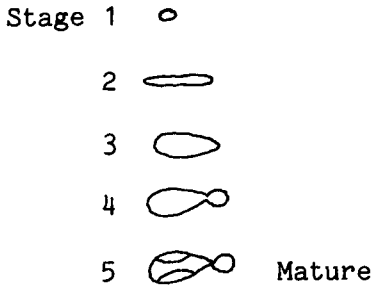
The infestation levels achieved in this study are unlikely to be reached in natural water bodies. Liao and Shih (1956) conducted

a detailed survey of the copepod fauna of fish rearing ponds along the Pearl River, China and found the maximum rate of infection be to 7%. Similar studies have found very low levels of infection of copepods by the coracidia of other Pseudophyllidea (e.g. Guttowa, 1963, Dubinina, 1966). Indeed in his accurate study of the population dynamics of B. rarus Jarroll (1980) estimated that only 0.8% of the copepods in an artificial pond were infected.

4) Effect of the age of the coracidium when consumed on the subsequent development of the procercoid.

Method

C. agilis were infected with 1-6 hour old coracidia using the procedure outlined in section 3. 10 copepods containing single procercoids were used for each experiment. The copepods were maintained individually, and fed daily on one drop of mature hay infusion. The procercoids were examined daily, and the stage of development was described using the following easily distinguishable stages.



Results

The results presented in figure 4a and b indicate that the age of the coracidium when consumed by Cyclops agilis affects the subsequent development of the procerocid. Comparison of the time taken for the procercoid to reach maturity using a Newman-Kuels multiple range test showed there to be no significant difference between procercoids originating from 1 and 2 hour old coracidia (1hr vs 2hr $q = 2.38$ $p > 0.05$) but a significant difference between those originating from 3, 4 and 6 hour old coracidia (2hr vs 3hr $q = 6.89$ $p < 0.001$; 2hrs vs 4hrs, $q = 4.596$, $0.005 > p > 0.001$; 4hrs vs 6hrs $q = 5.64$ $p < 0.001$). The data followed a linear relationship (Fig. 4a), which can be described by the equation $Y = 2.88x + 13.79$ $r^2 = 0.81$ (where Y = Age of procercoid when mature (days), X = age of coracidia when consumed in hours) and shows that procercoids originating from younger coracidia require a shorter period of time to develop to maturity (other things being equal).

In section 3 it was suggested that the coracidia have a finite energy supply which is utilised in swimming and infection of the copepod host (Dubinina, 1966; Kuperman, 1973). Therefore there appears to be a correlation between the age of the coracidium, its energy reserves, and the time taken for the resultant procercoid to develop to maturity.

The initial stages of development within the copepod haemocoel involves the development of the microvilli (Dubinina, 1966; Kuperman, 1973) enabling the procercoid to absorb nutrients. This is presumably an energy dependant process, and as such will be completed more rapidly by coracidia with abundant energy reserves. Therefore growth of procercoids originating from young coracidia would commence before those originating from old coracidia. In

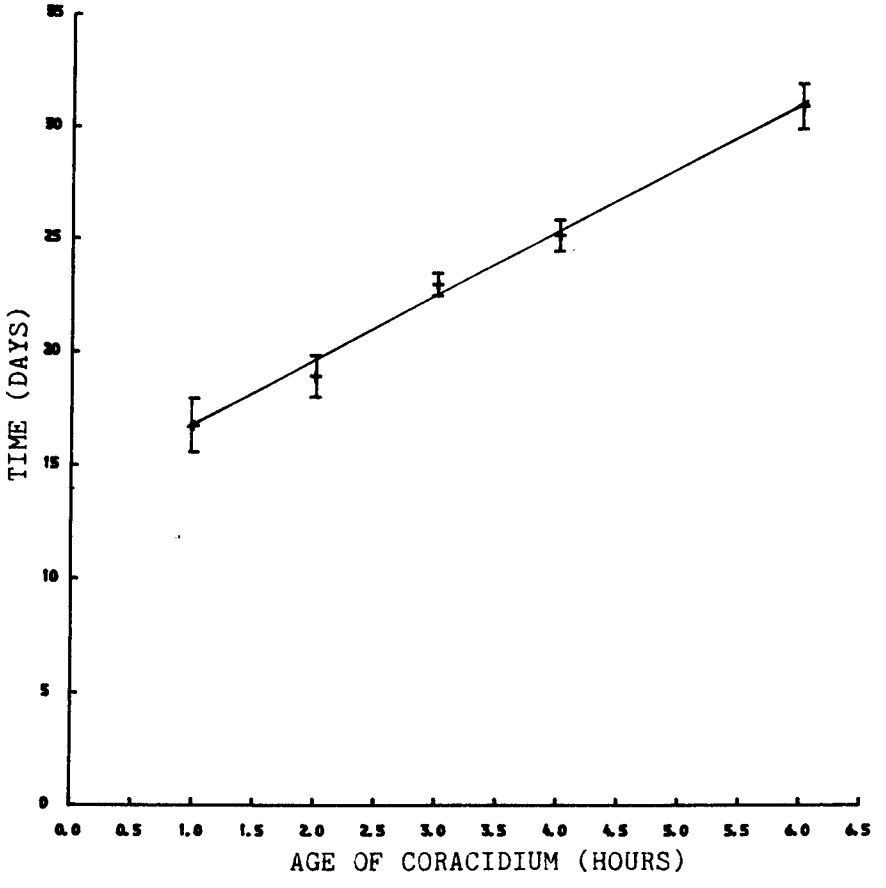


Figure 4a: The effect of coracidium age when consumed by C. agilis on the time taken for the procercoid to reach maturity. ($\bar{x}$ ± SE)

$Y = 2.88X + 13.79 \quad r^2 = 0.81$

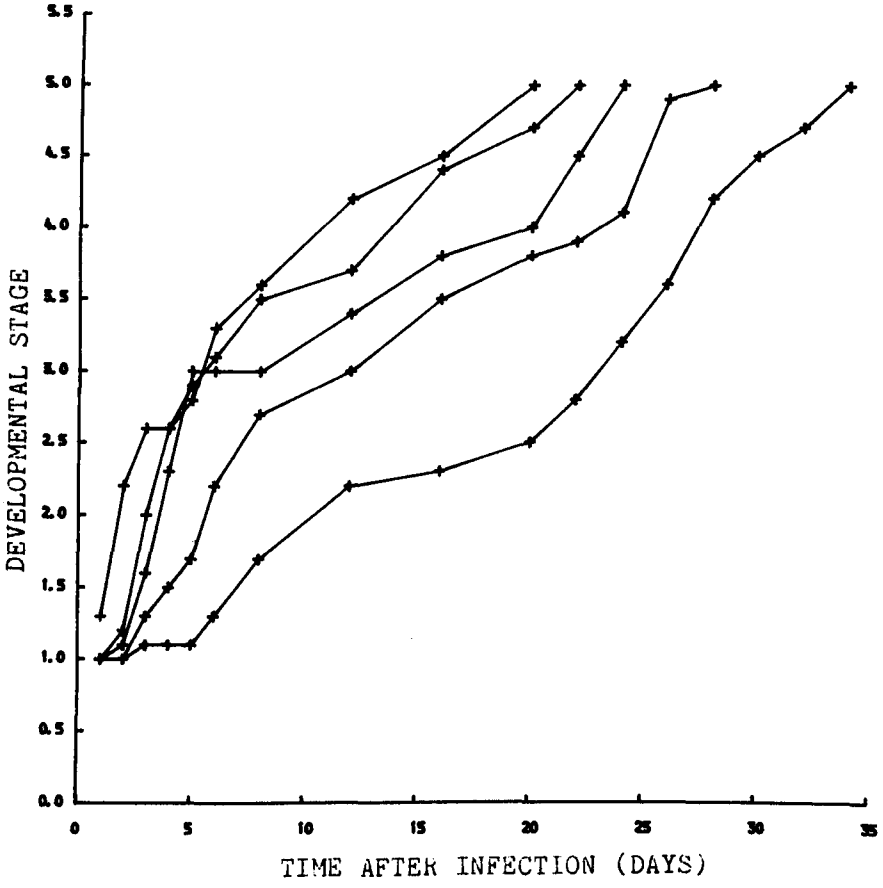


Figure 4b: The effect of coracidium age on subsequent procercoid development. (Error bars omitted for clarity).

addition Dubinina (1966) observed that the copepod host can show a protective reaction to the penetration of the onchosphere. The severity of this reaction was shown to vary depending on the condition of the host and the viability of the onchosphere. In terms of the present study the young onchospheres would be more viable than those originating from old coracidia, and so would be better able to withstand the host reaction resulting in a more rapid initial growth rate.

Comparison of the stage of development 3 days after entry into the copepod (Fig. 4b) using Newman-Kuels analysis indicates that the procercoids originating from 1, 2, 3 and 4 hour old coracidia differ significantly ($p < 0.05$) whereas those from 4 and 6 hour old coracidia do not ($p > 0.1$). Therefore it appears that procercoids from young coracidia develop more rapidly.

This variation in initial development has no significant effect on the overall development of the procercoids, as indicated by the length of the mature procercoids (mean length of those originating from 1 hour old coracidia = 0.17mm, SE = 0.007; 2 hour old coracidia x = 0.18mm, SE = 0.008; 3 hour old coracidia x = 0.18mm, SE = 0.006; 4 hour old coracidia x = 0.17mm, SE = 0.005; 6 hour old coracidia x = 0.16mm, SE = 0.003. Mann-Whitney test between lengths of coracidia originating from 2 and 6 hour old coracidia (greatest difference), $U_1 = 72$ $n_1 = 10$, $n_2 = 10$, $0.2 > p > 0.1$).

The rates of development of the procercoids of Eubothrium salvelini and Spirometra mansonoides were studied by Boyce (1974) and Mueller (1959) respectively. They noted that the procercoids developed at different rates under identical conditions of temperature, time, intensity of infection and host species.

Although this variation was attributed to intrinsic differences among individual metacestodes, it may also have resulted from the procercoids originating from coracidia of different ages.

5) The effect of temperature and age of the procercoid on establishment within a C. carpio definitive host

Methods

The experiment was conducted at 4, 10, 14, 18, 25, 30 and 35°C. Eggs were hatched at each temperature, and 1-2 day old C. agilis were infected with 0-1 hour old coracidia. 10 copepods containing single procercoids were fed to each of 10 uninfected 2.5cm C. carpio.

Infection of the fish was accomplished by placing a single C. carpio and 10 infected C. agilis into a 300ml crystalising dish. The dish was placed on a black surface to make the copepods conspicuous. Consumption of the copepods usually occurred within 2 hours. Fish which had not consumed all of the copepods within 6 hours were not included in the experiments.

Preliminary experiments indicated that the procercoids were unable to establish in the C. carpio intestine prior to development of the cercomer and bothria. The procercoids were therefore examined at daily intervals until development of the cercomer was evident they were then fed to the C. carpio at 5 day intervals (10 and 14°C) or 2 day intervals (18, 25 and 30°C).

24 hours after infection the C. carpio were sacrificed and the number of plerocercoids within the intestine was recorded.

Results

Temperature has a pronounced effect on procercoid development, with an increase in temperature (within the range 10-30°C) resulting in an exponential decrease in the time taken to become infective, the total time when the procercoid was infective and the maximum infectivity (Fig. 5a, b and c).

The relationship between temperature and the time taken for the procercoid to become infective can be described by the equation $\log_{10} Y = 0.064 x + 3.98$, $r^2 = 0.95$ (where Y = time taken for the procercoid to become infective in hours and x = temperature in °C). Previous authors have not established a precise relationship, but have given developmental times at a range of temperatures. Thus Liao and Shih (1956) noted that the procercoids of B. acheilognathi became mature in 4 days at 25-33°C, 5 days at 20.3°C and 21 days at 13.8°C; whereas Bauer et al. (1969) found that the procercoid became infective after 10-12 days at 16-19°C, 5-7 days at 22-25°C and 4 days 25-30°C. For comparison the present study found that at 10°C the procercoids were infective after 110 days, at 14°C after 60 days, at 18°C after 17 days, at 25°C after 12 days and at 30°C after 5 days. Therefore in the present study the procercoids required a longer period of time to reach maturity. The different size of the copepod intermediate host used by the various authors may account for the observed variation in the rate of development (Dubinina, 1966).

Development of the procercoid to an infective stage was only observed over the temperature range 10-30°C. At 4°C no development of the procercoid was observed despite incubation of the infected copepod of up to 80 days. Above 30°C procercoid development occurred rapidly, but the infected C. agilis died before the

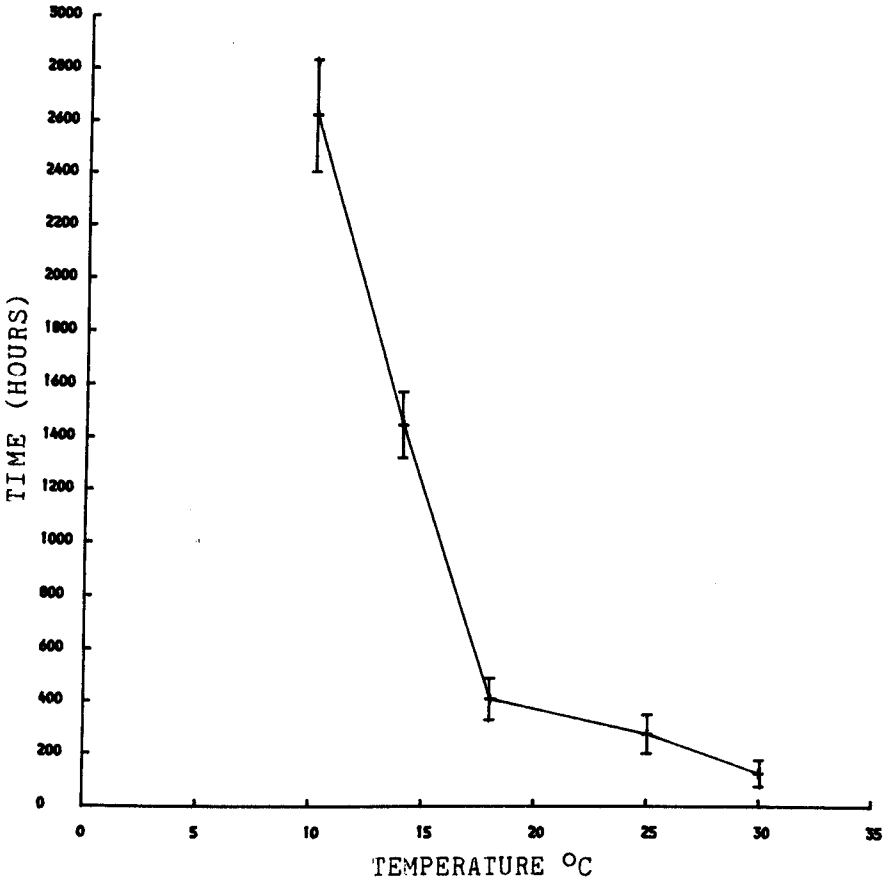


Figure 5a: The effect of temperature on the time taken for the procercoid to develop to an infective stage. ($\bar{x} \pm SE$)

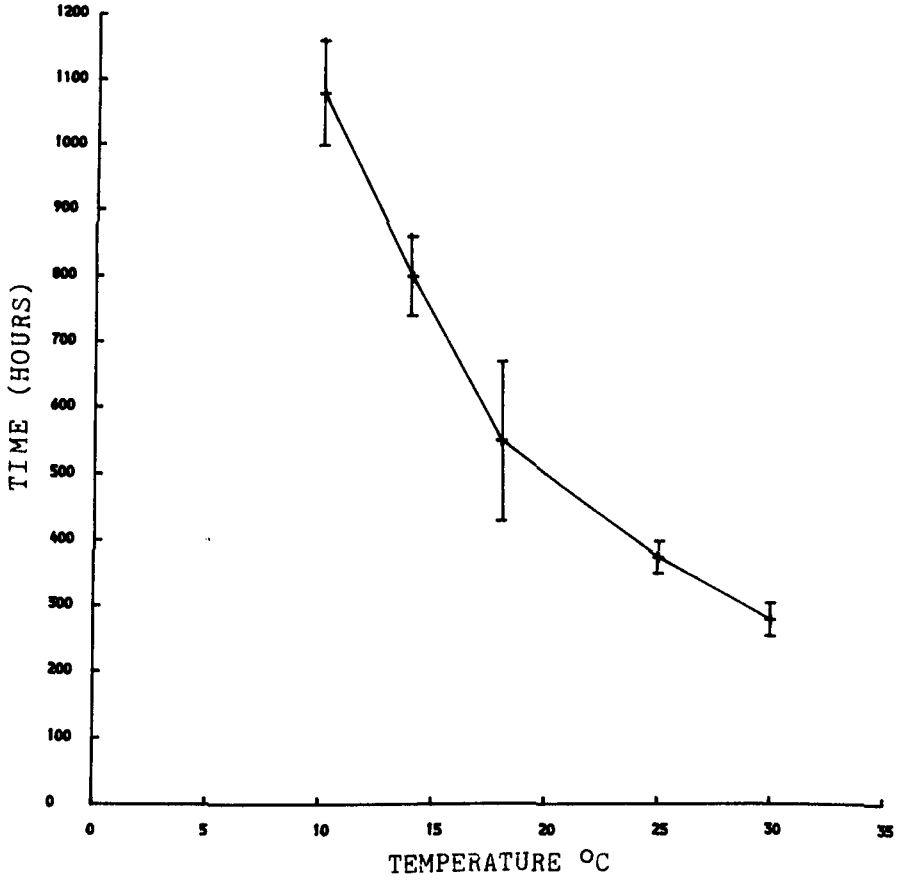


Figure 5b: The effect on temperature on the total time when the procercoids were infective. ($\bar{x} \pm SE$)

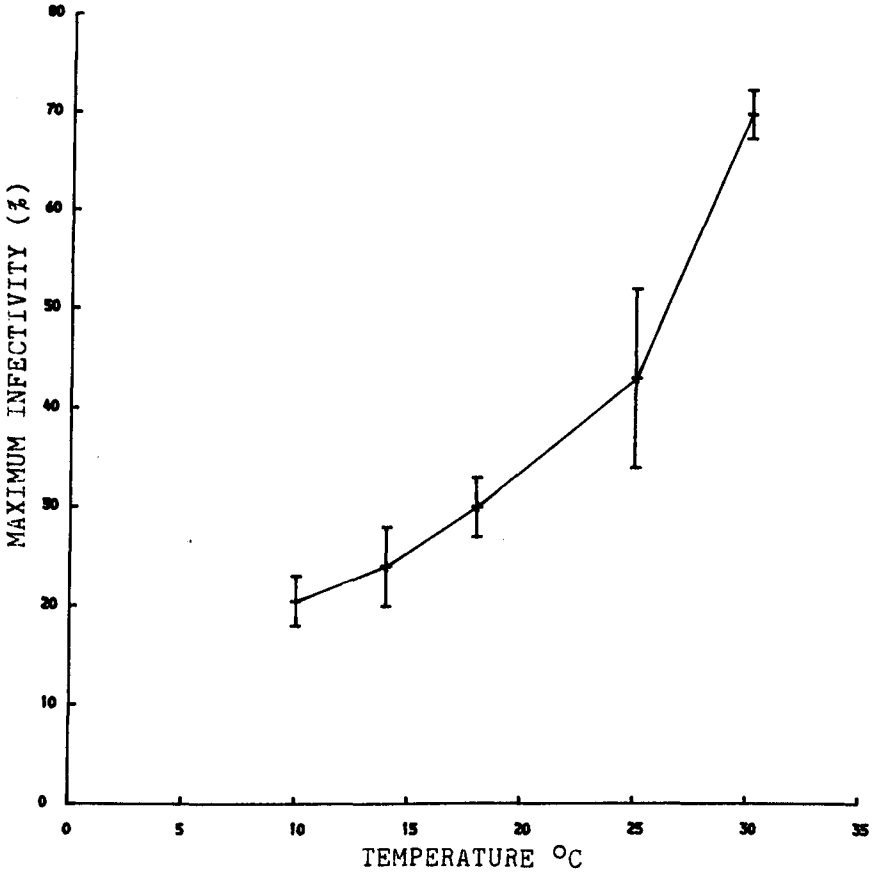


Figure 5c: The effect of temperature on the maximum infectivity of B. acheilognathi procercoids. ($\bar{x} \pm SE$)

procercoid reached maturity. Liao and Shih (1956) observed procercoid development at 8 and 33°C although they do not indicate if they became infective. Granath and Esch (1983a and b) observed the recruitment of B. acheilognathi at temperatures up to 40°C, however their experiments were conducted in natural conditions which would be subject to intermittent and diurnal fluctuations in temperature. Therefore establishment and/or development of the procercoid may have occurred at temperatures considerably lower than 40°C.

Data concerning the development times for the procercoids of other pseudophyllideans is sparse. However, that provided in the reviews of Dubinina (1966) and Kuperman (1973) show that the time taken for development to maturity is similar to that of B. acheilognathi over the range of temperatures examined. No data is available for development times at temperatures greater than 22°C. Triaenophorous nodulosus procercoids have a lower thermal limit than B. acheilognathi and can develop to maturity at 4°C (taking over 30 days) (Kuperman, 1973).

The total time when the procercoids were infective was also exponentially related to temperature (Fig. 5b) being described by the equation $\log_{10}Y = -0.03 x + 3.28$, $r^2 = 0.99$ (where Y = time when procercoid was infective in hours, and x = temperature in °C). Death of the procercoid was usually associated with the death of its C. agilis host, although in a number of cases dead or dying copepods were found containing active procercoids suggesting that it is the death of the host which is limiting the procercoid lifespan (Kuperman, 1973). There is considerable disagreement regarding the effect that procercoids have on their copepod intermediate hosts.

Kuperman (1973), Michajlow (1953) and Watson and Price (1960) suggested that the procercoids have no effect on the copepod, whereas Amman (1955) and Dubinina (1966) believed that there was a direct or indirect relationship between the level of infestation of the copepod and its survival and longevity. These observations were made on densely infected copepods. It is unlikely that the single procercoid infections used here will have a great effect on copepod longevity. Instead the observed results may reflect the effect of temperature on the life span of the intermediate host.

The procercoids were first able to establish in the fish intestine upon development of the bothria. As the bothria developed the procercoids were better able to establish until a maximal level was attained after which the percentage establishment remained similar until the death of the procercoid (Fig. 5d). Associated with the development of the bothria was the presence of calcareous granules within the procercoid. The function of these granules is unknown, although Dubinina (1966) believed them to be involved in the establishment of the procercoid within the intestine of the fish host by preventing the action of the digestive enzymes.

Comparison of the maximal level of establishment at the range of temperatures examined shows there to be an exponential relationship, with the procercoids having a higher maximum establishment at higher temperature. This relationship can be expressed in the formula $\log_{10}Y - 0.025 x + 1.02$, $r^2 = 0.97$ (where Y = maximum percentage establishment of procercoids and X = temperature in ^{o}C) (see Fig. 5c). This is presumably due to the increased activity of the procercoids at the higher temperatures, resulting in a higher probability of them coming into contact with, and attaching to the villi within the intestine.

Halvorsen (1966) noted that for Diphyllobothrium norvegicum (=D. dendriticum) the average size of the procercoids was smaller at the lower temperatures tested. This, he sugested was a result of the unfavourable conditions, and mirrored the situation in densely infected copepods. In the present study it is suggested that procercoid density, size and infectivity are associated (section 6). In the case of temperature, the size of 10 procercoids from 30°C and 10°C was 0.21 (SE = 0.01) and 0.15 (SE = 0.008) respectively. Comparison of these means using a Mann-Whitney U test showed them to differ significantly (U_1 = 91, U_2 = 9, N_1 and N_2 = 10 $0.002 > p > 0.001$) suggesting that the size of the procercoids at different temperatues may be responsible for the observed temperature dependent establishment.

Previous work on parasite establishment has shown it to be temperature independent e.g. Pomphorhynchus laevis (Brown, 1984) or reduced at raised temperatures, e.g. Caryophyllaeus laticeps (Kennedy and Walker, 1969) and Echinorhynchus truttae (Awachie 1963). Anderson (1976) found that at raised temperatures greater establishment of C. laticeps occurred due to the increased feeding rate of the host. In the case of B. acheilognathi the increased percentage establishment of the procercoid together with the increased feeding rate at higher temperatures would result in a great increase in the numbers of parasites entering the intestine at raised temperatures in a natural situation.

6) The relationship between the density of the procercoids and establishment in a Cyprinus carpio definitive host

Methods

Approximately 10 0.1 hour old coracidia were fed to 1-2 day old Cyclops agilis at a temperature of 18°C. After approximately 10 days the copepods were examined and divided according to the numbers of procercoids within them.

Upon development of the bothria, the copepods were fed to 2.5cm C. carpio (see section 5). Approximately 10 procercoids from equally infected copepods were fed to each of 10 replicates i.e. 10 C. agilis containing 1 procercoid each (=10x1), 5x2, 3x3, 2x4, 2x5, 2x6, 1x7, 1x8, 1x9 or 1x10 procercoids. A constant meal size of 10 copepods was given to each fish by the addition of uninfected copepods.

The fish were dissected 24 hours after infection, and the percentage of plerocercoids which established was calculated.

Results

The percentage establishment of the procercoids of *B. acheilognathi* in the *C. carpio* definitive host decreases exponentially as the number of procercoids per copepod increases (Fig. 6a) and is described by the equation $\log_{10} Y = 0.15x + 2.05$, $r^2 = 0.95$ (where Y = % establishment and X = number of procercoids per copepod). Several factors may be responsible for this observation.

Within the *C. carpio* intestine the procercoids are released from the copepod host by means of the mechanical action of the pharangeal teeth and the digestive enzymes of the host (Anderson, 1976, Dubinina, 1966). It might be speculated that a greater proportion of the procercoids from a densely infected copepod would be damaged by the mechanical action of the pharangeal teeth, resulting in fewer procercoids establishing.

In addition, several authors have reported that the procercoids from singly infected copepods were larger than those from multiple infections (e.g. Dubinina, 1966; Kuperman, 1973; Halvorsen, 1966). In the present study this was also found to be so, with a linear decline in procercoid length as procercoid density increased (Fig. 6b). This can be represented by the equation $Y = -0.006 x + 0.191$ $r^2 = 0.84$ (where Y = Procercoid length in mm and X = the number of procercoids per copepod.

The size of the procercoid may influence its ability to establish in two ways. Firstly the larger procercoids will have larger bothria, enabling them to grasp the intestinal villi more easily (see section 8). Secondly, the size of a procercoid may influence its energy reserves. Large procercoids might have greater energy reserves, and therefore be better able to move against the

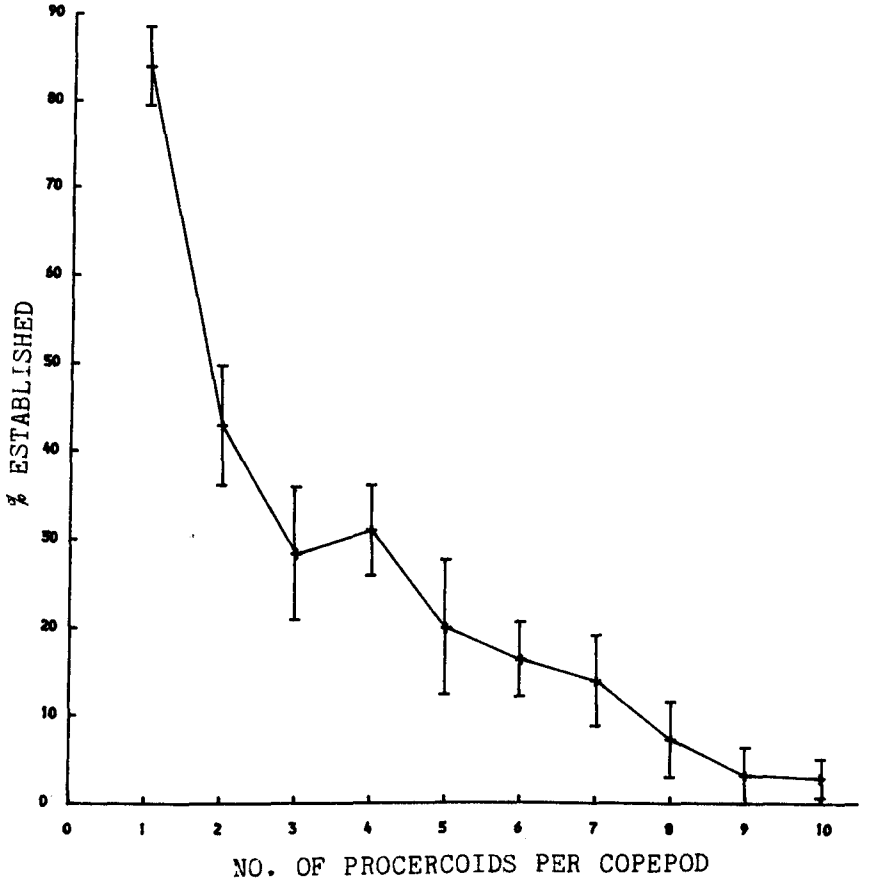


Figure 6a: The effect of procercoid density on the establishment of B. acheilognatni procercoids in a C. carpio definitive host. ($\bar{x} \pm SE$)

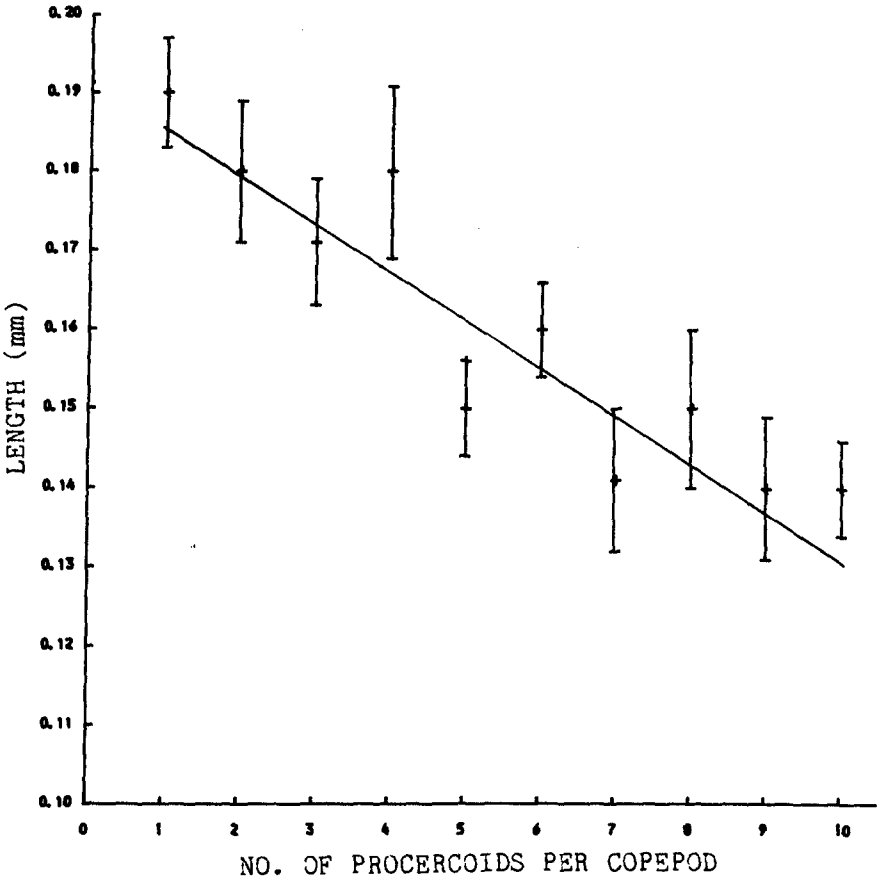


Figure 6b: The effect of procercoid density on the length of the procercoids of B. acheilognathi in a C. agilis intermediate host. ($\bar{X}$ + SE)

$Y = -0.006X + 0.191 \quad r^2 = 0.84$

peristaltic flow of the intestinal material, and come into contact with the intestine wall. Dubinina (1966) disagrees with the idea of size dependent establishment, indicating that different sized procercoids are all equally infective.

Several authors have observed that in densely infected copepods the rate of development varied considerably (Dubinina, 1966; Kuperman, 1973; Michajlow, 1953; Körting, 1975) this being reflected in the time taken for the procercoid to reach the infective stage. Indeed Kuperman (1973) noted that when 5 or more procercoids were present in a single copepod density dependent effects became apparent. In the densely infected copepods used in this experiment the procercoids may not have been equally infective, resulting in the reduced establishment. This effect was minimised by careful observation of the infected copepods to ensure all of the procercoids were at an infective stage before feeding to the definitive host. However, at increased procercoid densities it was more difficult to see all of the procercoids therefore errors might have occurred.

In natural conditions the number of copepods will be considerably lower than in the present study. The maximum infestation levels of Triaenophorous nodulosus were found to be 1-3 procercoids per copepod in Heming Lake (Watson and Lawler, 1965) therefore the density dependent effects noted here, and by Kuperman (1973) and Dubinina (1966) are unlikely to be apparent.

7) The effect of temperature on the development and growth of the plerocercoid and adult stages.

Method

1-2 day old Cyclops agilis were fed 5 0.1 hour old coracidia at 18°C (see section 3). 48 hours after infection the copepods were examined, and those containing one procercoid were transferred at the rate of 2°C per day to be incubated at 10, 14, 18, 25 and 30°C. When infective a single procercoid was fed to a naive 2.0-2.5cm C. carpio. Single worm infections were used to minimise the pathological effects on the fish (Par, 1978) and remove the effects of intraspecific competition (Granath and Esch 1983a and b; Read, 1951; Roberts, 1961 etc.)

Infected C. carpio were maintained at the appropriate temperature of infection, and fed daily on Mainstream Salmon Fry 02 crumb or Omega No. 4 trout pellets (depending on their size). Regular feeding was important to prevent potential destrobilation (Thomas, 1937) or a reduction in the growth rate of the worms due to starvation (Davydov, 1978).

C. carpio were sacrificed at a range of times after infection and the following information was collected from any B. acheilognathi recovered.

1) Percentage position of scolex in intestine (0% = mouth and 100% = anus).
2) Length after relaxation in water until dead.
3) Total number of segments.
5) Number of mature proglottides. (As indicated by the presence of eggs in the uterus).
6) Number of eggs released when relaxed in distilled water.

7) Dry weight.

For comparison C. carpio infected with single B. acheilognathi were maintained at ambient temperature from July 1st 1984 until 3rd June, 1985. Samples were taken at approximately 50 day intervals and the information described above was obtained.

Results

The data collected is presented in figures 7a-d. To avoid overlap and confusion the error bars have been omitted.

The survival of the parasites within the host intestine is shown in Fig. 7a. At temperatures of 18, 25 and 30°C there was a gradual decline with time in the number of fish infected indicating that the parasites were progressively passing out of the intestine. The rate at which this occurred was related to the temperature, being faster at higher temperatures.

A similar increased loss of cestodes at higher temperatures has been observed by many authors (e.g. Chubb 1980, 1982; Kennedy, 1969, 1971; Hopkins 1980; Andreassen and Hopkins, 1980; Awachie 1963) and has been explained in terms of the reduced availability of nutrients or the increased peristaltic action of the intestine (Awachie, 1963), a temperature dependent immune response by the fish (Hopkins, 1980; Andreassen and Hopkins, 1980), an alteration in the physiological condition of the host (Aho and Kennedy, 1984) or due to some other temperature dependent rejection response (Kennedy, 1969).

The experimental evidence provided in this study is not suficient to identify the mechanism by which the worms are lost

Granath and Esch (1983a and b) noted a similar decline in the density of B. acheilognathi as the water temperature increased, in a thermally altered reservoir (Belews Lake) in North Carolina (U.S.A.). However they worked with multiple infections and concluded that intraspecific exploitative competition was responsible. In the present study no competitive effects were observed due to the use of single worm infections.

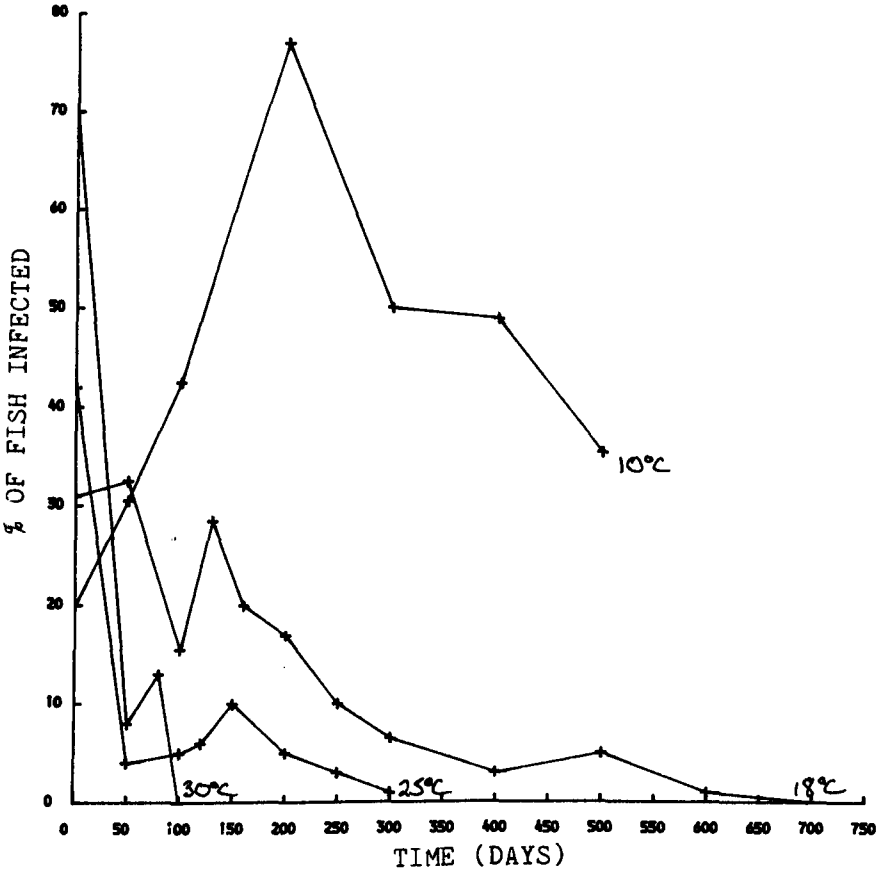


Figure 7a: The effect of temperature on the survival of B. acheilognathi in a C. carpio definitive host.

The maximum longevity of B. acheilognathi was inversely related to the temperature. Thus at 18°C the worms survived for a maximum of 670 days, at 25°C for 300 days and at 30°C for 100 days. The longevity of the worms was influenced by the temperature related loss of worms from the intestine mentioned previously, and directly by their increased metabolism and rate of development causing the more rapid completion of the life cycle (Strazhnik and Davydov, 1975).

The longevity values given here differ from those provided by other authors. Bauer et al. (1969) suggested that B. acheilognathi survived for approximately 1 year, although they do not give a temperature at which this occurred.

Chubb (1981) noted that in indoor aquaria at temperatures ranging from 8-27°C B. acheilognathi survived for at least 2.5 years. This long survival time is probably a result of the retarded growth and development that occurs at low temperatures (see later in this section).

The growth rate of B. acheilognathi at different temperatures can be compared by examining the increased length (Fig. 7b), weight (Fig. 7c) or number of segments (Fig. 7d). At each temperature and using each parameter the 3 phases of development described by Liao and Shih (1956), namely establishment, rapid growth and egg production, can be clearly seen.

When egg production commences (after 130 days at 18°C and 100 days at 25°C) the rate at which the parasites increase in size decreases (although there is still a significant variation at the 5% level shown by the use of Kruskal-Wallis analysis). Examination of the faeces of C. carpio containing mature worms showed that mature proglottides were shed by the worms (pseudopolysis) (see chapter 4).

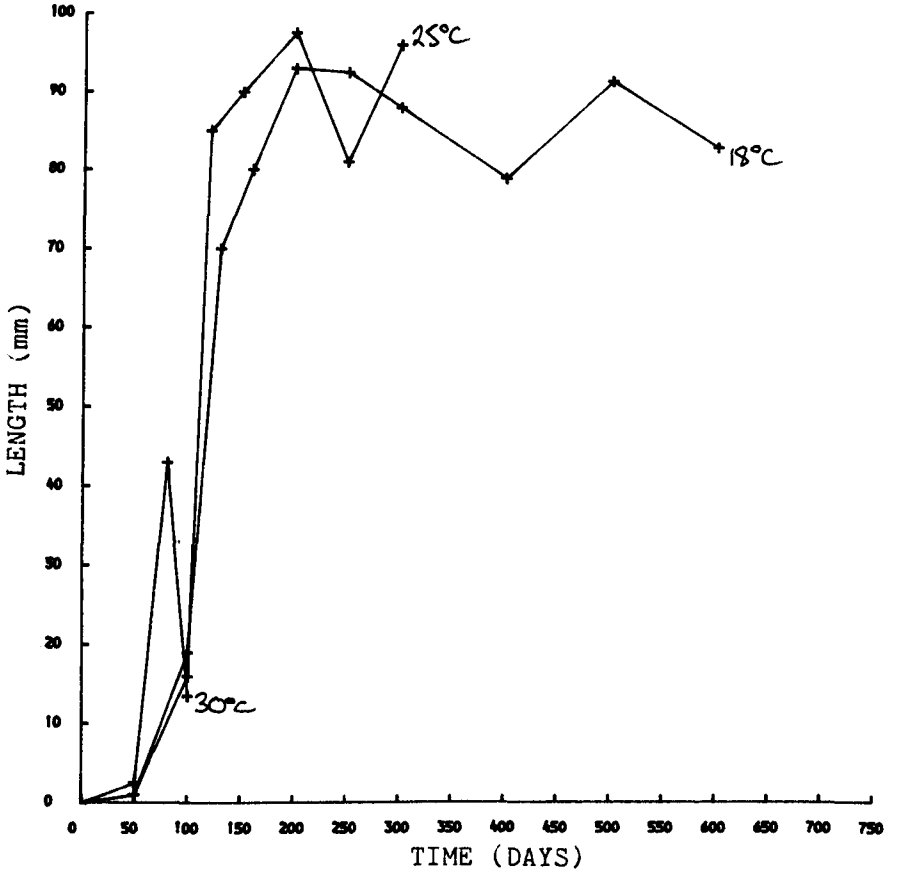


Figure 7b: The increase in length of B. acheilognathi within a C. carpio definitive host at a range of temperatures.

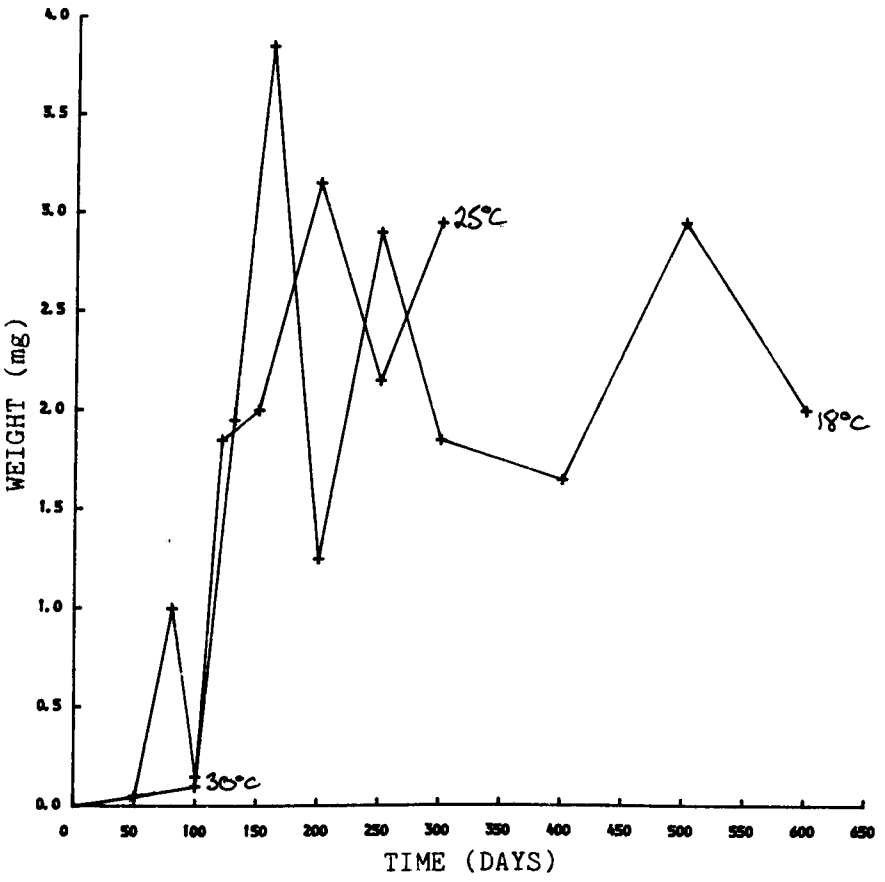


Figure 7c: The increase in dry weight of B. acheilognathi within a C. carpio definitive host at a range of temperatures.

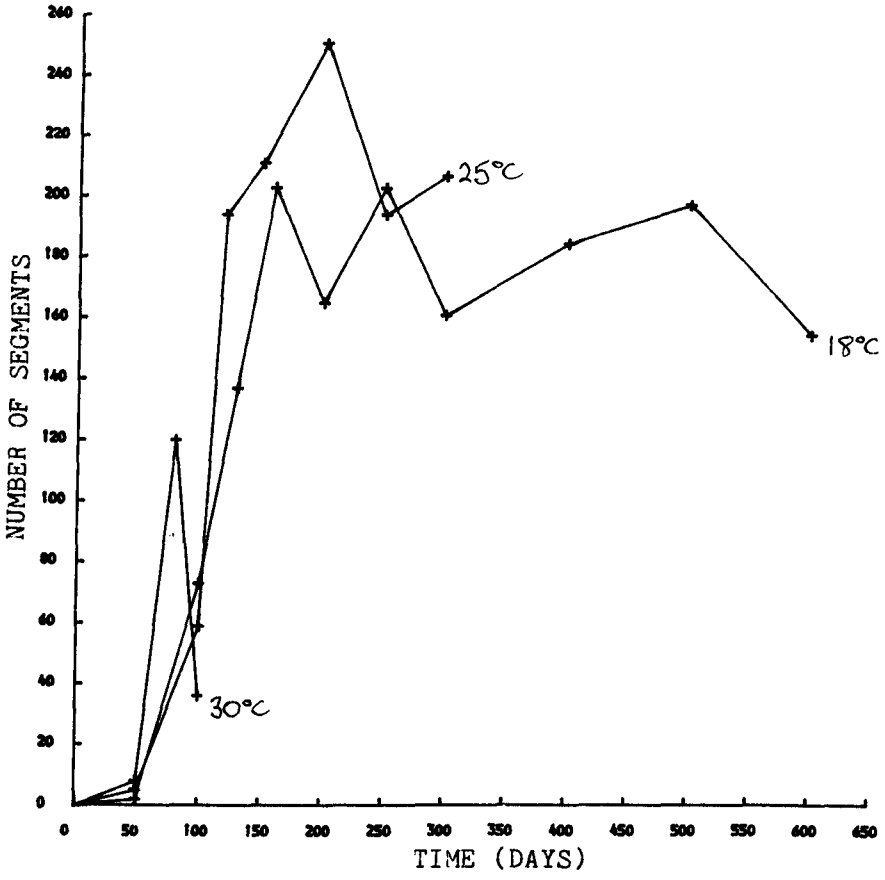


Figure 7d: The increase in the number of segments of B. acheilognathi within a C. carpio definitive host at a range of temperatures.

In order for the worms to remain approximately the same size the rate of pseudopolysis must approximate to the rate at which new segments were formed, and as such, it closely follows the pattern observed in the Cyclophyllidea (Kennedy, 1983), Diphyllobothrium latum (Wardle and McLeod, 1952) and Eubothrium salvelini (Boyce, 1974; Smith, 1973).

At 30°C B. acheilognathi shows an abnormal development pattern. In days 1-80 the rate of growth of individual parasites varied greatly, with some developing to maturity, whereas others clearly showed a retardation in growth. After 80 days an increasing proportion of the worms showed signs of retarded growth and degeneration (destrobilation) and after 100 days all of the parasites had passed out of the intestine. The possible reasons for the loss of worms were covered earlier in this section. It is interesting to note that Bauer et al. (1969) provide no information for the time taken for B. acheilognathi to reach maturity at 30°C, suggesting that in their studies the worms did not reach maturity. Granath and Esch (1983b) also reported that the lowest densities and prevalence of B. acheilognathi were recorded when the water temperature was highest, i.e. between 30 and 40°C.

At temperatures ranging from 18 to 30°C, the time taken for the plerocercoid to develop into a mature adult (producing eggs) is inversely related to temperature. Several authors have provided similar information. Although they find a similar inverse relationship, the times quoted are considerably shorter than in the present study (120 days at 18°C, 100 days at 25°C and 80 days at 30°C). Thus Bauer et al. (1969) observed that B. acheilognathi was mature after 20-21 days within the fish host at 19°C and 12-14 days

at 25°C, whereas Davydov (1978) found it took 22-25 days at 15-18°C and 12-14 days at 22-25°C. This discrepancy may result from these authors describing 'maturity' as the presence of proglottides, as opposed to the presence of eggs within the proglottides.

At 10°C there was no significant loss or growth (Mann-Whitney U analysis $U = 47$ $p < 0.05$) of B. acheilognathi over the 300 day experimental period. When a sample of infected fish maintained at 10°C for 300 days were transferred to 18°C, growth of the worms resumed and 120 days later they produced viable eggs. Therefore it appears that at 10°C development and growth was suspended, but that this did not affect subsequent development when the temperature was increased. This behaviour would obviously be advantageous in a temperate climate, where it would allow the worms to overwinter.

Statistical examination of the percentage position of attachment of the scolex of B. acheilognathi in the intestine of C. carpio shows there to be no significant variation at the 5% level at different temperatures, or with different aged parasites (using Kruskal-Wallis test). This suggests that this site (11-14% of the distance along the intestine) is optimal for the uptake of nutrients by the worm (Crompton, 1973). A more detailed examination of the site occupied by adult B. acheilognathi is provided in chapter 4.

The growth of B. acheilognathi specimens maintained at ambient temperatures is illustrated in table 2. Throughout the experimental period there was no significant loss of the worms ($X^2 = 4.22$ $df = 5$ $0.5 > p > 0.2$). This differs considerably from the pattern of survival of the parasites maintained at constant temperatures. It may be a result of the large fluctuations in temperature resulting in the parasites not being exposed to warmer temperatures for long periods; and the very low temperatures (10°C or less) for much of

Table 2. The growth of *B. acheilognathi* maintained at the ambient temperature from 1-7-1984 to 3-6-1985

Date sampled	Age (Days)	Number of worms	% of fish infected	length (mm) $\bar{x}$	length (mm) σ	weight (mg) $\bar{x}$	weight (mg) σ	No. of segments $\bar{x}$	No. of segments σ	Egg Production	Temperature $\bar{x}$	Temperature max	Temperature min
3-9-1984	50	27	27.0	1.60	1.08	0.026	0.014	3.3	2.8	No	20.8	29	14
1-11-1984	102	21	21.0	14.8	9.97	0.084	0.048	63.0	24.3	No	15.8	26	8
2-1-1985	164	22	22.0	28.0	18.5	0.335	0.299	139.0	37.9	No	10.1	18	2
9-4-1985	261	20	18.2	100.4	79.5	2.116	2.790	186.1	114.3	No	8.3	17	0
3-6-1985	311	18	30.7	66.8	13.2	1.931	0.650	169.5	25.6	Yes	18.2	29	7

the experimental period (during which the constant temperature experiment indicated that no loss of parasites occurs).

Throughout the experimental period the B. acheilognathi increased in size (length, weight and number of segments), even when the mean temperature fell below 10°C (1-11-1984 to 9-4-1985). From the constant temperature experiments we would expect no growth to occur at temperatures of 10°C and lower. Indeed a preliminary experiment at 14°C showed there to be very little growth over the first 100 days (Mean length after 100 days = 1.25mm, SE = 0.32; mean weight = 0.021ug, SE =0.009; mean no. of segments = 2, SE = 0.32). Therefore any growth that occurred during this period must have taken place when the water temperature rose above 14°C. The observed growth is still considerably greater than might be expected from the constant temperature experiment at 18°C considering the short time period when the water would be at a temperature sufficient for growth to occur. Therefore it appears that the compensatory growth described by Davydov (1978) is occurring when the water temperature rises to a suitable level. In his experiments Davydov found that B. acheilognathi specimens transferred from natural winter conditions (5-7°C) to laboratory conditions (14-16°C) showed a very rapid increase in weight over the following 3 day period.

Despite the growth of the B. acheilognathi specimens throughout the winter period (1-11-1984 to 9-4-1985), it was noted that in the 9-4-1984 sample the proglottides had fully developed uteri, but that they were empty of eggs, indicating that egg production was not occurring. Nakajima and Egusa (1977) also observed that egg production did not occur at low temperatures (0.2-12°C). While

supporting Nakajima and Egusa's observation, the present study also shows that egg production did not occur in the short periods when the water temperature rose to 18°C. In a separate study eggs were produced from worms maintained at a constant temperature of 14°C. Therefore it appears that for egg production to occur, B. acheilognathi has to be maintained at a temperature suitable for egg production for a certain minimum period of time.

It is interesting to note that the B. acheilognathi specimens examined in the 9-4-1985 sample did not show any signs of pseudopolysis, whereas in the 3-6-1985 sample all of the worms that were producing eggs had discarded spent proglottides. This phenomenon is presumably a result of the worms in the 9-4-1984 sample not having produced eggs. It would be interesting to see how large the B. acheilognathi specimens would grow if prevented from producing eggs but, allowed to grow by keeping them at a low fluctuating temperature; and if specimens approaching the size of those observed by Körting (Chapter 2) could be obtained.

8) The influence of the size of *C. carpio* on establishment of the plerocercoids of *B. acheilognathi*

Method

Using the method outlined previously (section 5) naive *C. carpio* ranging in size from 20 to 100mm in length were infected with 10 procercoids. 12 hours after feeding the fish were sacrificed and the number of plerocercoids established was recorded. The temperature throughout the experiment was maintained at 18°C and the photoperiod at 16L:8D.

Results

A significant negative correlation exists between the length of C. carpio and the percentage of plerocercoids that established within the intestine. This data is shown in figure 8 and can be described by the equation $Y = -1.40x + 92.5$, $r^2 = 0.70$ (where Y = % establishment of the procercoid and X = fork length of C. carpio in mm).

To ensure as far as possible that the C. carpio were equally susceptible to infection they were obtained from the same batch of fry (eliminating the effects of race described e.g. by Brown, 1984 pers. comm. for Pomphorhynchus laevis in Salmo gairdneri) and the fish were grown to the required size instead of taking fish of a range of sizes from the population at one time, in which case the smaller individuals may have been stunted, and/or less resistant to parasitic infection.

From Fig. 8 it appears that C. carpio above 63mm could not be infected with B. acheilognathi. Further evidence for a size dependent effect on establishment of B. acheilognathi has been provided by several authors. Liao and Shih (1956) observed that in natural conditions the incidence of parasitization of Ctenopharyngodon idella declined when the fish were greater than 100mm in length, suggesting that the maximum size at which C. idella can be infected is greater than in C. carpio. Žitňan (1984) reported that B. acheilognathi infected C. carpio soon after hatching and thereafter the infection gradually declined. In general B. acheilognathi appears to infect underyearling fish with fewer and larger worms being found in 2 and 3 year old fish (Körting, 1974; Davydov, 1978; personal observations).

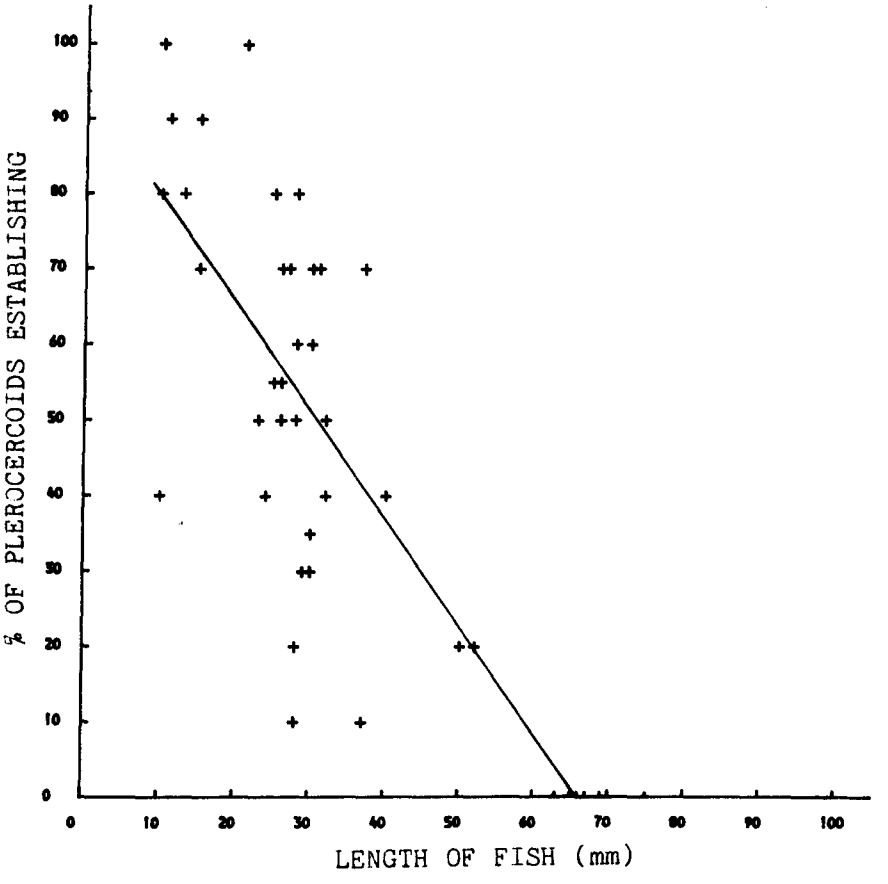


Figure 8: The influence of the length of C. carpio on the percentage of B. acheilognathi plerocercoids establishing within the intestine.

$Y = -1.4X + 92.5 \quad r^2 = 0.70$

The mechanism operating within the host intestine preventing the establishment of B. acheilognathi is unclear. Specific antibody formation is unlikely to be responsible since there had been no previous infection. Previous studies in fishes have shown that at least 7 days are required to produce antibodies to a parasite or injected antibody (Cushing, 1942; Orr et al., 1969). Antibodies specific to other parasites or other antigens have also been suggested as a means of rejecting intestinal parasites (McVicar and Fletcher, 1970), however the period of time between infection and examination was sufficiently short to prevent them causing the rejection of the plerocercoids.

The response by the C. carpio preventing the establishment or causing the rejection of the B. acheilognathi plerocercoids would have to be very quick acting since the fish were examined 12 hours after infection. Therefore it seems likely that it was a physical or physiological characteristic of the intestine of larger C. carpio that is affecting establishment.

It was thought that in the larger C. carpio the procercoids may not have been released by the maccerating action of the pharangeal teeth. To test this procercoids were released from the copepod by hand and introduced by stomach tube into the fore-intestine of small (10-30mm fork length) and large (70-80mm fork length) C. carpio. 43% of the procercoids established in the small C. carpio, whereas no establishment occurred in the larger fish, indicating that artificial release of the procercoids was having no effect on establishment.

Within the intestine, the plerocercoids and adult Pseudophyllidea are known to attach to the intestine by engulfing a fold in the intestinal wall using the bothrium (Wardle and McLeod,

1952). Rees (1958) while studying the attachment of Clestobothrium crassiceps (which has a very similar scolex to B. acheilognathi) suggested that the bothria function by drawing a plug of the hosts mucosa in by suction, and then the sphincter muscle running around the bothrium margin would contract, so holding onto the intestine. She doubted that the musculature of the adult was sufficiently strong to pull the mucosa up from a flat surface. From this work it seems likely that the plerocercoids of B. acheilognathi have to attach to the intestinal folds using their bothria. Examination of the plerocercoids in situ supported this idea. Therefore the physical size of the intestinal folds in larger fish may prevent the bothria from becoming attached. Examination of the width of the intestinal folds in C. carpio showed them to be positively related to the size of the fish (30mm C. carpio, mean fold width = 0.164mm, SE = 0.012; 50mm C. carpio width = 0.183, SE = 0.016; 80mm C. carpio width = 0.300mm, SE = 0.018). Measurement of the bothrium width of 10 plerocercoids relaxed in water until death gave a mean value of 0.057mm, SE = 0.004. This value is misleading since the bothria were not opened to their maximum extent. This information supports the above idea, and it may be that C. carpio above 63mm in length have intestinal folds which are too large to be grasped by the bothria of a B. acheilognathi plerocercoid. Further work in this area is obviously necessary.

Discussion

It is interesting to compare the data obtained in this experimental study with the seasonal studies conducted by a number of authors and reviewed by Chubb (1982).

Nakajima and Egusa (1977) noted that the eggs of B. acheilognathi died rapidly at temperatures below 7°C, and were not produced at temperatures of less than 12°C. They therefore concluded that the life cycle was only continued overwinter in the adult stage. The present study has demonstrated that the eggs can hatch at temperatures of 4°C, and a temperature of 0°C does not kill them. In addition a temperature of 4°C was not lethal to the other stages of the life cycle, although development was greatly retarded. Therefore it appears the B. acheilognathi could overwinter in the egg, procercoid or adult stages.

The seasonal dynamics of B. acheilognathi have been studied by Liao and Shih (1956), Klenov (1972), Nakajima and Egusa (1977), Davydov (1978) and Žitňan (1984). The observed pattern of incidence can be explained using the results obtained in the present study. The overwintered worms would become mature, and egg production would commence when the temperature rose above 12°C (Nakajima and Egusa, 1977). The production and release of eggs would therefore coincide with the increase in water temperature and the resultant zooplankton bloom which occurs in spring and early summer. The hatching of the eggs of the cyprinid definitive host also tends to coincide with the zooplankton bloom, consequently there would be high densities of both intermediate and definitive hosts during this period resulting in a high level of efficiency of infection.

Throughout the summer period it is probable that the parasites are in an approximate state of dynamic equilibrium between recruitment of plerocercoids and loss of established worms (e.g. Kennedy, 1975; Chubb, 1982), with many of the worms being lost from the intestine before reaching maturity (e.g. Hopkins, 1959; Kennedy, 1983). In the autumn, however, the decreased water temperature would result in a reduced feeding rate by the fish, together with a reduction in the rate of egg production and development of *B. acheilognathi*. Consequently fewer plerocercoids would be available for infection. The scarcity or absence of infective procercoids over the winter period could explain the gradual decline in the intenstiy of infection (also observed for *Proteocephalus filicollis* by Hopkins, 1959). Below a temperature of approximately 10°C no loss of parasites was observed in experimental conditions, therefore the infection intensity might be expected to remain approximately the same throughout the winter period.

The lack of feeding by the definitive host at low temperatures may result in destrobilation or even loss of some worms (Davydov, 1978), and as such may explain the large loss of *B. acheilognathi* throughout the winter period described by Nakajima and Egusa (1977).

By combining the data presented in this chapter the time taken for *B. acheilognathi* to develop from an egg to an adult, and from an egg to a mature adult (having eggs in the uterus), can be estimated (see Table 3). As expected the time taken for completion of the life cycle decreased at higher temperatures. Comparable data presented by Davydov (1978) and Bauer *et al.* (1969) is consistant with the present study in the time taken to develop to an adult worm, but is considerably lower in the time taken to develop to maturity (as a result of the low values for adult development

Table 3. The effect of temperature on the time taken for the completion of the life cycle of *B. acheilognathi*.

Temp. °C	Time for development (hours)					
	Egg	Coracidium	Procercoid	Adult (mature)	Egg to Adult	Egg to sexually Mature adult
0	No development	-	-	-	-	-
4	450-1686	No data	-	-	-	-
10	301-1307	0.12	2640-3720	-	2941-5039 (122-210 days)	-
16	136-616	No data	-	-	-	-
18	40-433	0.9	408-960	3120	448-1402 (19-58 days)	3568-4522 (149-188 days)
25	40-262	0.6	288-660	2880	328-928 (14-39 days)	3208-3808 (133-159 days)
30	40-144	0.5	121-408	1920	161-557 (7-23 days)	2081-2477 (87-103 days)
35	27-117	No data	-	-	-	-
40	No development	-	-	-	-	-

indicated in section 7). Thus Davydov (1978) estimated that the egg developed to an adult in 42-56 days at 15-22°C and 168-224 days at 4-8°C, and to a mature adult in 64-81 days at 15-22°C. Bauer et al. (1969) found that development to an adult required 13-22 days at 16-19°C; 7-15 days at 22-25°C and 5.5-8 days at 25-30°C; and to a mature adult in 33-43 days at 22-25°C, and 19-29 days at 22-25°C. It is interesting to note that Davydov (1978) found development occurred at 4-8°C, whereas in the present study no development occurred at 10°C even after 300 days. The reason for this difference is unclear.

From the data presented it is possible to estimate the probability that a particular individual will complete each stage of the life cycle, and so calculate the probability that a parasite will develop from an egg into a mature adult. This was done for a range of temperatures, and is presented in table 4.

The data indicates that the major losses of parasites from the population occur at different stages of the life cycle at different temperatures. Thus at 4, 10 and 40°C the major loss ocurs in the egg to coracidium stage, at 35°C in the coracidium to procercoid stage, at 18°C in the coracidium to plerocercoid stage and at 25 and 30°C in the plerocercoid to mature adult stage. This differs from the findings of Jarroll (1980) for B. rarus and Dronen (1978) for Haematolechus coloradensis who found the major losses in natural conditions were during the egg to early larval stages (procercoid and miracidium respectively). This may reflect the differences between natural and experimental conditions. The reasons for the temperature related mortality at each stage of the life cycle has been covered in the appropriate results section.

Table 4. The effect of temperature on the maximum probability of a B. acheilognathi individual developing to the next life cycle stage under experimental conditions.

Life cycle stage	Temperature							
	0	4	10	18	25	30	35	40
Egg Coracidium	0	0.097	0.247	0.722	0.921	0.962	0.614	0
Coracidium procercoid	-	0	0.387	0.300	0.350	0.343	0	-
Procercoid plerocercoid	-	-	0.200	0.300	0.400	0.700	-	-
Plerocercoid mature adult	-	-	0	0.919	0.119	0.186	-	-
Egg Plerocercoid	-	-	0.019	0.069	0.129	0.231	-	-
Egg Mature adult	-	-	-	0.063	0.015	0.043	-	-

Using the fecundity estimate obtained at 18°C and combining it with the probability of an egg developing to a mature adult from table 4, it can be estimated that under experimental conditions a single B. acheilognathi at 18°C would give rise to approximately 11,400 mature adults in the next generation.

It is important to note that these values were obtained under experimental conditions, and as such take no account of losses due, for example, to consumption of eggs, coracidia and procercoids by unsuitable hosts; coracidia and procercoids being consumed at an uninfective stage, or not at all; and over infection of intermediate or definitive hosts causing their death.

Therefore infection intensities in natural conditions would be considerably lower than those found in the present study. In natural lakes the incidence of infected copepods is very low, even during severe parasitic outbreaks. For example Guttowa (1963) found only 0.8% of the copepods in a Finnish lake were infected during a severe outbreak of Diphyllobothrium latum. Similarly Watson and Lawler (1965) found 1-2% of the copepods in Lake Heming, Canada, were infected with Triaenophorus sp. with only 1-3 procercoids per copepod. In addition they noted that of the 10 species of copepod which could be experimentally infected only 1 species was infected in the wild. Even in aritifical conditions such as fish farms the percentage of copepods infected is considerably lower than in experimental conditions. For example Pimenova (1973) observed that 9.1% of the copepods in a fish farm pond in the U.S.S.R. were infected with B. acheilognathi; and Liao and Shih (1956) estimated that this value was 7% in fish ponds along the Pearl River, China; whereas in the experimental conditions of the present study up to 35% of the coracidia established with around 90% of the copepods

being infected.

Transmission rates will also be much lower in natural conditions than those estimated in the present study. In his study of the population dynamics of B. rarus in Notophthalmus viridescens, Jarroll (1980) calculated that the probability of an egg survivng to a procercoid was 0.022, of a procercoid surviving to a juvenile worm in a larval newt was 0.029, and in an adult newt was 0.038. Therefore the probability of an egg developing to a juvenile worm in a larval newt was 0.00064 and in an adult newt was 0.00084. These values are considerably lower than in the experiment of B. acheilognathi C. carpio system and are largely a result of the loss of parasites in a natural system (mentioned earlier), and the careful manipulation of the life cycle under laboratory conditons so that at each stage in the life cycle suitable succeptible hosts were available allowing maximal transmission rates.

What does the information presented in this chapter indicate about the survival of B. acheilognathi in the British Isles? figure 9 illustrates the seasonal fluctuation in water temperature in Crose Mere, Shropshire (Courtesy of Dr. J.O. Young), a typical eutrophic lake in which the cyprinid definitive hosts of B. acheilognathi might be found. The water temperatures recorded fall within the thermal limits of B. acheilognathi (0-30^{o}C), therefore if introduced the parasite could survive.

Under constant experimental conditions egg release was observed to occur at temperatures above 12^{o}C. At Crose Mere this would result in egg release occurring from April until mid October, a total of 196 days. The eggs would survive throughout the year, however they would hatch most rapidly from June to August, when the

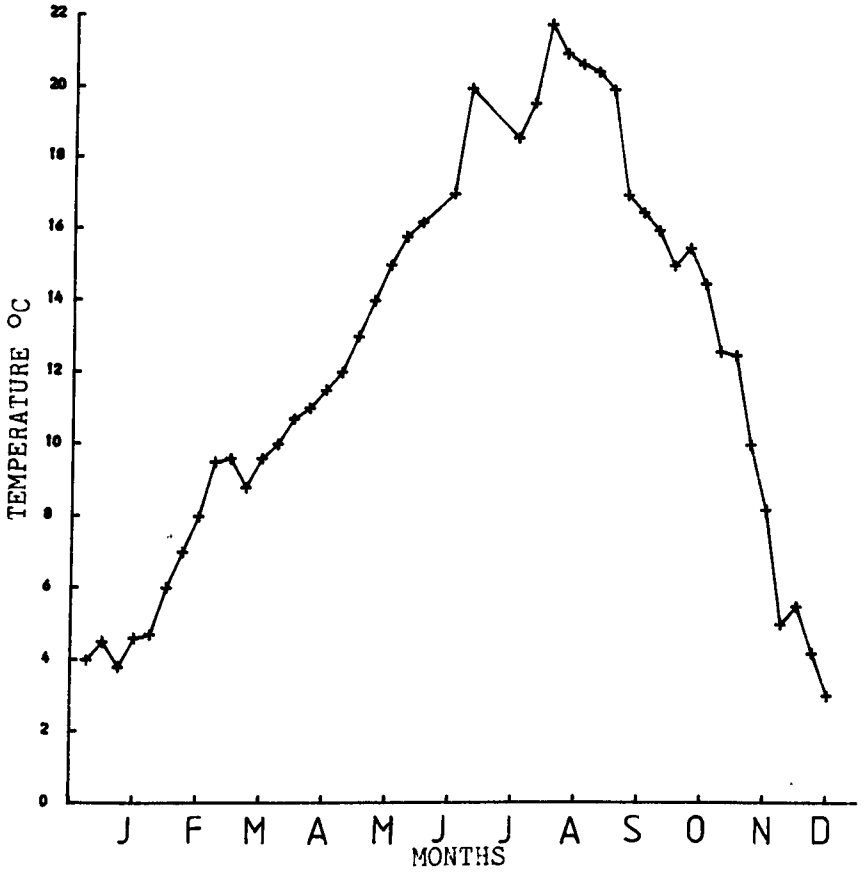


Figure 9: Seasonal changes in the water temperature at Crose Mere, Shropshire during 1977. Each point represents an average weekly value. (Courtesy of Dr. J. O. Young).

highest temperatures were recorded. Procercoid development would occur from March until late October (temperature greater than 10°C), and adult development would occur when the temperature rose above 14°C, i.e. a total of 154 days from mid April until October. Obviously development would also occur when the water temperature rose above these temperatures even when the mean value was lower.

In table 4 it was calculated that B. acheilognathi required 122-210 days at 10°C and 19-58 days at 18°C to develop from an egg to an adult worm; and 149-188 days at 18°C to develop to maturity. At Crose Mere the mean water temperature rose above 10°C for 203 days, and above 18°C for 84 days. Therefore the eggs released in April and May would develop into adult worms and possibly to maturity within a single year. The water temperature was not sufficiently high to allow the life cycle to be completed more than once within each year.

Therefore in a natural lake, such as Crose Mere, B. acheilognathi could survive, but as a result of the slow generation time and large losses throughout the life span it is unlikely to be present in large numbers.

In aquaculture the situation would be different. In cyprinid culture it is a common policy to raise the water temperature to 20-25°C by the use of shallow ponds or by artificially increasing the temperature (Huet, 1973) in order to promote spawning and rapid fish growth. The raised temperatures would result in the more rapid completion of the life cycle (Table 4) resulting in one or possibly two generations of worms occurring within one year. This together with the increased probability of an egg developing to an adult at the increased temperature (Table 5), and the high densities of zooplankton (promoted by fertilizing the ponds) and cyprinid fry,

would lead to a rapid increase in the population size of *B. acheilognathi*.

Conclusions

The establishment, development and mortality of each stage in the life cycle of Bothriocephalus acheilognathi was observed to be temperature dependent. In addition the infectivity of the coracidium and procercoid was age dependent, with coracidium infectivity decreasing with age, and the procercoid being infective upon development of the bothria. Development of the procercoid was influenced by the age of the coracidium when ingested by the Cyclops agilis intermediate host. The infectivity of the procercoid was found to be inversely related to the density within the intermediate host.

Estimation of the time taken for the completion of the life cycle at temperatures from 18-30°C showed it to be inversely related to temperature. Comparison of these values with the seasonal fluctuations in water temperature in a typical eutrophic lake indicated that B. acheilognathi could survive there, with completion of the life cycle occurring within one year.

The size of the Cyprinus carpio definitive host influenced the ability of B. acheilognathi plerocercoids to establish, with no establishment occurring in C. carpio greater than 63 mm fork length. It was suggested that the size of the intestinal folds in fish greater than 64 mm may prevent attachment by the bothria.

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Chapter 4

The movement of *Bothriocephalus acheilognathi* within the intestine of the cyprinid host, and its relationship with egg release

Introduction

Periodic activity is a well known phenomenon which has been widely reported in the animal kingdom. Perhaps the commonest and most important biological rhythms are those occurring on an approximately 24 hour cycle. Such rhythms, variously referred to as diurnal, circadian or diel, have been comprehensively reviewed by Hawking (1975).

In the course of other experiments using mature Bothriocephalus acheilognathi, it was noted firstly that the worms appeared to be in different regions of the host intestine at different times of the day, and secondly that egg release varied throughout the day.

The movement of parasites from one site to another within the host has been the subject of a large amount of research (reviewed by Crompton 1973; Holmes, 1973; and Hawking, 1975). Following the definitions proposed by Heape (1931) and Allee et al. (1949), Crompton (1973) suggested that this movement be divided into emigration and migration: emigration being a movement involving a change in site without a return journey, and migrations being movements involving a regular phase of coming and going between sites or within a site.

Emigratory activity is exhibited by many parasites during the process of the initial site selection within the host, and occasionally as a result of inter- or intraspecific interactions,

Migratory activity is less frequently observed among parasites. Mackenzie and Gibson (1970) noted the the trematode Podocotyle sp. and the nematodes Cucullanus heterochrous, C. minutus and Contracaecum aduncum moved from the anterior intestine to the rectum in association with the movement of food. Similar migrations occur

in the intestine of Gadus morhua by Contracaecum aduncum; the stomach of the rays Raia radiata and R. naevus by the trematode Otodistomum sp.; and the spiral valves of the same rays by the cestode Grillolia sp. (Williams et al., 1970).

A large body of literature exists on the emigratory and migratory activity of the cyclophylidean cestode Hymenolepis diminuta in its rat definitive host (see Arai, 1980 for review). The emigratory behaviour of H. diminuta was first recorded by Chandler (1939). He noted that 24 hours after infection the newly attached scolices were located approximately halfway along the intestine, but on the 7th to the 10th days post infection the worms moved forward to the anterior part of the small intestine. Later workers confirmed this anterior movement to the favoured site by means of surgical transplantation of the young adults (e.g. Braten and Hopkins, 1969).

The circadian migration of H. diminuta was described by Read and Kilejian (1969) and Hopkins (1970). They observed a periodic change in the distribution of the total worm tissue and in the location of the scolices in the rat intestine. The worms were situated in the anterior of the small intestine when food was present in the stomach, but moved to a more posterior position when the stomach was empty. Bailey (1971) confirmed this migration in rats fed ad libitum, and showed that the migration did not occur in starved rats, suggesting that it was exogenous in nature (a response to an environmental change which did not persist under constant conditions (Harker, 1958)).

The migration of H. diminuta of varying ages was investigated by Chappell et al. (1970). They observed three age dependent phases

of migration: from day 5 to day 7 post infection the worms moved anteriorly when the intestine was devoid of food and posteriorly when food was present - the opposite to the normal adult migration; a brief period when no migrations occurred (days 7-8) was followed by the normal adult migration as described by Read and Kilejian (1969).

The studies of Read and Kilejian (1969), Hopkins (1969, 1970), Bailey (1971), Chappell et al. (1970) and Mettrick (1971a, 1972) indicate that the circadian migration exhibited by H. diminuta is related to host feeding. Subsequently it was demonstrated that organic or inorganic constituents of a liquid meal, when fed via a stomach tube, did not explain the previously described migratory activity (Mettrick 1971a). Similarly a number of physical (Mettrick 1971b, 1971c) and chemical (Mettrick 1971a, 1972, 1975) gradients within the intestine could not be correlated with the rhythmic movements of H. diminuta.

Mettrick (1973) demonstrated that stimulation of the vagal nerve induced anteriad migration of the worms in the small intestine. Vagal stimulation mimics the normal vagal response to host feeding, suggesting that the migratory behaviour was not directly caused by the presence of nutrients within the intestine, but indirectly via the parasympathetic nerve stimulation of gastro-intestinal function (Mettrick and Cho 1981a, 198ab; Cho and Mettrick 1982). These studies also provided circumstantial evidence that a major factor in the worm response was the changing levels of intestinal serotonin (5 Hydroyxtryptamine). Cho and Mettrick (1982) indicated that the circadian migration shown by H. diminuta was correlated with similar changes in luminal serotonin levels in the small intestine, which in turn was related to the pattern of host

feeding. Intraperitoneal, intramuscular, subcutaneous and oral administration of serotonin resulted in a dose-dependent anteriad migration by H. diminuta (Mettrick and Cho, 1981b). Further evidence that serotonin was directly involved in the migratory behaviour of H. diminuta was provided by Mettrick and Cho (1982). They introduced two serotonin inhibitors (methysergide hydrogenmaleinate and magnesium sulphate) into the intestine via a stomach tube, and observed that the migratory activity was reduced or stopped.

The release of eggs by certain parasites has also been reported to follow a circadian cycle. Phillipson (1973) observed that the mouse nematode Aspiculuris tetraptera released most of its eggs during the hours of darkness, reaching a peak just before dawn. Egg output was not always proportional to faeces production, but often it was greatest when faeces production was highest.

A similar situation was reported to occur in the trematode Schistosoma haematobium. In this parasite the eggs were discharged in the urine of the infected human. The number of eggs passed out was not in proportion to the volume of urine, but showed a distinct peak at around 10.00 in the morning. Since the snail vectors are more active during the daytime, cyclical activity such as this would facilitate transmission of the parasite (Dukes and Davidson, 1968). No information is available concerning the mechanisms responsible for this rhythmic discharge of ova.

Not all parasites show a circadian rhythm in the production of their eggs. The trematode Phyllodistomum folium showed alternating periods of ovarian activity and rest with, in any fourteen day period, two resting sequences (Johnston, 1967).

The purpose of this chapter is to examine in greater detail, the preliminary observations concerning the movement of Bothriocephalus acheilognathi in the C. carpio intestine and the daily variation in the release of eggs in order to determine a) if the movement within the intestine is emigratory, migratory or a simple contraction and expansion of the strobila; b) if the two phenomena follow a circadian rhythm, c) the effect of altering the feeding regime, d) the rate at which the worms respond to the entry of food into the intestine, and e) if there was any relationship between worm movement and egg production.

Method

In all of the experiments 1 year old Cyprinis carpio infected with 1-12 Bothriocephalus acheilognathi per fish were used. The fish were acclimated for a period of 30 days in aquaria maintained at 18°C, with a 16 light:8 dark photoperiod (light from 6.00 a.m. to 10.00 p.m.) and fed daily at 9.00 a.m. with Omega number 4 trout pellets.

Movement of B. acheilognathi within the host intestine

5 infected C. carpio were killed by means of a sharp blow on the head 1, 6, 12, 18 and 24 hours post feeding. The intestine was quickly removed, unravelled and carefully slit open. The most anterior and posterior position occupied by the cestodes was recorded as a percentage of the total intestine length (0% being the anterior of the intestine and 100% the anus). The intestine was flushed with 0.9% saline and the percentage position of each of the scolices was recorded.

Altering the feeding regime

As a control to the above experiment, and in order to demonstrate that the movement of B. acheilognathi was dependent on the passage of food through the intestine, a second batch of infected C. carpio were starved throughout the 24 hour period. The percentage position of the worms and scolices was recorded as above.

Two further experiments were conducted to determine the relationship between worm movement and the time when the host was fed. The fish were maintained in the acclimated conditions, but were fed 12 hours later (at 9.00p.m.) or at 12 hourly intervals (at

9.00 a.m. and 9.00 p.m.). Fish were sacrificed at a range of times post feeding and the percentage position of the worms and the scolices was recorded.

Rate of reaction of the worms to the presence of food in the intestine

Preliminary observations suggested that the cestodes occupied a more anterior position when food was present in the intestine. To determine the reaction time of the worms to the entry of food into the intestine 5 C. carpio were killed and processed as above 10 minutes before feeding and 5, 10 and 30 minutes after feeding.

Rate of passage of food through the intestine

Uninfected C. carpio of the same size as the infected fish were acclimated to the conditions described previously. To determine the rate of passage of food through the intestine the fish were given a single meal at 9.00 a.m. consisting of trout pellets impregnated with a carmine dye (Rosin & Mayor, 1961). At 2 hourly intervals post feeding a sample of 5 fish were sacrificed and the percentage position of the dyed pellet material was recorded. In addition the gut was divided into 5 equal sections, and the contents placed on a preweighed, dry filter paper. After drying for 2 days at 50-60°C the weight of the faeces was recorded.

Egg release by B. acheilognathi

Infected C. carpio were maintained singl y in aquaria for a 14 day acclimation period in the conditions described previously. On day 15 the C. carpio were fed at 9.00 a.m. and faeces produced were

collected at hourly intervals for 24 hours, and at 2 hourly intervals for the subsequent 24 hours. To facilitate the collection of faeces, the aquaria were inclined at an angle of approximately 30° allowing the faecal material to be removed using a siphon without excessively disturbing the fish.

A faecal squash was prepared for each sample and the number of eggs present was counted using a light microscope at x100 magnification. The weight of the faeces was determined after drying for 2 days at 50-60°C.

Faecal egg counts were also taken from C. carpio starved for the 48 hours period, and from carp fed at 12 hourly intervals.

Results

1) Movement of B. acheilognathi in host intestine

In experiment 1 the percentage mid point position of the worms showed a significant movement along the intestine during the 24 hours period after feeding (t between positions 1 and 24 hours after feeding = 5.861 $p < 0.001$) (Table 1). This movement was linear, and can be described by the equation $Y = 0.73X + 27.61$, where Y = the percentage mid point of the worm and X = the time after feeding (Fig. 1a).

1 hour after feeding the C. carpio a second time the percentage mid point position of the worms had returned to the position occupied previously 1 hour after feeding ($t = 0.1611$, $p > 0.5$), indicating that the movement was cyclical.

Throughout the 25 hour period the scolices of the worms showed no significant movement (using Kruskal-Wallis test, $H = 5.3686$, $0.5 < p < 0.25$) suggesting that they remained attached to the intestine. The movement of B. acheilognathi cannot therefore be described as a migration or an emmigration (as defined by Crompton, 1973), but as a cyclical contraction and elongation of the strobila. The worm contracts its strobila within 1 hour of food entering the intestine, and then gradually extends the strobila along the intestine until 24 hours after feeding, it is at its maximum extension (see Plate 1a and b).

One hour after feeding the worms were observed to occupy their most anterior position in the intestine. In many cases the strobila of the worm was folded in addition to being contracted, which resulted in its extending to the anterior of the scolex.

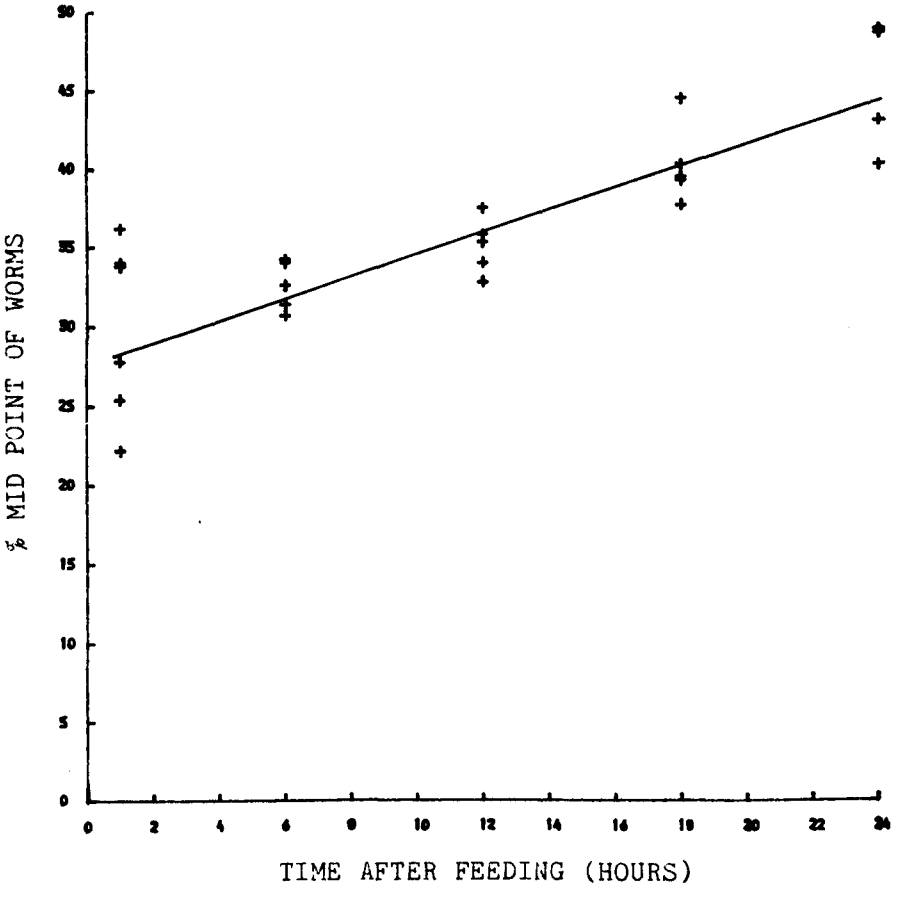


Figure 1: The relationship between the percentage mid-point position of B. acheilognathi specimens in the intestine of C. carpio and the time after feeding.

a. C. carpio fed at 9.00am.

Y = 0.73X + 27.61 Corr. coef. = 0.8886

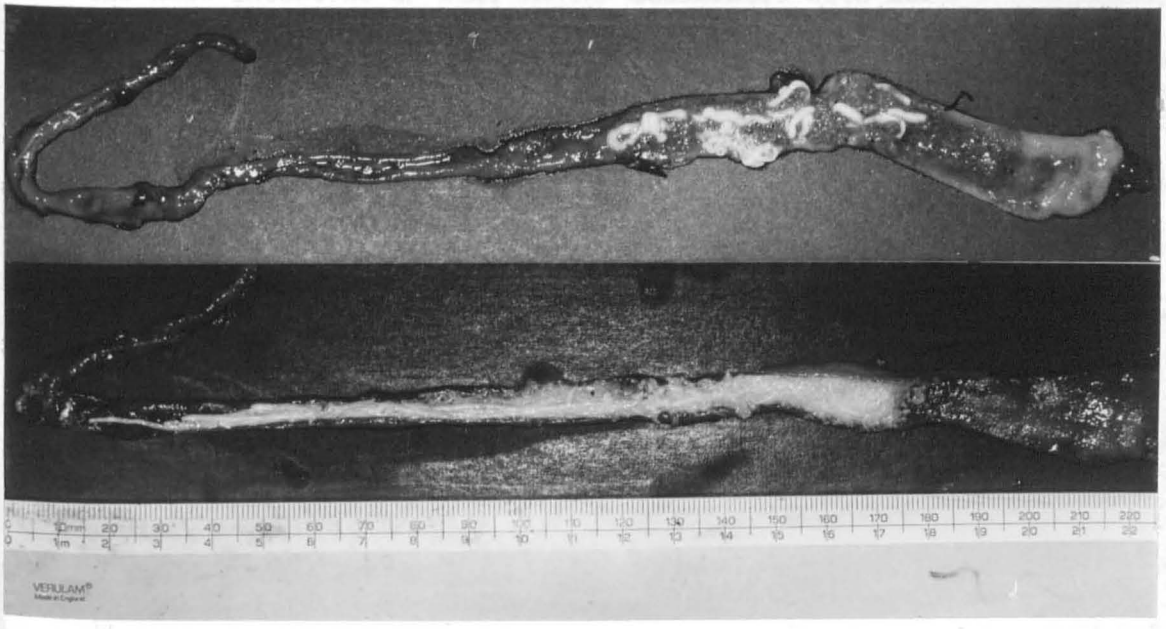


Plate 1: The position of B. acheilognathi within the C. carpio intestine, fed at 24 hour intervals.

a. 1 hour after feeding.

b. 24 hours after feeding.

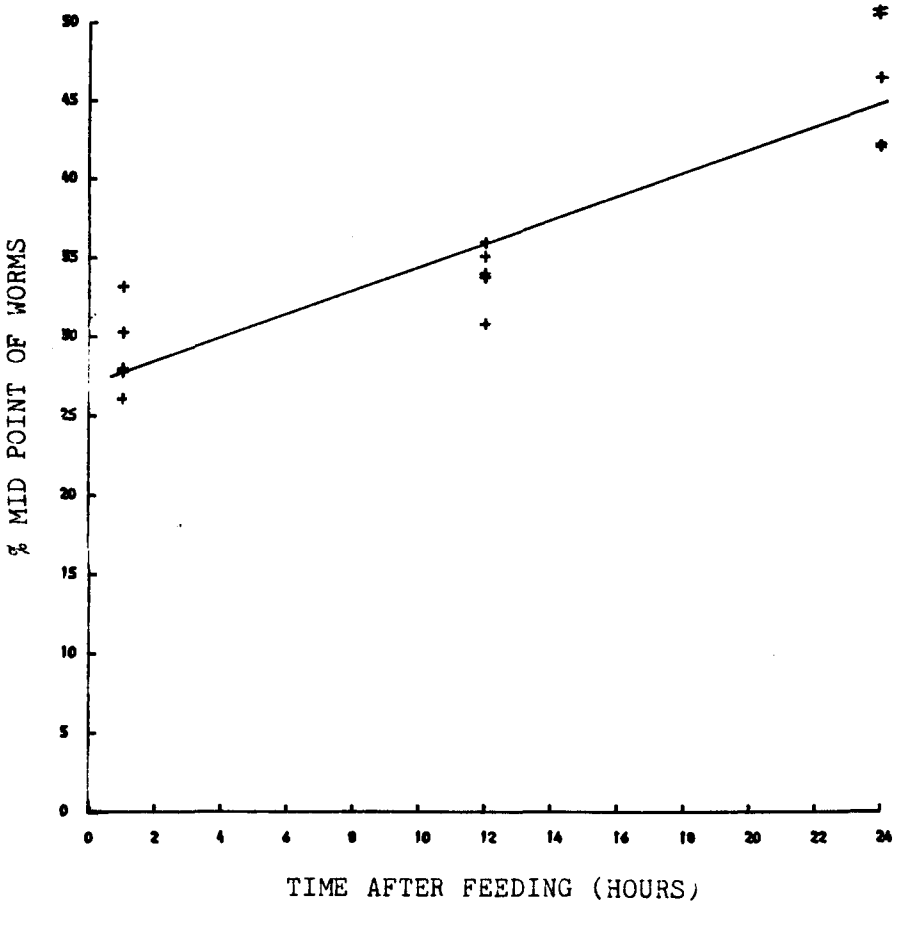
When starved for the 24 hour experimentation period the percentage position of the scolices and the percentage mid point position of the worms did not vary significantly ($H = 1.5845$, $0.9 < p < 0.75$). Therefore the cyclical movements observed in experiment 1 are exogenous in nature, being a response to the presence of food in the host intestine or some factor associated with it.

Experiment 3 provides additional evidence for this. When the fish were fed 12 hours later than during the acclimation period, the movement of the strobila was delayed by 12 hours (Fig. 1b) It was still linear, described by the equation $Y = 0.77X + 27.10$ and significant (t between position 1 and 24 hours after feeding = 7.475, $p < 0.001$).

When the carp were fed at 12 hour intervals the strobila moved along the intestine in two distinct phases (Fig. 1c). In each case, in the 12 hour period after the fish were fed, the extension of the strobila was linear and significant (from 1-12 hours after 9.00 a.m. feeding $Y = 0.51X + 28.44$, t between 1 and 12 hours after feeding = 6.882, $p < 0.001$; and from 1-12 hours after 9.00 p.m. feeding $Y = 0.42X + 29.89$, t between 1 and 12 hours after feeding = 4.4399, $p < 0.005$). The contraction and elongation of the strobila is therefore not circadian, but is associated with the host feeding activity.

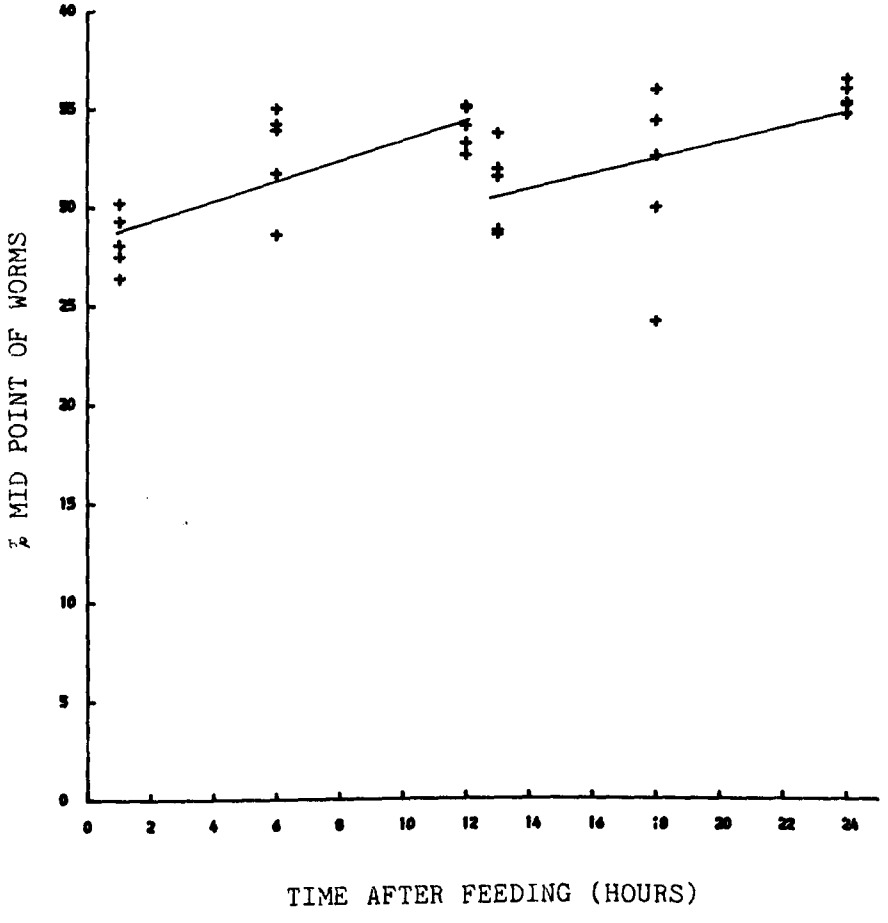
The movement of the strobila was cyclical, with the worms occupying the same position 1 hour after feeding ($t = 2.205$, $p < 0.05$). No significant variation in the percentage position of the scolices was observed (using Kruskal-Wallis test, $H = 1.585$, $p < 0.5$).

Comparison of the slopes of the four regression lines obtained in these experiments using analysis of covariance indicated that there was no difference between the slopes, i.e. between the rates at which the strobila extended along the intestine ($F = 2.914$, $p >$



1b. C. carpio fed at 9.0pm.

Y = 0.77X + 27.10 Corr. coef. = 0.9075



1c. C. carpio fed at 9.0am and 9.0pm.

After feeding at 9.0am: Y = 0.84X + 27.41 Corr. coef. = 0.8891

After feeding at 9.0pm: Y = 0.81X + 25.96 Corr. coef. = 0.8246

0.05) or between the elevations, i.e. between the most anterior positions occupied by the worms ($F = 1.612$, $0.5 < p < 0.2$).

The rate of passage of food through the intestine of the C. carpio is illustrated in figure 2. Intestinal material stained with carmine was present in the rectum after 9 hours and was completely eliminated from the intestine after 24 hours. The distribution of the carmine stained material at various times after fee ding is illustrated in figure 3.

There appears to be a close association between the position of the main mass of the carmine stained material (Fig. 3) and the position occupied by B. acheilognathi (Table 1). So that, for example, 1 hour post feeding the main mass of the food is in the anterior 40% of the intestine, and the position occupied by the worms extends from 10% to 50% of the way along the intestine. After 12 hours the food is situated from 0 to 60% of the way along the intestine, and the worms extended from 10 to 59% of the way along the intestine. After 24 hours the small amount of material present in the intestine was in the posterior 40%, and the worms extended from 16.4 to 79% of the way along the intestine.

Rate of reaction of worms to presence of food in intestine

Examination of the percentage mid point of the worms 5 minutes after feeding indicated that the strobila had significantly contracted towards the anterior of the intestine ($t = 4.051$, $0.005 < p < 0.002$). This anterior movement continued until 10 minutes post feeding when the most anterior position occupied by the worms was observed. No posterior movement of the strobila was observed between 10 and 60 minutes post feeding ($t = 0.4614$ $p > 0.5$). The

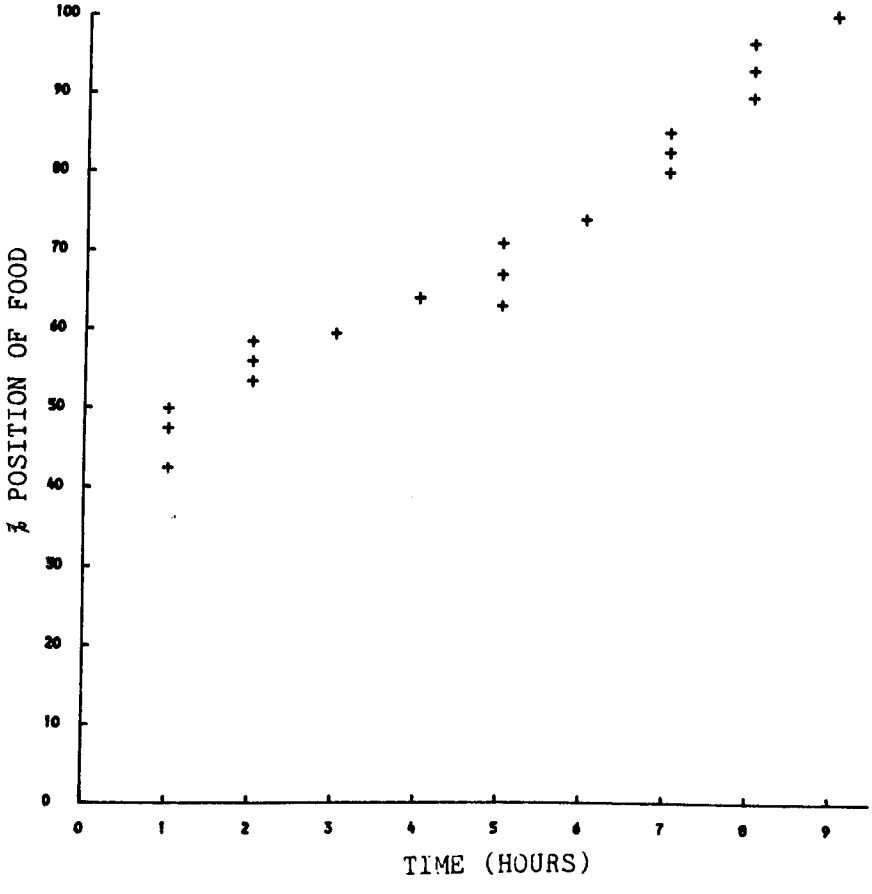


Figure 2: The passage of carmine stained trout pellets through the intestine of C. carpio at 18 °C.

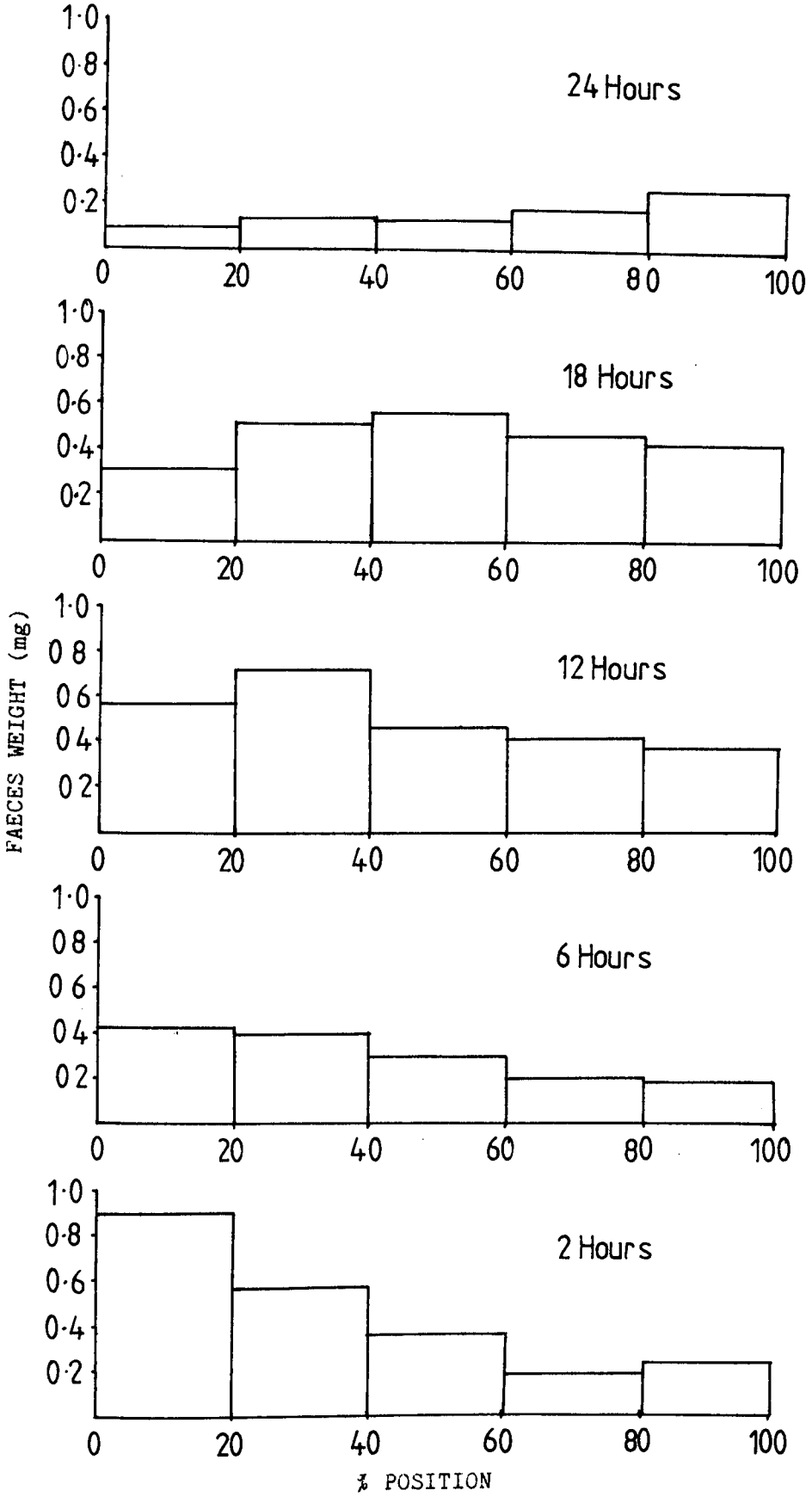


Figure 3: The distribution of carmine stained trout pellets in the intestine of C. carpio at 18 °C.

Table 1. The position occupied by *B. acheilognathi* over a 24 hour period, when the *C. carpio* host was fed at 24 hourly intervals.

Time after feeding (hours).	Maximum and minimum % worm position.	Mean % worm position.	SE
1	10.0 - 50.0	28.6	2.32
6	13.7 - 54.7	32.6	0.69
12	10.0 - 59.0	35.1	0.80
18	14.0 - 74.1	40.2	1.14
24	16.4 - 79.0	45.9	1.80
1	15.3 - 49.5	29.1	1.18

contraction of the strobila after food enters the intestine is shown in figure 4.

Egg release by B. acheilognathi

The faecal egg counts of 4 fish infected with between 1 and 7 worms are illustrated in figure 5a. In each case there is a large, and obviously significant increase in the numbers of eggs recovered from faeces 1 to 4 hours after the fish were fed. No correlation was observed between faecal egg count and faecal weight (Spearmans rank correlation $r = 0.1032$, $0.5 < p < 0.2$).

When the fish were starved for the 48 hour period the number of eggs recovered from the faeces showed a significant variation (Fish 1, $x^2 = 116.89$. Fish 2, $x^2 = 133.95$. Fish 3, $x^2 = 150.04$. Fish 4, $x^2 = 141.52$. Ho = no variation in faecal egg count $p < 0.001$) however there were no large peaks in the faecal egg count as observed when the fish were fed (Fig. 5c).

The effect of feeding the carp at intervals of 12 hours is presented in figure 5b. The faecal egg counts showed a significant increase between 6 and 10 hours after feeding. Therefore egg release does not follow a circadian pattern, but is related to the time at which the fish host was fed.

It is interesting to note that mature proglottides were recovered from aquaria containing fish 1 and 4. Similar proglottides have been recovered from aquaria containing infected fish on a number of previous occasions. In each instance the shed proglottides contained very few eggs. Examination of the terminal proglottides of mature B. acheilognathi collected from the intestine of the carp host revealed them to contain fewer eggs than more anterior mature proglottides and in many instances contained a

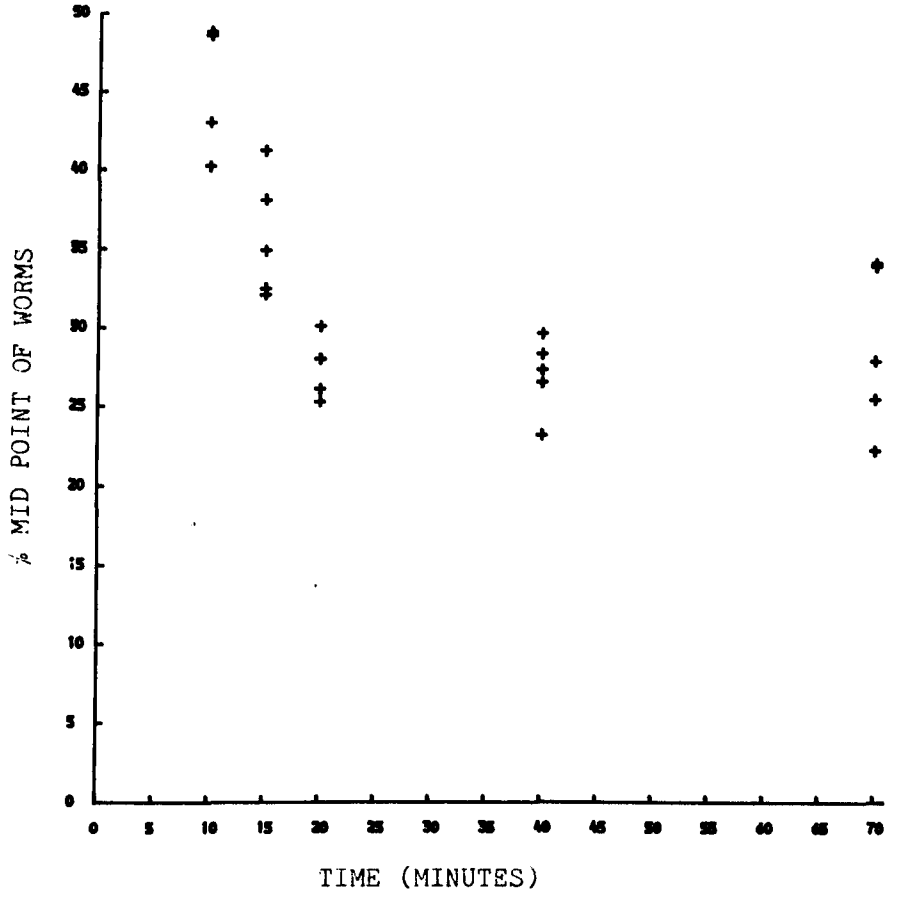


Figure 4: The relationship between the percentage mid-point position of B. acheilognathi specimens in the intestine of C. carpio.and the time after feeding.

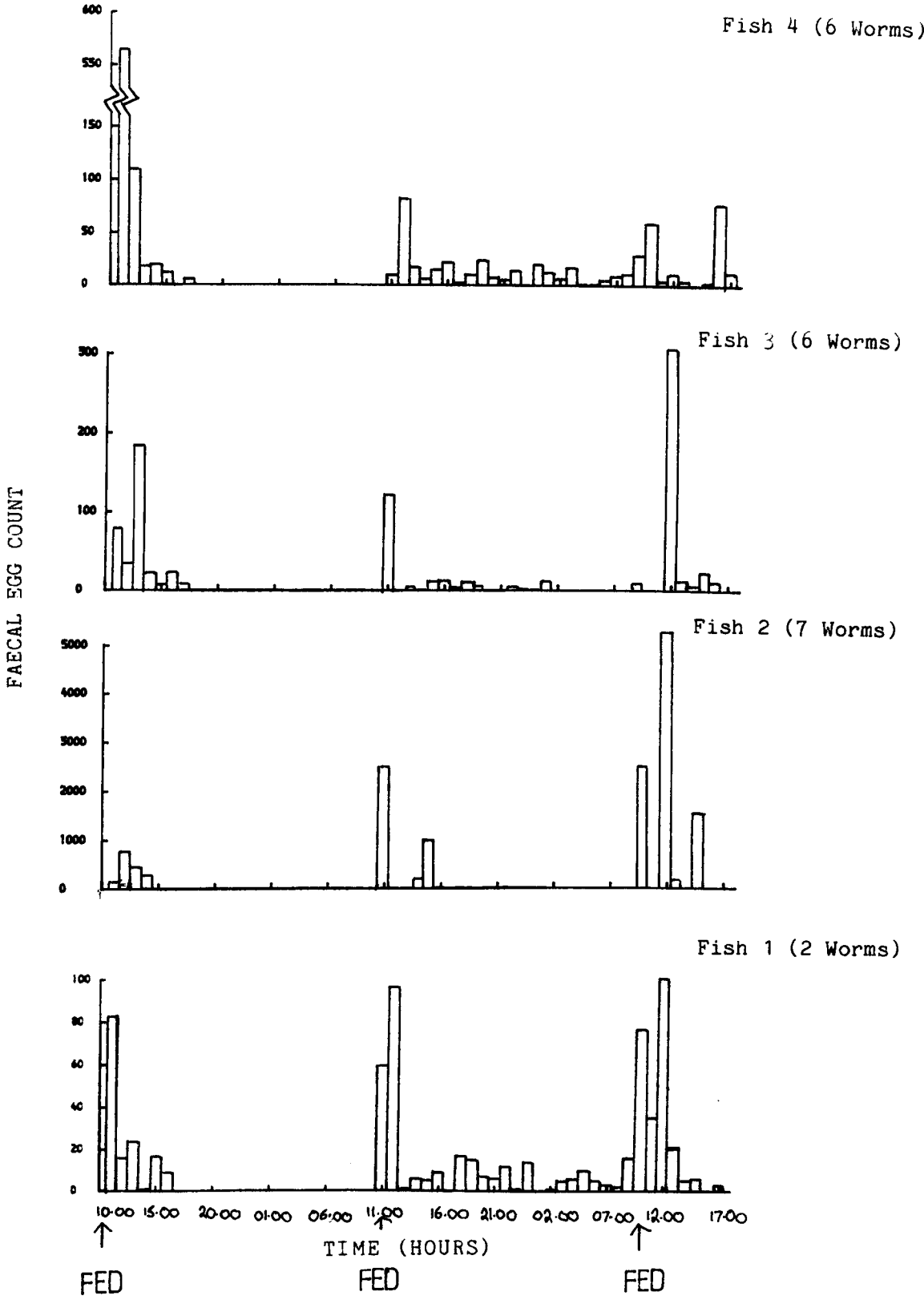
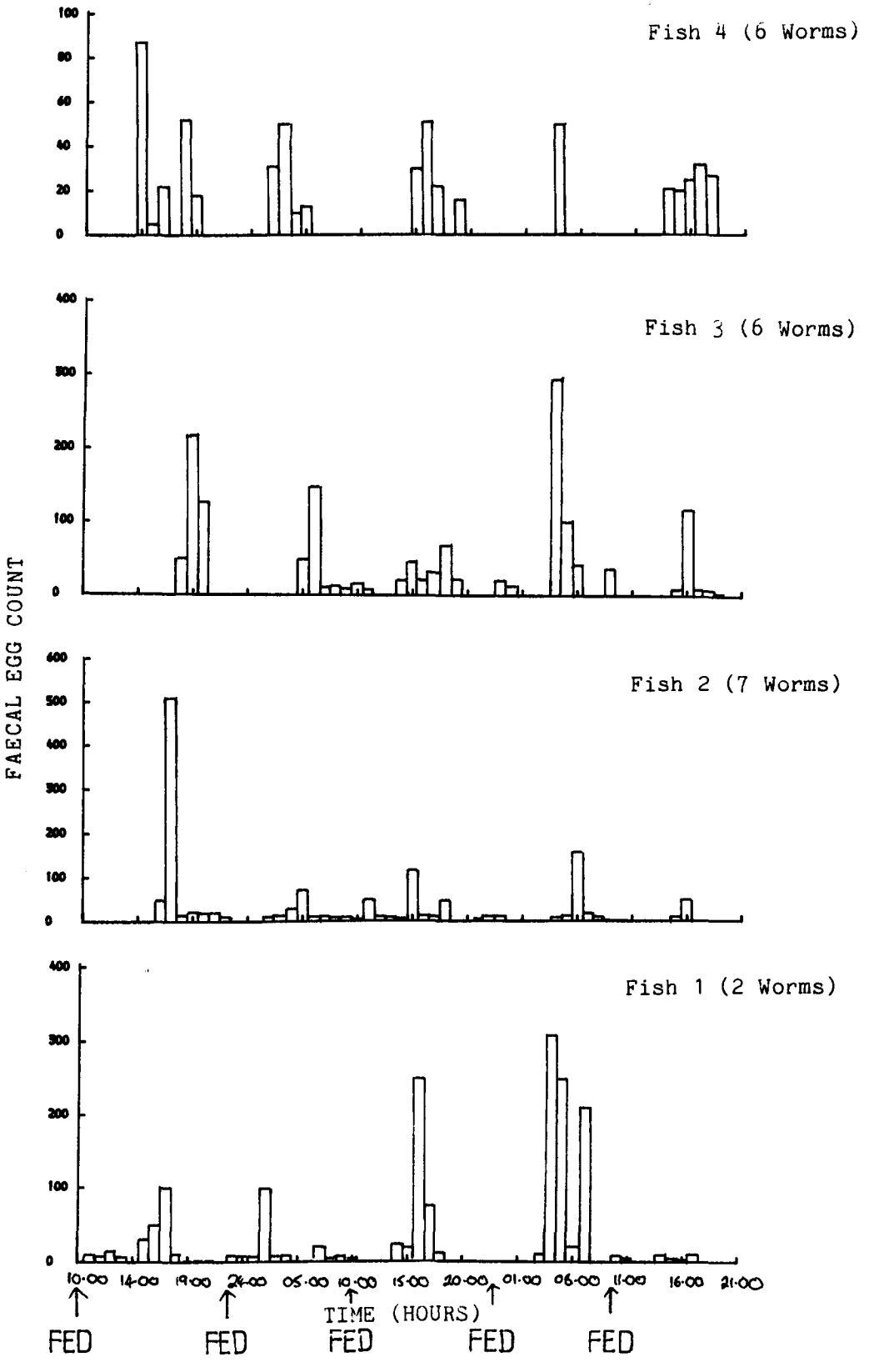
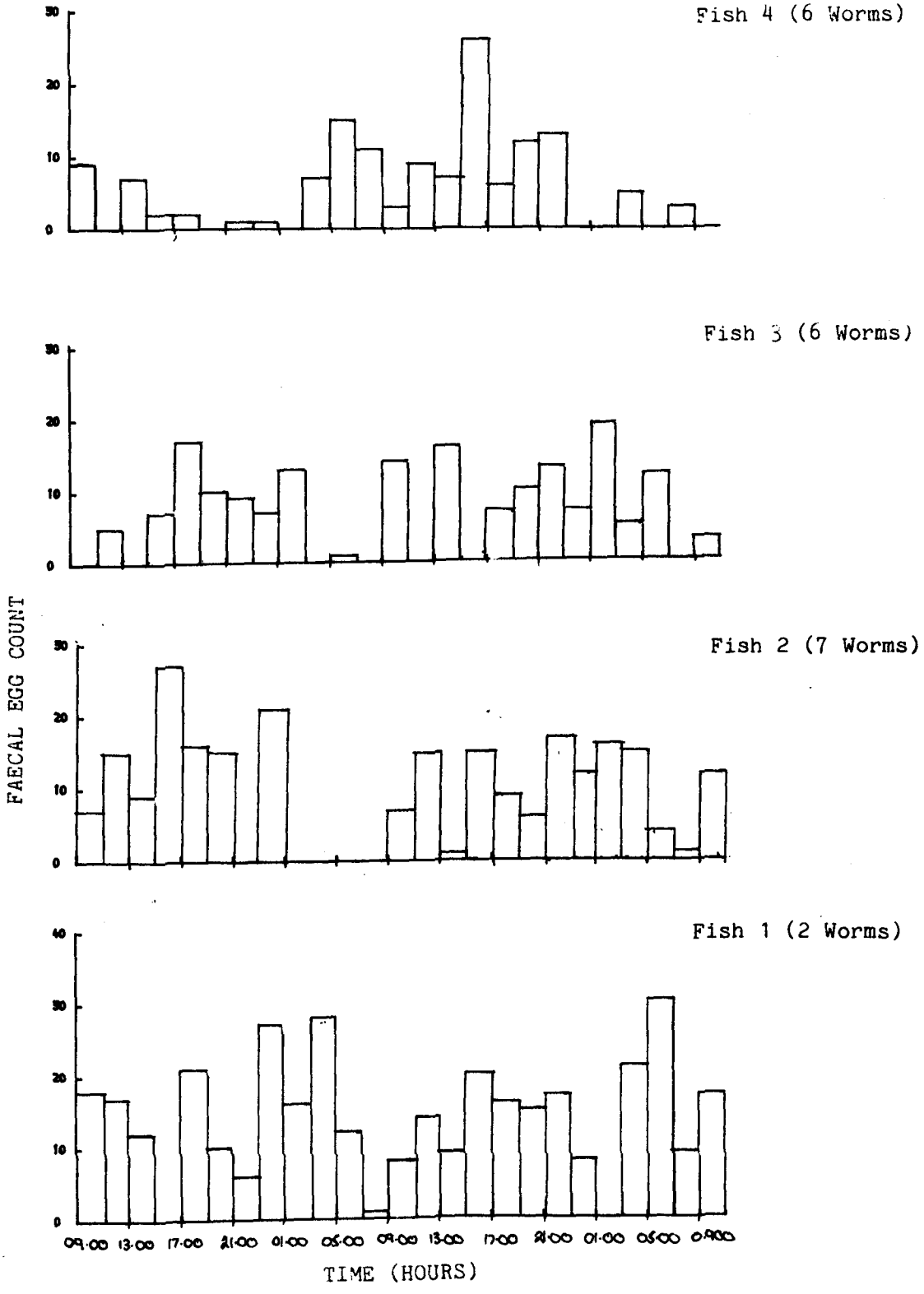


Figure 5: Faecal egg counts from C. carpio infected with B. acheilognathi.

a. C. carpio fed at 24 hour intervals.



5b. C. carpio fed at 12 hour intervals.



5c. C. carpio starved for a 48 hour period.

similar number of eggs to the expelled proglottides. The evidence suggests that in B. acheilognathi exhausted proglottides break from the strobila and are expelled from the fish intestine. This phenomenon, termed pseudopolysis by Nybelin (1922) (translation Wardle and Mcleod, 1952), has also been reported to occur in other pseudophyllidean species, for example in Dibothriocephalus latus (=D. latum) (Wardle and Mcleod, 1952) and Eubothrium salvelini (Boyce, 1974). Nybelin (1952) noted that members of the genus Bothriocephalus have internal divisions between groups of genitalia enabling proglottides to be shed without disrupting the internal organisation of the worm. It is unlikely that the number of eggs release from the shed proglottides will affect the observed pattern of the faecal egg counts.

When examining the data for the faecal egg counts it is important to note that there is a potentially large error resulting from the dissociation of some eggs from the faeces. These eggs would not be collected by siphoning and therefore would not be counted. The size of this error is probably related to the number of eggs present in the faeces and is therefore unlikely to affect the observed pattern of the faecal egg counts.

Discussion

Throughout this investigation C. carpio containing between 1 and 12 B. acheilognathi were used therefore intraspecific interactions may be influencing the results. Hopkins and Allen (1979) suggested that the position of Hymenolepis diminuta in multiple infections would be more variable because individual worms would be screened from location stimuli. In addition Mettrick and Podesta (1974) noted that H. diminuta could alter their environment, resulting in a change in behaviour. The effect of worm burden was investigated by Read and Kilejian (1969) who observed the migration of H. diminuta in rat intestines containing 1, 10 and 30 worms. They concluded that the migratory behaviour was independent of worm burden.

In this investigation the similarity of the results obtained from carp containing different numbers of worms indicates that worm burden had an insignificant effect on worm movement.

The data presented clearly demonstrate a cyclical contraction and extension of the strobila of B. acheilognathi within the C. carpio intestine. However Hopkins (1970), when working with H. diminuta in rats, noted that the size of the worm may affect the distance that the strobila could stretch down the intestine, and that because of this it was difficult to unequivocably demonstrate that the position of the strobila changed through the day. To minimise the effects of differences in worm size in the present study, the fish to be sacrificed at any particular time were taken at random from a population of fish infected with worms of the same age. Therefore there should have been a randomly distributed variation in worm length throughout the experiments. The

consistancy of the results suggests that the size of the worms did not have a significant effect.

By altering the feeding period and by starving the carp, it has been demonstrated that the described patterns of movement by B. acheilognathi were associated with the passage of food through the intestine. The movement of H. diminuta in the rat intestine are also reported to be related to the feeding of the host (e.g. Read and Kilejian, 1969; Hopkins, 1970; Bailey, 1971), however it differs in that the scolex also shows a cyclical variation in the position of attachment.

It was noted that the infected fish showed a peak in activity when fed. It was possible that mechanical stimulation resulting from this activity could account for the observed worm movements. The peak activity was also induced in the starved control carp when other fish in the aquarium room were fed, however no resultant contraction of the strobila was observed, indicating that activity alone could not cause the observed movements.

In the present investigation no further research was carried out aimed at determining the exact stimulus (or stimuli) required to produce the observed movements of B. acheilognathi. Research centered around H. diminuta, by Mettrick and his colleagues (see introduction) indicates that serotonin plays an important part in controlling intestinal migration. It would be interesting to repeat the experiments of Mettrick and Cho (1981b) (aimed at determining the effects of serotonin on worm migration), and of Mettrick and Cho (1982) (aimed at blocking the natural release of serotonin in the intestine using inhibitors) using B. acheilognathi in the C. carpio intestine in order to determine if serotonin was responsible for the

movements described in this investigation. Davydov et al. (1974) noted that the glycogen content of B. acheilognathi could be altered using serotonin, and Teremina (1983) found serotonin in the tissues of B. acheilognathi but no workers have examined its effect on motility.

Throughout this investigation the infected C. carpio were subject to artificial feeding regimes, with food being available only at specified times. Would the extension and contraction of the strobila occur if the C. carpio were fed ad libitum? Bailey (1971) examined the migrational activity of H. diminuta in rats fed ad libitum and concluded that the worms still showed the migrations because of the increased feeding activity of the hosts during the period from 1400 hours until dawn (08.00 hours). A similar situation would probably occur in the C. carpio with peak feeding activity, and therefore the worms at their most anterior position at dawn and dusk.

Egg release by B. acheilognathi

Egg release by B. acheilognathi shows a cyclical pattern. The frequency of this pattern appears to be related to the frequency at which the fish were fed (Figs. 5a and b).

By utilising the rate at which food passes along the intestine (Fig. 2) it is possible to determine the position of the intestinal material containing a high egg density at the time at which the C. carpio were fed. Thus, when the C. carpio were fed at 24 hour periods the peak faecal egg counts were recorded 1-4 hours post feeding. This intestinal material would have been 60-80% of the way along the intestine at the time of feeding. Similarly when the C. carpio were fed at 12 hour periods, the peak faecal egg counts

occurred 6-10 hours post feeding. This intestinal material would have been 40-60% of the way along the intestine at the time of feeding. If these positions are compared with the position occupied by the mature proglottides at the time of feeding (Table 1) they can be seen to coincide.

The results seem to indicate that the peak egg release from the mature proglotiddes occurs at the time when food enters the intestine. Associated with the entry of food into the intestine is the rapid contraction of the strobila which might be expected to cause pressure to be exerted in the uterus within each proglottid resulting in the expulsion of eggs. Boyce (1974) was also of the opinion that eggs would be expelled from the strobila (of Eubothrium salvelini) under the pressure resulting from contortions of the worm. Alternatively the stimulus initiating the contraction of the strobila (possibly serotonin) may also initiate the release of eggs from the uterus.

Since the presence of food in the intestine, serotonin levels and strobila contraction are closely associated, it is difficult to distinguish the causal factor of egg release. Further work in this area is obviously necessary.

Conclusions

The strobila of Bothriocephalus acheilognathi was observed to undergo a cyclical pattern of contraction and extension within the host intestine. The frequency of this behaviour was associated with the feeding behaviour of C. carpio host. The strobila contracted to its most anterior position 10 minutes after food had entered the intestine, this was followed by a linear extension as the food moved along the intestine. 24 hours post feeding the bulk of the food material had been expelled from the intestine, and the strobila of B. acheilognathi was fully extended. No significant variation in the scolex position was observed.

Egg release by B. acheilognathi was also associated with the entry of food into the intestine. It is suggested that the contraction of the strobila causes pressure to be exerted on the uterus resulting in the expulsion of the eggs.

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Part III:

The Anthelmintic Treatment of Bothriocephalus acheilognathi

Introduction.

The pathological effects of Bothriocephalus acheilognathi on it's cyprinid definitive host have been well documented. In intensive and semi-intensive cyprinid pond culture systems in Europe, U.S.S.R. and the Far East the prevalence of infection may reach 100%, with up to 467 worms per fish being recorded (eg. Liao & Shih, 1956; Körting, 1974). At low levels of infection (1-7 B. acheilognathi) no effects on the health and metabolic activity of the fish were apparent (Par, 1978). However, heavy infestations may cause intestinal blockage, inflammation and perforation of the intestine wall, generalized emaciation, reduced growth rates and death (Liao & Shih, 1956; Bauer et al., 1969; Edwards & Hine, 1974; McDaniel, 1979; Scott & Grizzle, 1979; Hoffman, 1980).

In areas where it has become established B. acheilognathi has caused severe economic losses. For example in the German Democratic Republic, where movement of infected fishes is restricted, and extensive prophylactic measures are practised, 14.5% of the Cyprinus carpio produced are infected with B. acheilonathi; the average weight of infected fishes is 10% lower than in uninfected individuals; 1-1.5% of the C. carpio are lost as a result of bothriocephaliasis; and the cost of feeding infected fishes is increased by 5% compared with uninfected fishes (Wierowski, 1984).

The economic importance of B. acheilognathi has resulted in considerable research being aimed at discovering a suitable anthelmintic. A wide range of substances have been utilised to treat infected fishes. These range from natural plant products, for example ground coniferous needles (Klenov, 1969), tobacco dust (Avdos'ev, 1973) and Lupin seed (Balatskii et al., 1976), through a

variety of anthelmintics including devermin (eg Molnár, 1970), Phensal (eg Muzikovski, 1971) and Yomesan (eg Fijan et al., 1976). The effectiveness of these anthelmintics, and many others, varies considerably depending on the circumstances of their use. In addition many (eg Mebendazole, (Andrews, 1983 Yorkshire Water Authority report); Mansonil and Depifen, (Fijan et al., 1976); Yomesan, (Jezek, 1979)) are toxic to the fish at the concentrations necessary for successful treatment.

Praziquantel (Droncit, Bayer), a pyrazinoisoquinoline derivative, has a very broad spectrum of activity against parasitic cestodes and trematodes, and has been extensively used for the treatment of human schistosomiasis (Andrews et al., 1983). The highly efficacious nature of praziquantel, together with the tol.eration shown to it by fishes make it an ideal anthelmintic for the control of many fish parasites. Studies have shown it to be effective against Diplostomum spatheceum in Salmo gairdneri (Bylund and Sumari, 1981); Valipora campylancristrota in Cyprinus carpio (Suvorov, 1981); Bunodera lucioperca in Perca fluviatilis, Eubothrium rugosum in Lota lota, Ligula intestinalis in Rutilus rutilus and Sanguinicola inermis in Cyprinus carpio (cited Andrews et al., 1983; Proteocephalus osculatus in Siluris glanis and Bothriocephalus acheilognathi in Ctenopharyngodon idella (Andrews and Riley, 1982).

In this part of the thesis the use of praziquantel for the control of B. acheilognathi will be examined. The in vitro effect of praziquantel on B. acheilognathi is investigated in chapter 5 using scanning and transmission electron microscopy. Chapter 6 describes how praziquantel was used in a field situation to treat a batch of 30,000 newly imported Ctenopharyngodon idella which were

found to be infected with B. acheilognathi. This latter work was carried out in conjunction with Dr. C. Andrews (Yorkshire Water Authority) and Mr K. Ryder (Humberside Fisheries).

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Chapter 5:

The effect of praziquantel on *Bothriocephalus acheilognathi* in vitro.

Introduction.

Praziquantel (Droncit, Bayer) has been shown to be an effective anthelmintic against Bothriocephalus acheilognathi in naturally infected Ctenopharyngodon idella (Andrews and Riley, 1982; see Chapter 6).

Although there have been numerous studies on the effects of praziquantel on the morphology of various cestodes and trematodes of medical and veterinary importance (eg Andrews and Thomas, 1979; Becker et al., 1980, 1981; Conder et al., 1981; Coles, 1979) none have described the effects on the parasites of fishes. The present study was designed to study the in vitro effects on the fine structure of the integument of B. acheilognathi using scanning and transmission electron microscopy.

Materials and Methods

Immature and mature Bothriocephalus acheilognathi (specimens) were obtained from 0+ grass carp (Ctenopharyngodon idella). The parasites, individually and attached to the intestinal mucosa, were exposed for 5, 15 and 60 minutes to concentrations of 0 (control), 0.1, 1.0 10 and 100 ug praziquantel per ml of 0.9% saline at 18°C.

For scanning electron microscopy the worms were fixed in a 4% formaldehyde solution for 1 hour, washed twice in distilled water and dehydrated using an ethanol series. The worms were treated in a Polaron E3000 critical point drier, followed by cooling with 60% gold palladium using a Polaron E5100 sputter coater and viewed using a Philips 501B scanning electron microscope.

Worms to be studied using the transmission electron microscope were fixed for 5 hrs in a 2% paraformaldehyde and 2.5% gluteraldehyde solution in 0.1M sodium cacodylate buffer, adjusted to a pH of 7.2 using 0.1 m HCL. Calcium chloride was added to the buffer to give a final concentration of 2.5 mM to reduce membrane swelling. The worms were washed twice, in 0.1 M sodium cacodylate buffer, pH 7.2; cut into sections of approximately 2 proglottides, and post-fixed for 3 hours in 1% osmium tetroxide in a sodium cacodylate buffer, pH 7.2. After washing in a cacodylate buffer, the worms were dehydrated in an ethanol series, and gradually infiltrated with a standard mixture of spur low epoxy resin. The specimens were cured for 16 hours at 60°C. 90 nm sections were cut using a Reichert OMU3 ultramicrotome and stained using saturated uranyl acetate in 50% ethanol for 20 minutes and lead citrate for 5 minutes at 20°C. Sodium hydroxide crystals were added to the lead citrate to prevent lead carbonate formation. The sections were

washed in 0.02 M sodium hydroxide and distilled water; and viewed using a Kratos Conneth 500 transmission electron microscope (Smith 1980).

Results.

The tapeworms treated with 1.0, 10.0 and 100 ug praziquantel per ml saline contracted immediately upon being placed in the drug solution, the intensity of the response increased with the concentration of the drug. After 15 minutes the worms placed in a 0.1 ugml solution showed areas of contraction and relaxation along the strobila. After 24 hours exposure to the drug solutions, the worms were still attached to the intestine, and showed a weak response when touched. Control worms were still active but had released their hold on the intestine.

Scanning electron microscopy:

Treated and untreated worms, as observed by the scanning electron microscope are shown in figures (1-6). The tegument of the control worms was uniformly covered by microtriches. Dome shaped tumuli were evenly spaced over the scolex, but decreased posteriorly, being absent from mature and gravid proglottides (Fig. 2). Adjacent proglottides were a similar size and shape (Fig. 1).

Worms exposed to 0.1 ug praziquantel per ml saline were morphologically identical with the control after 5 and 15 minutes.

After 60 minutes exposure occasional small 'blebs' (1-10 ug) were apparent on the outer surface of the bothria, and on proglottide margins in the neck region.

At a praziquantel concentration of 1.0 ugml the 'blebs' were more numerous particularly on the scolex, and appeared after only 5 minutes exposure (Fig. 3). Increased exposure to the drug resulted in many of the blebs bursting (Fig. 4). The mature proglottides were swollen after 15 and 60 minutes exposure (Fig. 5).

Worms exposed to 10 ug praziquantel per ml saline solution

Legends

Figure 1: Mature proglottides from Bothriocephalus acheilognathi incubated in the control solution (0.9% saline) for 15 minutes. Viewed using a scanning electron microscope. Marker = 100 um.

Figure 2: S.E.M. of B. acheilognathi tegument from the neck region incubated in the control solution for 60 minutes. Note microtriches (M) and tumuli (T). Marker = 1 u.

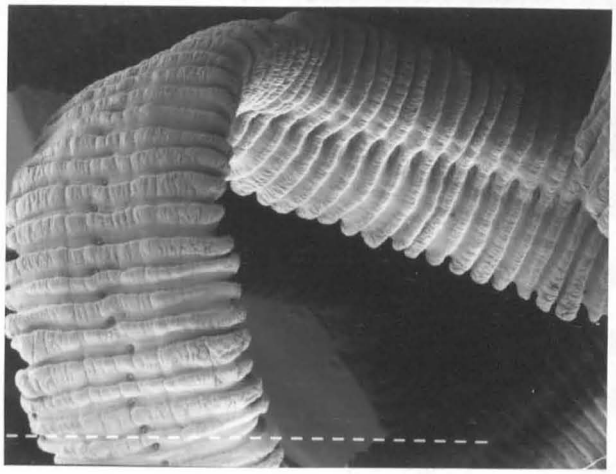
Figure 3-4: S.E.M. of B. acheilognathi tegument from the neck region exposed to a solution of 1.0 ug praziquantel per ml 0.9% saline. **Fig. 4:** Incubated for 5 minutes. **Fig. 5:** Incubated for 15 minutes. Marker = 10 um.

Figure 5: S.E.M. of swollen mature proglottides from B. acheilognathi incubated in 1.0 ug praziquantel per ml 0.9% saline for 60 minutes. Marker = 100 um.

Figure 6: S.E.M. of large swelling on mature proglottide of B. acheilognathi incubated in 10 ug praziquantel per ml 0.9% saline for 60 minutes. Marker = 10 um.

Figure 7: Transmission electron micrograph (T.E.M.) of the tegument in the neck region of B. acheilognathi incubated in the control solution for 15 minutes. Note Microtriches (M) Distal cytoplasm (DC) Basal layer (BL) and circular musculature (CM). x5000

Figure 8: T.E.M. of B. acheilognathi tegument from the neck region incubated for 5 minutes in 1.0 ug praziquantels per ml 0.9% saline. Note vacuolisation (V) and ballooning of tegument (B). x3000.



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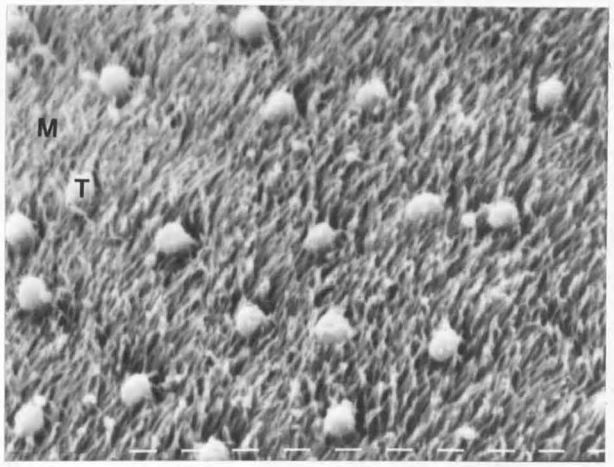

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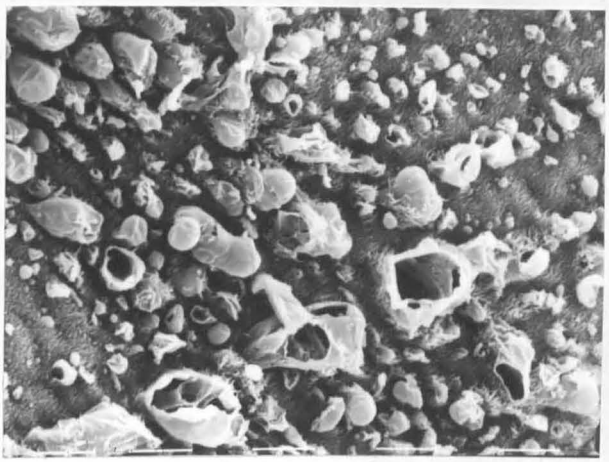

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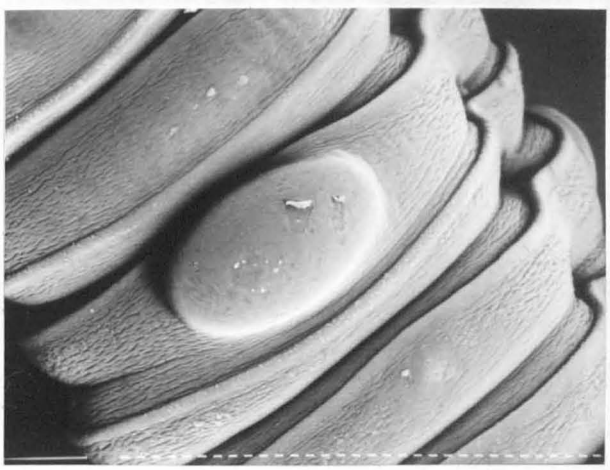

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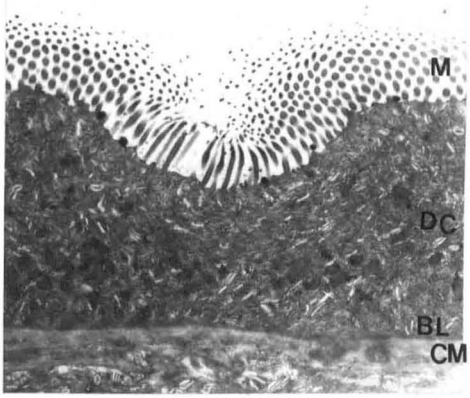

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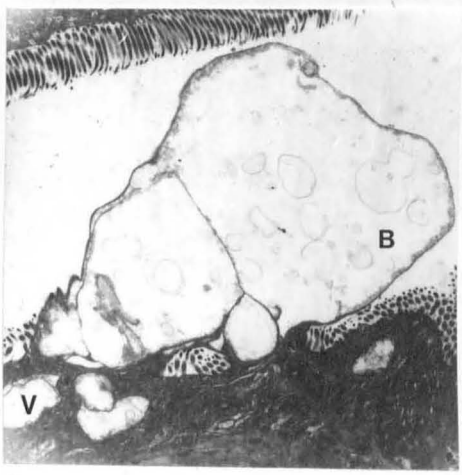

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showed the most intense reaction. Large areas of the scolex were covered in 'blebs'(1 u-10 u diameter) after only 5 minutes exposure. Swelling and distortion of the mature proglottides occurred after 15 minutes. Large vacuoles (280 u x 140 u) formed on some mature proglottides after 1 hour (Fig. 6).

Exposure to 100 ug ml^{-1} praziquantel solution resulted in fewer blebs on the scolex, but a higher density on the neck region of the strobila. Mature segments were swollen.

Transmission electron microscopy:

The structure of the tegument of B. acheilognathi is similar to that described for other cestodes (e.g. Lumsden, 1966; Becker et al., 1980) and has been described in detail by Granath et al. (1983). The distal cytoplasm is covered in numerous 1-2 um microtriches, and is rich in mitochondria and vessicles. The basal lamina separates the muscle layer from the distal cytoplasm (Fig. 7).

Examination of the 'blebs' observed using the scanning electron microscope reveals them to be continuous with the distal cytoplasm. The 'blebs' diameter 1-10 um were filled with cytoplasm and granules, indicating that leakage of the tegument had occurred (Fig. 8).

Vacuoles (2-5 u diameter) were apparent after 15 minutes in the distal cytoplasm of worms exposed to a 1.0 ugml or greater praziquantel solution. After 1 hour these vacuoles were larger (2-10 u diameter) and more extensive. Cellular leakage into the vacuoles had occurred (Fig. 8). The microtriches appear to be unaffected by the drug.

Discussion

The effect of praziquantel on the tegument of Bothriocephalus acheilognathi is similar to that described for other cestode species (e.g. Conder et al., 1981; Becker et al., 1980, 1981).

Praziquantel concentrations as low as 0.001 ugml^{-1} have been shown to affect adult hymenolepid and echinococcus adult cestodes, while concentrations of 0.01 ugml^{-1} can cause contraction and paralysis (Andrews & Thomas, 1979). B. acheilognathi appeared to show a greater degree of tolerance to the drug, a concentration of 0.1 ugml^{-1} for 15 minutes or 1 ugml^{-1} for 5 seconds being required to produce contraction. Paralysis of the strobila did not occur in the drug concentrations tested. Previous in vitro studies were conducted with mammalian cestodes (e.g. Becker et al., 1980, 1981) at a temperature of 37°C. The lower temperature (18°C) employed in this study may explain the apparent reduced sensitivity.

A similar reduced succeptibility to praziquantel was observed with Diphyllobothrium latum larvae and adults treated in vitro (Bylund et al., 1977). Although the mode of action of praziquantel is poorly understood, Andrews et al. (1983) have suggested that one pre-requisite for its action is the presence of a syncytial tegument such as that found in many cestodes and trematodes. The observed reduction in succeptibility by the two pseudophyllidean cestodes may therefore be a result of the integument being slightly different to that possesed by other cestodes.

Considerable variation in the effect of the drug occurred along the length of the worm. The scolex and neck region of the tegument showed the characteristic ballooning and vacuolization, previously recorded in a number of cestode species (Andrews et al., 1983).

Becker et al. (1981) suggest that this is a result of the high metabolic activity in this area. Unlike other cestodes, the mature proglottides of B. acheilognathi were affected by praziquantel being grossly distorted with occasional large swellings. This response resulted in the mass expulsion of eggs, which hatched normally to produce viable coracidia. Control worms released very few eggs. The non ovicidal activity of praziquantel has previously been recorded for Echinococcus granulosus (Thakur et al., 1979) and stresses the need for care when disposing of water and faeces after treatment.

The in vivo studies of Andrews and Riley (1981) and in chapter 6 found praziquantel to be completely effective in eliminating B. acheilognathi from the fish host. It is interesting to note that in the present study in vitro exposure of the worms to praziquantel concentrations of up to 100 $ugml^{-1}$ for 24 hours failed to kill the worms or cause them to release their hold on the intestine. Further in vivo studies have shown that expulsion of the worms from the gut occurs approximately 72 hours after treatment. In view of the rapid elimination of praziquantel from the body (Steiner and Garbe, 1976, Andrews & Thomas, 1979) it is probable that some other factor, such as the action of the host digestive enzymes on the damaged tegument, and the peristatic action of the intestine contributes to the expulsion of the worms from the gut.

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Chapter 6:

The control of *Bothriocephalus acheilognathi* in *Ctenopharyngodon idella* using praziquantel under field conditions.

Introduction.

In November, 1982 approximately 30,000 0+ Ctenopharyngodon idella (length 10-16cm, weight approximately 20g each) were imported into the Yorkshire Water Authority region from a Ministry of Agriculture, Fisheries and Food approved scource in Yugoslavia. Examination of the C. idella revealed that 50-80% were infected with Bothriocephalus acheilognathi, with 1-30 worms per fish.

B. acheilognathi was considered to be a potentially dangerous pathogen, therefore movements of the C. idella were prevented by the Yorkshire Water Authority under section 30 of the 1975 Salmon and Freshwater Fisheries Act 1975. Because of their high value it was necessary to treat the fishes in order to remove the B. acheilognathi.

In order to select an anthelmintic which could be used to treat the C. idella trials were carried out in the laboratory by C. Andrews, using three anthelmintics: mebendazole (Mebenvet, crown chemicals), niclosamide (Mansonil, Bayer) and praziquantel (Droncit, Bayer). The anthelmintics were suspended in 0.9% saline and administered as a single dose via stomach tube to groups of 10 fishes maintained at 18-20° C (after Andrews and Riley, 1982).

At dose rates of 35-500 mg kg body weight^{-1} mebendazole was ineffective at removing the B. acheilognathi, and in addition, at dose rates of 50 mg kg body weight^{-1} (and above) there were signs of toxic effects on the fish. Niclosamide, at a dose rate of 150 mg kg body weight^{-1}, did not achieve satisfactiry control and there were also signs of toxic effects on the fish. At dose rates of 35-100 mg kg body weight^{-1} praziquantel was completely successful at eliminating the tapeworm infestation. There were no signs of toxic

effects on the fish, and all of the worms passed out of the gut within 7 days.

In view of these results, and the large number of fish to be treated it was decided to use praziquantel, and to administer it in the form of a medicated feed.

Methods and Results

The infected C. idella were divided into two batches of approximately 15,000 fishes.

The first batch of fishes were netted from an outside pond (temperature 4°C), and placed into an indoor recirculating system consisting of eight 1600 litre fibreglass tanks and a large biological filter. The temperature was increased at the rate of 2° C day^{-1} up to 24°C. At this temperature it was hoped that the fishes would feed actively on the medicated feed, and that the praziquantel would be as effective as in the laboratory trials.

The C. idella were offered trout pellets (Fingerling 1, B.P. nutrition) for several days, during which time any lethargic or moribund (and hence non-feeding) fish were removed and destroyed. As soon as the fishes were feeding well, they were starved for 3 days and then offered anthelmintic-medicated feed. To help ensure that all the fishes had access to the feed, automatic feeders were used to provide a 2 minute feed every 30 minutes during daylight hours. The medicated feed was prepared by binding praziquantel powder (obtained from Bayer AG.) to the pellets using a small quantity of vegetable oil. Sufficient praziquantel powder was added to the three days ration to achieve a dose rate (over the 3-day period) of 125 mg kg body weight^{-1}.

Nine days after the end of the treatment period 300 C. idella (ie sufficient to provide a 95% chance of detecting a 1% level of infection (Ossiander and Wedemeyer, 1973)) were examined for the presence of B. acheilognathi. Non were found to harbour intestinal tapeworms.

The temperature in the recirculating system was allowed to cool at the rat e of 2°C day^{-1} to ambient (winter) temperatures, and the

fishes were released into a previously disinfected pond.

The second batch of fishes were treated in the same manner, exept that sufficient praziquantel was used to acheive a dose rate of 105 mg kg body weight^{-1} over the 3 day period.

Discussion.

The dose rates of praziquantel employed in this treatment were approximateley 3 times greater than the minimum quantity found to be effective under the laboratory trials, and those used to control other cestodes (reviewed by Andrews et al., 1983). This was to allow for a certain amount of anthelmintic wastage (bearing in mind the method of administration), and to ensure that the fishes which were feeding only occasionally (probably the most heavily infected) received sufficient praziquantel to eliminate the parasite. Wastage was further reduced by the use of small but frequent feeds to ensure that the pellets were consumed rapidly, the acclimation of the C. idella to the indoor tanks and to the pelleted food, and the 3 day starvation period prior to treatment.

The removal of any obviously non-feeding fishes was important, as the B. acheilognathi in these fishes would not have been eliminated by the anthelmintic. Approximately 500 non-feeding fishes were removed. They were particularly obvious because of their dark colouration and because they congregated around the inlet pipes.

The ponds which contained the infected C. idella prior to treatment were drained, limed and left dry over the winter period to kill any eggs or infected copepods which may have been present (eg Babaev and Shcherbakova, 1963; Mitchell and Hoffman, 1980). Prior to treatment these ponds had been wired to prevent the transmission of the parasite to adjacent ponds via bird predation (Prigili, 1974, 1975).

Praziquantel is obviously completely successful at eliminating B. acheilognathi. Unfortunately, because of the high cost of

praziquantel and other proven anthelmintics, they are often not used by European cyprinid culturists. Instead cheaper, less effective drugs are used to reduce the worm burden, so preventing excessive growth reduction and death (K. Ryder pers.comm.). Over a short period of time these measures will be economically beneficial. However as they do not eliminate B. acheilognathi, recurrence of the infection at a later date, or in another locality may occur.

In the present situation treatment of the fishes was facilitated by the use of a large recirculating unit. However the cost of running this, together with the cost of the praziquantel and the labour, would have been prohibitively high if less valuable fishes had been infected. In a number of instances in the British Isles where B. acheilognathi has been diagnosed in Cyprinus carpio fry it has been economically beneficial to kill the fishes rather than treat the parasite.

Because of the high cost of effective treatment it is important that B. acheilognathi be prevented from entering the British Isles. In an attempt to prevent the introduction of novel, fish pathogens such as B. acheilognathi into the British Isles there is legislation under the Diseases of Fish Acts, 1937 and 1983 to control the importation of freshwater fishes and their eggs. However fishes for ornamental purposes are imported with no real restrictions. Such imports may include fish species which are, in theory, subject to the above mentioned legislative controls, for example Tinca tinca, Scardinius erythrophthalmus and Ctenopharyngodon idella (Andrews, 1984). On a number of occasions this has resulted in ornamental fishes such as golden orfe (Leuciscus idus) and Koi carp (Cyprinus carpio) which were infected with B. acheilognathi being introduced into British fish farms (Andrews, 1984; personal observations). The

effluent from these farms, fishes which escape and fishes kept on the same farms for restocking purposes could all result in the release of *B. acheilognathi* into British waters.

Conclusions

Praziquantel administered as a medicated feed is effective at eliminating Bothriocephalus acheilognathi from Ctenopharyngodon idella at dose rates of 105 and 125 mg kg body weight^{-1}. For completely successful treatment it was necessary to ensure that all of the fishes consumed sufficient quantities of the drug. This was acheived by using very high dose rates, removing non-feeding fishes, acclimation of the fishes to a pelleted food and raised temperatures, feeding small quantities of food frequently and by starving the fishes for 3 days prior to treatment.

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Appendix. Publications.

J. Fish Biol. (1984) **25**, 361–364

A scanning electron microscope study of the life cycle of *Bothriocephalus acheilognathi* Yamaguti, 1934

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The stages in the life cycle of *Bothriocephalus acheilognathi* have been studied with the aid of scanning electron microscopy. Emergence of the coracidium occurred after 3–5 days at 20°C. Soon after hatching the coracidium began to swell and the cilia became coiled and lost their locomotory function. The surface of the coracidium was covered in protuberances of unknown function. After consumption by the copepod intermediate host, the coracidium developed into a procercoid. Upon development of a cercomer the procercoid could infect the fish definitive host. Identification of adult *B. acheilognathi* should be made on specimens relaxed in cold water for 10 min, and be based on the heart shaped scolex and prominent square apical disc.

I. INTRODUCTION

A study of the pseudophyllidean cestode *Bothriocephalus acheilognathi* is being undertaken at the University of Liverpool. As an essential first stage to this research the life cycle was established in the laboratory, and the external morphological features of the various stages in the life cycle were examined using scanning electron microscopy.

II. METHOD

Mature *B. acheilognathi* specimens, obtained from a grass carp, *Ctenopharyngodon idella*, were placed in Petri dishes containing distilled water at 10° C, where the majority of the eggs were expelled from the gravid segments within 1 h. Coracidia emerged from the egg after 3–5 days at 20° C. Approximately 10 coracidia were placed in a drop of water containing a single copepod and left for 1 h. The infected copepods were placed in a Petri dish containing 20 ml dechlorinated tap water to which 0·5 ml of hay infusion was added daily. After 10 days at 20° C the procercoids had developed a cercomer and could be used to infect the fish definitive host. Five copepods containing 20 procercoids in total were placed in a beaker containing 100 ml dechlorinated tap water and one 2–3 cm carp, *Cyprinus carpio*. The carp were fed a small quantity of flaked food daily, and killed after 10 days, to obtain the plerocercoid, or after 30 days to obtain the young adult stage.

At each stage in the life cycle specimens were isolated from their respective environments and placed in a cone of filter paper to facilitate handling. After being fixed in 5% formaldehyde solution, washed twice in distilled water and dehydrated using an ethanol series, the contents of the cone were emptied onto double sided adhesive tape for attachment to a viewing stub, or the apex of the cone was mounted directly onto the stub. The specimens were then treated in a Poloron E3000 critical point drier, coated with 60% gold-palladium using a Poloron ES100 sputter coater and viewed using a Philips 501B scanning electron microscope.

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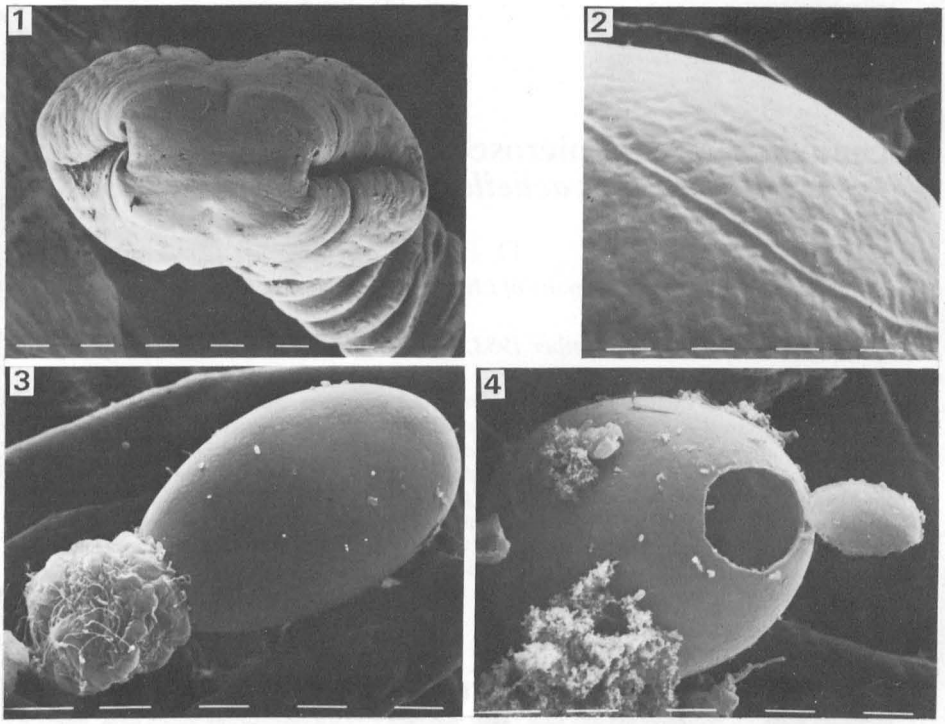

FIG. 1 Scolex of adult *B. acheilognathi* from *Ctenopharyngodon idella* showing square apical disc. Scale = 100 μm.

FIG. 2. Narrow pole of egg, showing operculum. Scale = 1 μm.

FIG. 3. Coracidium emerging from egg. Scale = 7·14 μm

FIG. 4. Hatched egg. Scale = 5·6 μm.

III. RESULTS AND DISCUSSION

Each stage in the life cycle is represented in Figs 1–10.

The life cycle of *Bothriocephalus acheilognathi* closely follows that of other *Bothriocephalidae*, e.g. Essex (1928) (*B. cuspidatus*), Thomas (1937) (*B. rarus*) and Jarecka (1959) (*B. claviceps*).

The ovoid eggs (measuring 0·05 × 0·062 mm) have a distinct operculum at the narrower end, typical of the pseudophyllidean cestodes (Wardle & McLeod, 1952).

Soon after hatching, the coracidium begins to swell and loses its motility as a result of water absorption (Thomas, 1937). Essex (1928) noted that after swelling the coracidium is 'exclusive of cilia', however reference to Fig. 6 suggests that the cilia become coiled and non-functional. Coiling of the cilia is not permanent and by altering the salt concentration of the solution surrounding the coracidium, the ciliary action can be 'stopped and started at will' (Thomas, 1937). The surface of the coracidium is covered in numerous protruberances which have an unknown function.

The procercoid characterizes the pseudophyllidean and proteocephalan life cycles. The cercomer of infective procercoids contains the rudimentary

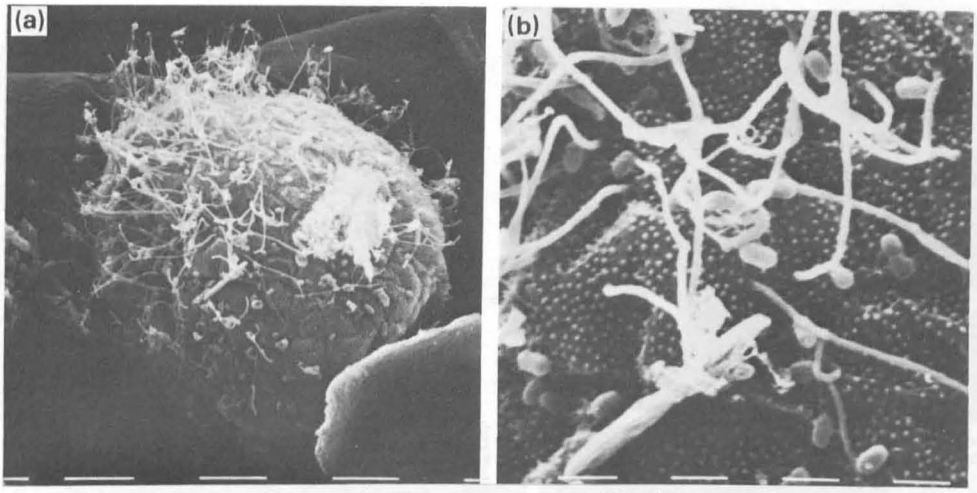

FIG. 5. Coracidium soon after emerging from egg. Note uncoiled cilia and surface protruberances. (a) Scale = 5·9 μm. (b) Scale = 1 μm.

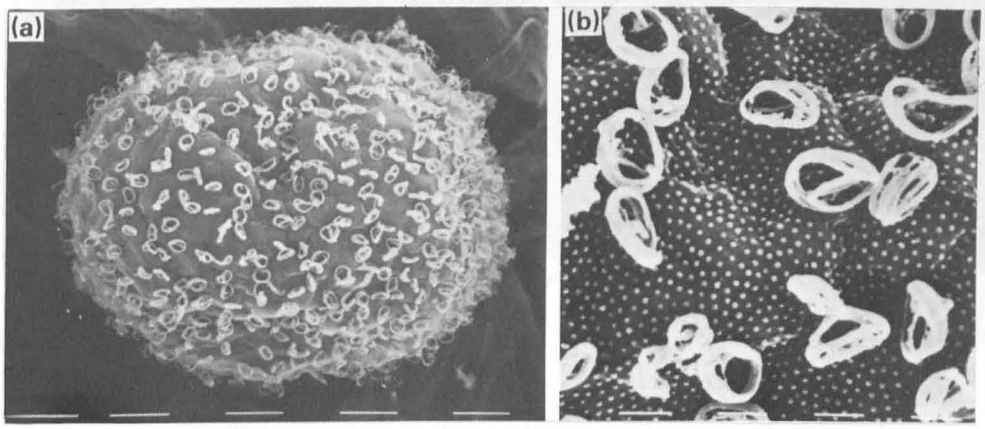

FIG. 6. Swollen coracidium 5 h after hatching. Note coiled cilia. (a) Scale = 4·9 μm. (b) Scale = 1 μm.

embryonic hooks which are revealed by removal of the outer membrane. Up to 11 procercoids have been recorded from a single copepod. Procercoids from heavily infected copepods have reduced rates of growth (Körting, 1975) and development (Thomas, 1937); infectivity is not affected.

Attachment of the plerocercoid to the fish intestine is by means of two bothria. Development of the bothria commences in the late procercoid stage at the end of the body opposite the cercomer (Thomas, 1937). The scolex is fully formed at the initiation of proglottization, after 15 days at 20° C.

Liao & Shih (1956), while maintaining the life cycle of *B. acheilognathi* at 29° C, observed egg production 20 to 21 days after entry into the intestine. Preliminary experiments by this author suggest that the time required to reach sexual maturity at 30° C, is much longer.

Identification of adult *B. acheilognathi* should be made on specimens relaxed in cold water for 10 min when the heart shaped scolex and prominent square

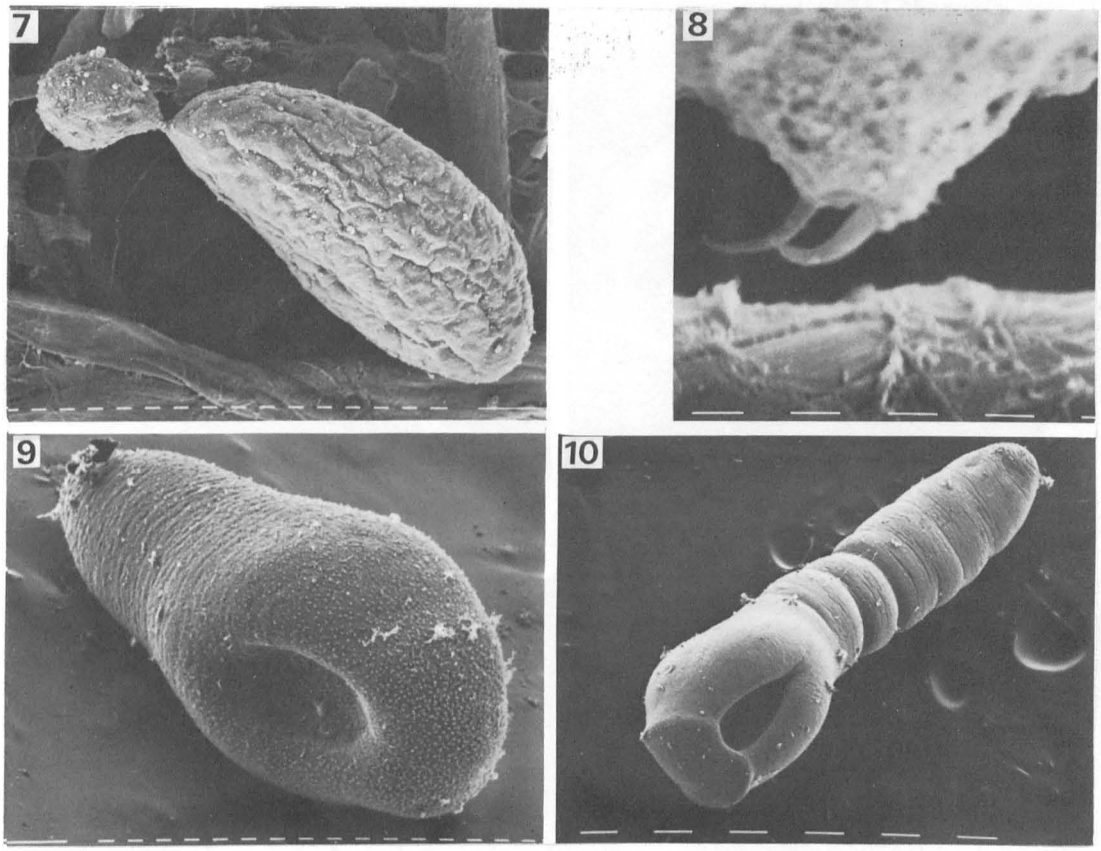

FIG. 7. Infective procercoid with well developed cercomer. Scale = 6·25 μm.

FIG. 8. Embryonic hooks on procercoid cercomer, revealed by removal of outer membrane. Scale = 1 μm.

FIG. 9. Plerocercoid 10 days after entering definitive host. Scale = 7·7 μm.

FIG. 10. Early adult worm 30 days after entering definitive host. Scale = 5·9 μm.

apical disc enable them to be distinguished from other cestodes infecting freshwater fish.

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The effect of praziquantel on the pseudophyllidean cestode *Bothriocephalus acheilognathi* in vitro

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Abstract. Adult *Bothriocephalus acheilognathi* were incubated in solutions containing 0 (control), 0.1, 1.0, 10.0 and 100 µg praziquantel per ml (0, 10^2, 10^3, 10^4 and 10^5 $\mu g l^{-1}$) of 0.9% saline for 5, 15 and 60 min at a temperature of 18° C. The worms contracted immediately upon being placed in the drug. Scanning and transmission electron microscopy revealed considerable tegumental damage particularly in the neck region. Vacuolization and 'bubbling' of the tegument occurred in all of the drug solutions tested. Exposure to drug concentrations of more than 1.0 $\mu g ml^{-1}$ (10^3 $\mu g l^{-1}$) praziquantel for 15 min or greater resulted in many of the 'bubbles' bursting and releasing their contents to the exterior. Mature proglottides were distorted and had occasional large swellings resulting in the mass expulsion of eggs. Praziquantel had no ovicidal activity. Exposure to drug concentrations of 100 µg (10^5 $\mu g l^{-1}$) praziquantel per ml saline for 24 h was not lethal to the worms.

Introduction

They highly efficacious nature of praziquantel (Droncit, Bayer) against numerous cestode and trematode species (Andrews et al. 1983); and the toleration to it shown by fish make it an ideal anthelmintic for the control of many fish parasites. Studies have shown it to be effective against *Diplostomum spathaceum* in rainbow trout (*Salmo gairdneri*) (Bylund and Sumari 1981), *Bunodera luciopercae* in perch (*Perca fluviatilis*), *Eubothrium rugosum* in burbot (*Lota lota*), *Ligula intestinalis* in roach (*Rutilus rutilus*) and *Sanguinicola inermis* in carp (*Cyprinus carpio*) (cited Andrews et al. 1983), *Proteocephalus osculatus* in the Wels catfish (*Siluris glanis*). (Andrews and Riley 1982) and *Bothriocephalus acheilognathi* in grass carp (*Ctenopharyngodon idella*) (Andrews and Riley 1982; Pool et al. 1984).

Previous workers have studied the effect of the drug on the morphology

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of parasites of medical and veterinary importance (e.g., Conder et al. 1981; Becker et al. 1981), but as yet none have concentrated on the parasites of fish.

As part of a larger investigation of the treatment of *Bothriocephalus acheilognathi* with praziquantel, worms were examined using scanning and transmission electron microscopy (SEM and TEM) after in vitro exposure to various drug concentrations.

Materials and methods

Immature and mature *Bothriocephalus acheilognathi* specimens were obtained from 0+ grass carp (*Ctenopharyngodon idella*). The parasites, individually and attached to the intestinal mucosa, were exposed for 5, 15 and 60 min to concentrations of 0 (control), 0.1, 1.0, 10 and 100 µg praziquantel per ml (0, 10^2, 10^3, 10^4 and 10^5 μgl^{-1}) of 0.9% saline at 18° C.

For SEM the worms were fixed in a 4% formaldehyde solution for 1 h, washed twice in distilled water and dehydrated using an ethanol series. The worms were treated in a Polaron E3000 critical point drier, followed by coating with 60% gold palladium using a Polaron E5100 sputter coater and viewed using a Philips 501B SEM.

For TEM worms were fixed for 5 h in a 2% paraformaldehyde and 2.5% gluteraldehyde solution in 0.1 *M* sodium cacodylate buffer, adjusted to a pH of 7.2 using 0.1 *M* hydrochloric acid. Calcium chloride was added to the buffer to give a final concentration of 2.5 m*M* to reduce membrane swelling. The worms were washed twice in 0.1 *M* sodium cacodylate buffer, pH 7.2; cut into sections of approximately 2 proglottides, and post-fixed for 3 h in 1% osmium tetroxide in a sodium cacodylate buffer, pH 7.2. After washing in a cacodylate buffer, the worms were dehydrated in an ethanol series, and gradually infiltrated with a standard mixture of spur low epoxy resin. The specimens were treated for 16 h at 60° C. Sections were cut 90 nm thick using a Reichert 0MU3 ultramicrotome and stained using saturated uranyl acetate in 50% ethanol for 20 min and lead citrate for 5 min at 20° C. Sodium hydroxide crystals were added to the lead citrate to prevent lead carbonate formation. The sections were washed in 0.02 *M* sodium hydroxide and distilled water; and viewed using a Kratos Conneth 500 TEM (Smith 1980).

Results

The tapeworms treated with 1.0, 10.0 and 100 µg praziquantel per ml (10^3, 10^4 and 10^5 μgl^{-1}) saline contracted immediately upon being placed in the drug solution, the intensity of the response increased with the concentration of the drug. After 15 min the worms placed in a 0.1 μgml^{-1} (10^2 μgl^{-1}) solution showed areas of contraction and relaxation along the strobila. After 24 h exposure to the drug solutions, the worms were still attached to the intestine, and showed a weak response when touched. Control worms were still active but had released their hold on the intestine.

Scanning electron microscopy

Treated and untreated worms, as observed by SEM are shown in Figs. 1–6. The tegument of the control worms was uniformly covered by microtriches. Dome-shaped tumuli were evenly spaced over the scolex, but decreased posteriorly, being absent from mature and gravid proglottides (Fig. 2). Adjacent proglottides were of a similar size and shape (Fig. 1).

Worms exposed to 0.1 µg (10^2 μgl^{-1}) praziquantel per ml saline were morphologically identical with the control after 5 and 15 min.

After 60 min exposure occasional small 'blebs' (1–10 μm) were apparent on the outer surface of the bothria, and on proglottide margins in the neck region.

At a praziquantel concentration of 1.0 μgml^{-1} (10^3 μgl^{-1}) the 'blebs' were more numerous, particularly on the scolex, and appeared after only 5 min exposure (Fig. 3). Increased exposure to the drug resulted in many of the blebs bursting (Fig. 4). The mature proglottides were swollen after 15 and 60 min exposure (Fig. 5).

Worms exposed to 10 μg praziquantel per ml (10^4 μgl^{-1}) saline solution showed the most intense reaction. Large areas of the scolex were covered in 'blebs' (1–10 μm diameter) after only 5 min exposure. Swelling and distortion of the mature proglottides occurred after 15 min. Large vacuoles (280 μm × 140 μm) formed on some mature proglottides after 1 h (Fig. 6).

Exposure to 100 μgml^{-1} (10^5 μgl^{-1}) praziquantel solution resulted in fewer blebs on the scolex, but a higher density on the neck region of the strobila. Mature proglottides were swollen.

Transmission electron microscopy

The structure of the tegument of *B. acheilognathi* is similar to that described for other cestodes (e.g., Lumsden 1966; Becker et al. 1980) and has been described in detail by Granath et al. (1983). The distal cytoplasm is covered in numerous 1–2 μm microtriches, and is rich in mitochondria and vesicles. The basal lamina separates the muscle layer from the distal cytoplasm (Fig. 7).

Examination of the 'blebs' observed using the SEM reveals them to be continuous with the distal cytoplasm. The 'blebs' diameter 1–10 μm were filled with cytoplasm and granules, indicating that leakage of the tegument had occurred (Fig. 8).

Vacuoles (2–5 μm diameter) were apparent after 15 min in the distal cytoplasm of worms exposed to a 1.0 μgml (10^3 μgl^{-1}) or greater praziquantel solution. After 1 h these vacuoles were larger (2–10 μm diameter) and more extensive. Cellular leakage into the vacuoles had occurred (Fig. 8). The microtriches appear to be unaffected by the drug.

Discussion

The effect of praziquantel on the tegument of *Bothriocephalus acheilognathi* is similar to that described for other cestode species (e.g. Conder et al. 1981; Becker et al. 1980, 1981).

Praziquantel concentrations as low as 0.001 μgml^{-1} (1 μgl^{-1}) have been shown to affect adult hymenolepid and echinococcus adult cestodes, while concentrations of 0.01 μgml^{-1} (10 μgl^{-1}) can cause contraction and paralysis (Andrews and Thomas 1979). *Bothriocephalus acheilognathi* appeared to show a greater degree of tolerance to the drug, a concentration of 0.1 μgml^{-1} (10^2 μgl^{-1}) for 15 min or 1 μgml^{-1} (10^3 μgl^{-1}) for 5 s being required to produce contraction. Paralysis of the strobila did not occur in the drug concentrations tested. Previous in vitro studies were conducted

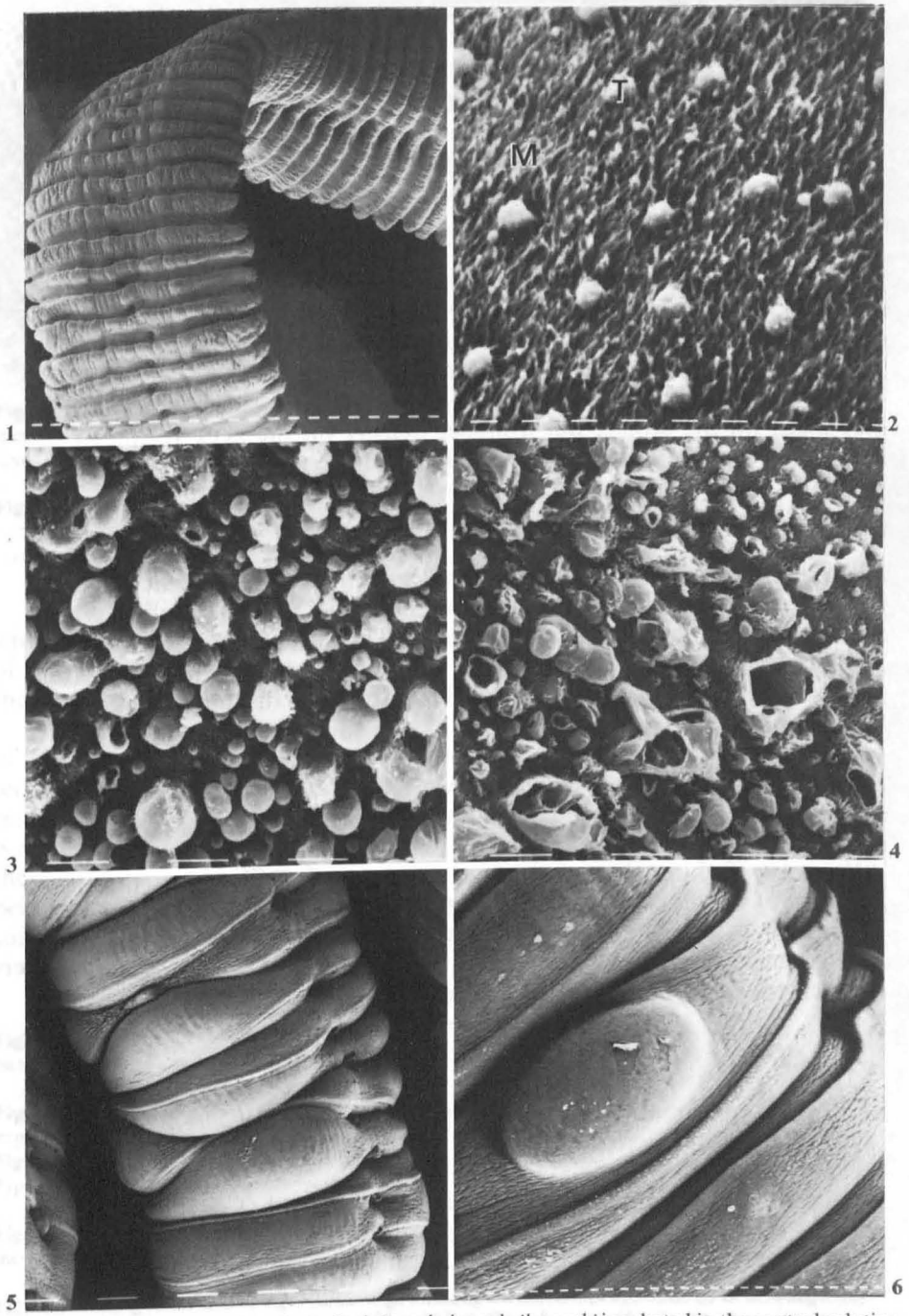
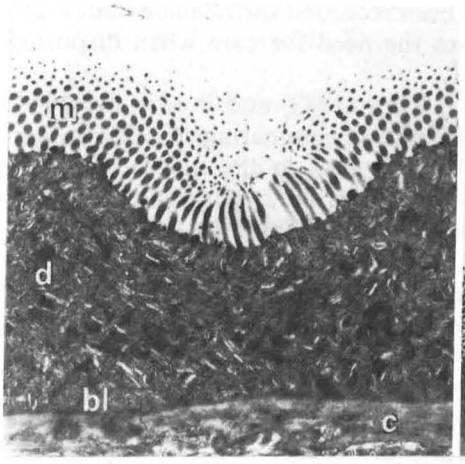


Fig. 1. Mature proglottides from *Bothriocephalus acheilognathi* incubated in the control solution (0.9% saline) for 15 min. Viewed with SEM. Marker = 100 μm



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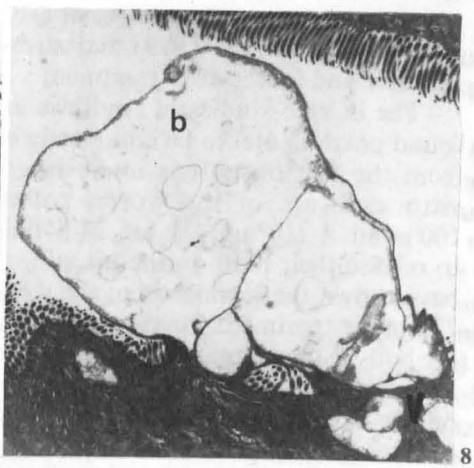

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Fig. 7. Transmission electron micrograph of the tegument in the neck region of *B. acheilognathi* incubated in the control solution for 15 min. Microtriches (*m*), distal cytoplasm (*d*), basal layer (*bl*), and circular musculature (*c*). × 5,000

Fig. 8. Transmission electron micrograph of *B. acheilognathi* tegument from the neck region incubated for 5 min in 1.0 μg praziquantel per ml (10^3 $\mu g l^{-1}$) 0.9% saline. Note vacuolisation (*v*) and ballooning of tegument (*b*). × 3,000

with mammalian cestodes (e.g., Becker et al. 1980, 1981) at a temperature of 37° C. The lower temperature (18° C) employed in this study may explain the apparent reduced sensitivity.

Considerable variation in the effect of the drug occurred along the length of the worm. The scolex and neck region of the tegument showed the characteristic ballooning and vacuolization, previously recorded in a number of cestode species (Andrews et al. 1983). Becker et al. (1981) suggest that this is a result of the high metabolic activity in this area. Unlike other cestodes, the mature proglottides of *B. acheilognathi* were affected by praziquantel being grossly distorted with occasional large swellings. This response resulted in the mass expulsion of eggs, which hatched normally to produce viable coracidia. Control worms released very few eggs. The non ovicidal

Fig. 2. Scanning electron micrograph of *B. acheilognathi* tegument from the neck region incubated in the control solution for 60 min. Note microtriches (*M*) and tumuli (*T*). Marker = 1 μm

Figs. 3 and 4. Scanning electron micrograph of *B. acheilognathi* tegument from the neck region exposed to a solution of 1.0 μg praziquantel per ml (10^3 $\mu g l^{-1}$) 0.9% saline.
Fig. 3. Incubated for 5 min.
Fig. 4. Incubated for 15 min. Marker = 10 μm

Fig. 5. Scanning electron micrograph of swollen mature proglottides from *B. acheilognathi* incubated in 1.0 μg praziquantel per ml (10^3 $\mu g l^{-1}$) 0.9% saline for 60 min. Marker = 100 μm

Fig. 6. Scanning electron micrograph of large swelling on mature proglottide of *B. acheilognathi* incubated in 10 μg praziquantel per ml (10^4 $\mu g l^{-1}$) 0.9% saline for 60 min. Marker = 10 μm

activity of praziquantel has previously been recorded for *Echinococcus granulosus* (Thakur et al. 1979) and stresses the need for care when disposing of water and faeces after treatment.

The in vivo studies of Andrews and Riley (1982) and Pool et al. (1984) found praziquantel to be completely effective in eliminating *B. acheilognathi* from the fish host. It is interesting to note that in the present study in vitro exposure of the worms to praziquantel concentrations of up to 100 $\mu g ml^{-1}$ (10^5 $\mu g l^{-1}$) for 24 h failed to kill the worms or cause them to release their hold on the intestine. Further in vivo studies by the author have shown that expulsion of the worms from the gut occurs approximately 72 h after treatment. In view of the rapid elimination of praziquantel from the body (Steiner and Garbe 1976, Andrews and Thomas 1979) it is probable that some other factor, such as the action of the host digestive enzymes on the damaged tegument and/or the peristatic action of the intestine contributes to the expulsion of the worms from the gut.

Acknowledgements. The author wishes to thank Drs. A.F. Brown and J.C. Chubb, University of Liverpool for critical reading of manuscript, Mr. C. Veltkamp, University of Liverpool for SEM, Mr. J. Smith, University of Liverpool for TEM and Dr. R. Sweeting, Thames Water Authority for providing praziquantel. The Natural Environment Research Council provided a studentship, without which the work could not have been completed.

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A critical scanning electron microscope study of the scolex of *Bothriocephalus acheilognathi* Yamaguti, 1934, with a review of the taxonomic history of the genus *Bothriocephalus* parasitizing cyprinid fishes

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Summary

Bothriocephalus specimens from one *Ctenopharyngodon idella* (O+) were fixed using three different techniques and the scolex morphology was examined using scanning electron microscopy. The scolices were compared with figures of the five species of *Bothriocephalus* and one species of *Schyzocotyle* previously described from cyprinid fishes. The taxonomic history of the *Bothriocephalus* species parasitizing cyprinid fish is reviewed. It is concluded that there is only one *Bothriocephalus* species parasitizing cyprinid fishes, and that continued use should be made of the name *B. acheilognathi* Yamaguti, 1934.

Introduction

The specific identity of *Bothriocephalus* species imported into fish farms in the British Isles has been a matter of conjecture since their first reported introduction (Andrews *et al.*, 1981).

Recently Dubinina (1982) has drawn attention to the confusion concerning the taxonomic status of the species of the genus *Bothriocephalus* parasitizing cyprinid fishes. She concluded that in the USSR there were two species: the widely distributed *B. opsariichthydis* Yamaguti, 1934 (synonyms *B. gowkongensis* Yeh, 1955 and *B. phoxini* Molnár, 1968) and the less prevalent *B. acheilognathi* Yamaguti, 1934 (synonym *Schyzocotyle fluviatilis* Akhmerov, 1960), which could be distinguished by their differing scolex morphology. This opposed the generally accepted view of Molnár (1977) that the four species of *Bothriocephalus* described from European cyprinid fishes were in fact one and, in agreement with Körting (1975), that priority should be given to the name *B. acheilognathi* with *B. opsariichthydis*, *B. gowkongensis* and *B. phoxini* as synonyms.

Thus there is considerable disagreement over the identity of species of *Bothriocephalus* in cyprinids: this may be owing to the extreme variation in the size of the living worms. Adult *Bothriocephalus* have no hard structures, and are active worms, capable of considerable mobility of the body, especially the scolex. Therefore considerable variation in morphology can be induced by the fixation process. The species described from cyprinids are very similar in morphology, so much so that it is a matter of conjecture whether the differences are specific, intraspecific or artefacts of fixation.

It is the purpose of the present paper to examine the suggestion of Dubinina (1982) that, for the identification of species of this genus, special attention should be paid to the structure of the scolex; and to clarify the taxonomic status of some members of the genus.

Materials and methods

In order to demonstrate consistency or variation in scolex morphology in our species of *Bothriocephalus* all the worms were obtained from a single O+ *Ctenopharyngodon idella* (Valenciennes) (Cyprinidae). Ten worms of various sizes were fixed in each of the following procedures: (i) *in situ* in the host intestine using a 10% formaldehyde solution; (ii) removed from the intestine and immediately placed in absolute alcohol at 25° C; and (iii) removed from the intestine, relaxed in distilled water at 10° C for 5 min followed by immersion in a 10% formaldehyde solution. After fixation specimens in formaldehyde were washed twice in distilled water and dehydrated using an ethanol series. All scolices were treated in a Polaron E3000 critical point drier, followed by coating with 60% gold-paladium using a Poloron E5100 sputter coater and viewed using a Philips 501B scanning electron microscope.

Bothriocephalus plerocercoid and early adult stages were reared in the laboratory using the methods detailed by Körting (1975). The plerocercoids and early adults were allowed to develop within the fish intestine for 16 and 40 days respectively at 20° C before removal. To facilitate handling owing to their small size these specimens were placed in a cone of filter paper for fixation using method (iii) (above). After drying, the contents of the cone were emptied on to double-sided adhesive tape for attachment to the viewing stub, or the apex of the cone was mounted directly on the stub. The specimens were sputter coated and viewed as indicated above.

Results

The results are presented as a series of photographs of representative scolices obtained using each of the three fixation procedures.

Figures 1–4 show scolices fixed *in situ* in the intestine of the host. They were rounded, the bothria were short and orientated towards the apex of the scolex (anterior) and the apical disc was very apparent, with, in some individuals (Figs. 2–4) four distinct lobes. Little variation in size or form of scolices fixed *in situ* was seen.

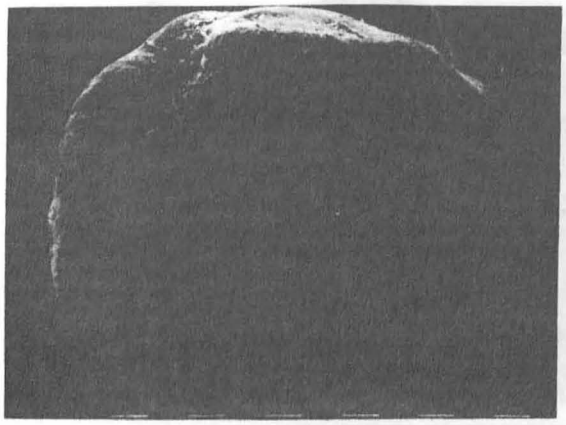
Figures 5–7 demonstrate scolices fixed immediately after removal from the intestine. In this instance the scolices were not completely relaxed, the bothria were open and the apical discs were not obvious. The shape of the scolex was a manifestation of the state of contraction or extension of the musculature at the moment of fixation. Accordingly, considerable variation in the form of the scolex resulted.

Figures 8–12 illustrate scolices relaxed in distilled water before fixation. These scolices were all an inverted heart-shape with a characteristic apical disc (Fig. 12). In most of these relaxed specimens the bothria were closed and were approximately two thirds the length of the scolex. The variation between the two bothria of one individual scolex is shown in Figures 10 and 11. One bothrium (Fig. 10) is contracted around a piece of intestinal tissue, while the other (Fig. 11) is relaxed. The scolices which were relaxed before fixation showed very little variation in morphology.

The apical disc, shown on adult *Bothriocephalus* fixed *in situ* in the intestine (Figs. 1–4) and in the relaxed individuals (Figs. 8–12), was also visible on late plerocercoid (Fig. 13) and early adult stages (Fig. 14) of the life-cycle.

Discussion

The results illustrated in this paper clearly demonstrate the well-known fact that the shape of soft-bodied helminths will vary according to their treatment during autopsy and fixation. To provide a controlled situation, gravid *Bothriocephalus* from a single species infection in one individual fish were used, and, therefore, any variation would be owing to intraspecific variation or artefacts of fixation. In fact, specified conditions and methods of fixation appeared more significant in affecting scolex morphology than intraspecific variation, which should not surprise anyone who has worked with living pseudophyllidean cestodes, as in many species the mobility of the scolex can be clearly seen *in vitro* (Brandt *et al.*, 1981).



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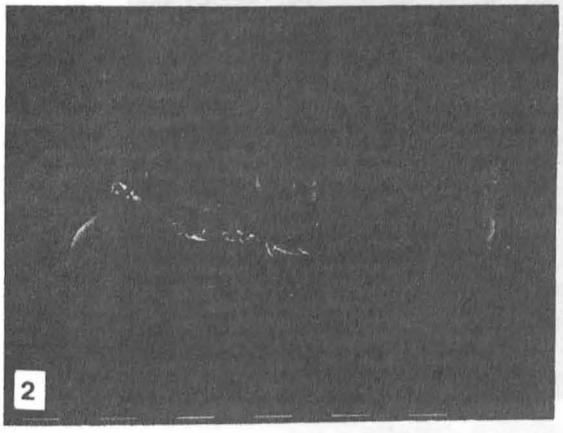

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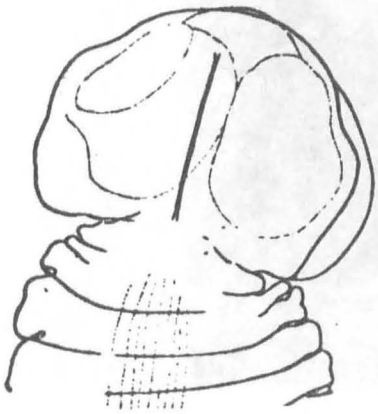

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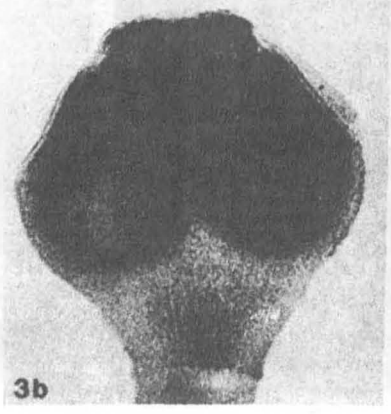


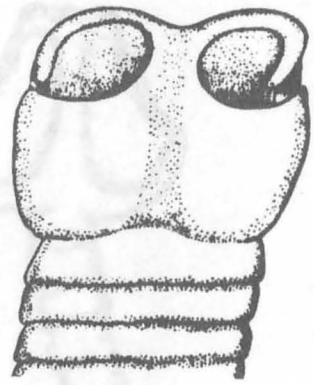
3b

Figs. 1, 2, 3. *B. acheilognathi* scolices from *C. idella* fixed *in situ* in the host intestine using 10% formaldehyde solution. 1. Viewed at 7.2 kv. Bar micron markers are 100 μm each. 2. Viewed at 30 kv. using back scattered electrons. Markers 76.92 μm each. 3. Viewed at 3.6 kv. Markers 100 μm each.

Fig. 1.a. *Schyzocotyle fluviatilis*. From Akhmerov, 1960: Fig. 4.1 p. 20.

Fig. 3a. *B. opsariichthydis*. From Dubinina, 1971: Fig. 10, p. 90.

Fig. 3b. *B. gowkongensis* or *B. opsalichthydis* (= *opsariichthydis*) From Körting, 1974: Fig. 2 p. 168.



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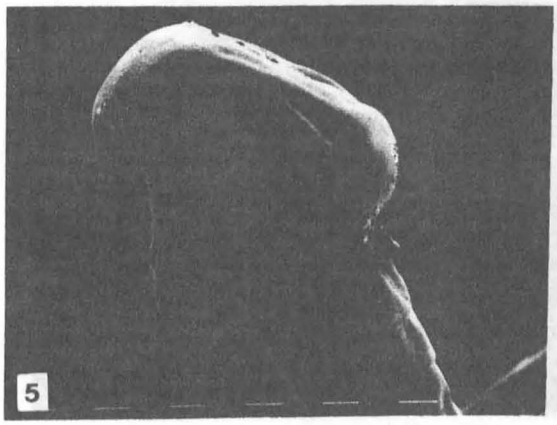

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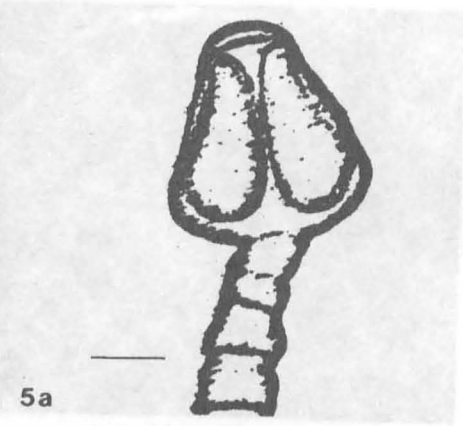

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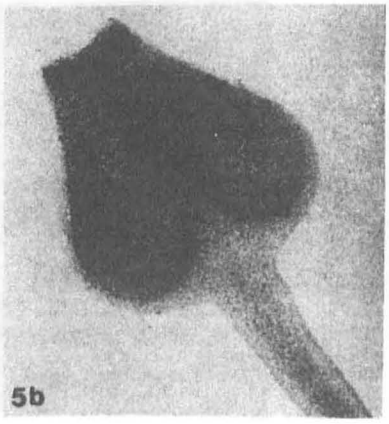


5b

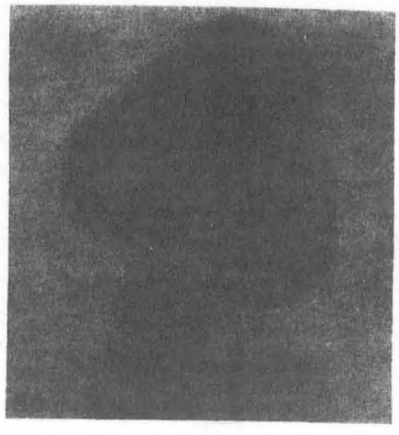


Fig. 4. *B. acheilognathi* scolex from *C. idella* fixed *in situ* in the host intestine using 10% formaldehyde solution. Viewed at 7.2 kv. Markers 83.33 μm each.

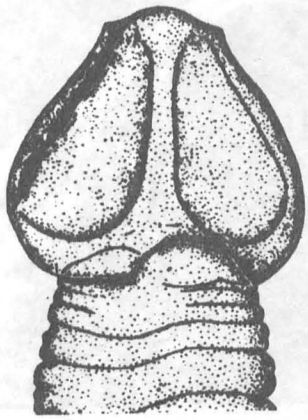
Fig. 4a. *Schyzocotyle fluviatilis*. From Akhmerov, 1960: Fig. 3,1. p. 20.

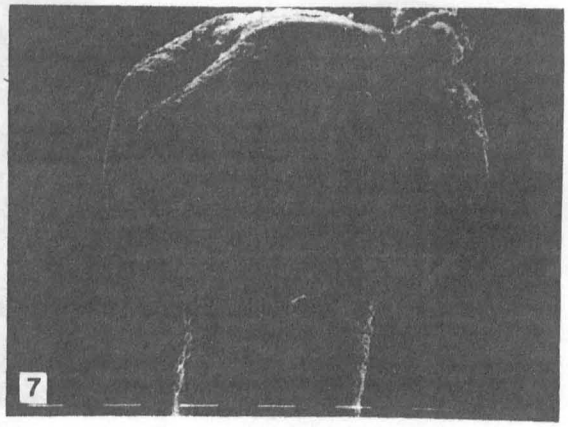
Fig. 5. *B. acheilognathi* scolex from *C. idella* fixed immediately after removal from intestine by immersion in absolute alcohol at 25° C. Viewed at 7.2 kv. Markers 71.43 μm each.

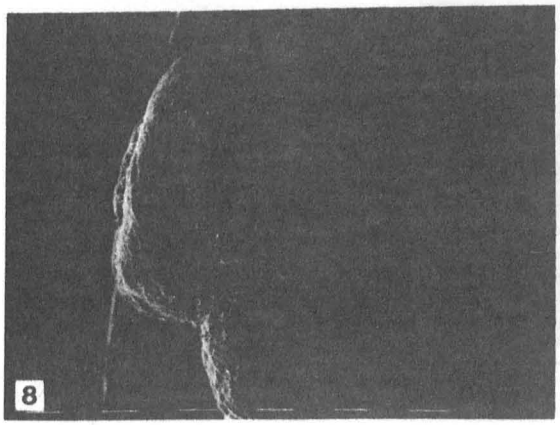
Fig. 5a. *B. gowkongensis*. From Yeh, 1955: Fig. 2 between pp. 73–74. Bar marker = 100 μm.

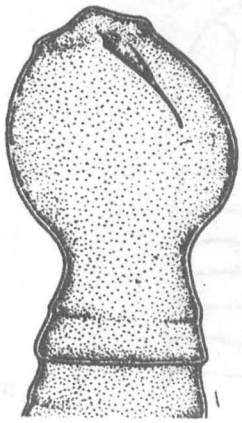
Fig. 5b. *B. gowkongensis* or *B. opsalichthydis*. (= *opsariichthydis*). From Körting, 1974: Fig. 1, p. 168.

Fig. 5c. *B. gowkongensis* or *B. opsalichthydis*. (= *opsariichthydis*). From Körting, 1974: Fig. 3, p. 169.









Figs. 6, 7. *B. acheilognathi* scolices from *C. idella* fixed immediately after removal from intestine by immersion in absolute alcohol at 25° C. Viewed at 3.6 kv. Markers 76.92 μm each. 7. Viewed at 7.2 kv. Markers 71.43 μm each.

Fig. 6a *B. gowkongensis*. From Yeh, 1955: Fig. 1 between pp. 73–74. Bar marker = 100 μm.

Fig. 6b. *B. opsariichthydis*. From Yamaguti, 1934: Fig. 23, p 17.

Fig. 8. *B. acheilognathi* scolex from *C. idella*, relaxed in distilled water at 10° C for 5 min before fixation in 10% formaldehyde solution. Viewed at 7.2 kv. Markers 100 μm each.

Fig. 8a. *B. acheilognathi*. From Yamaguti, 1934: Fig. 20, p. 15.

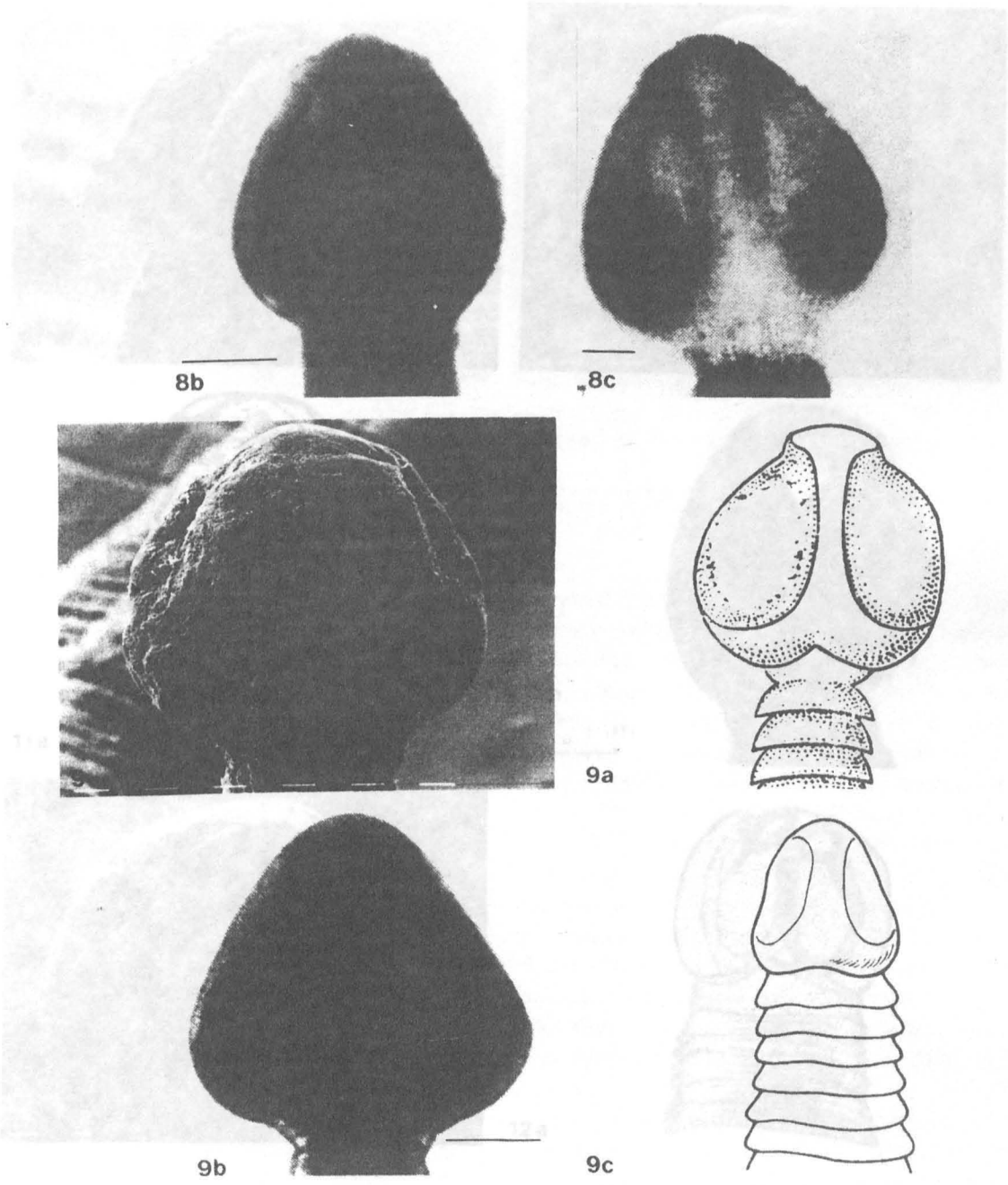
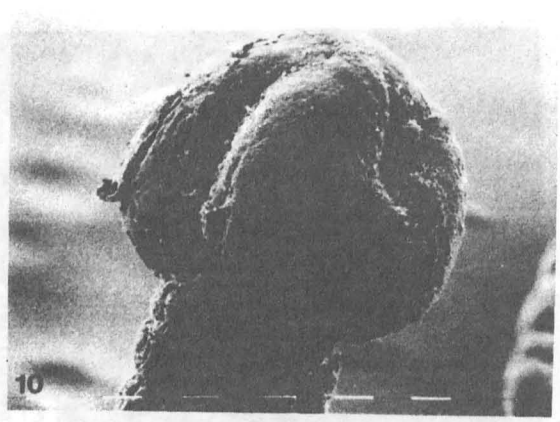
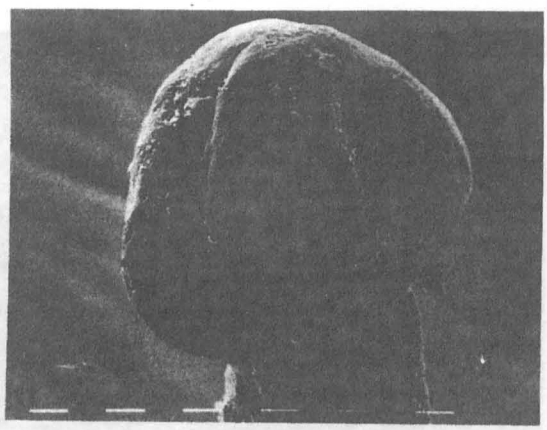
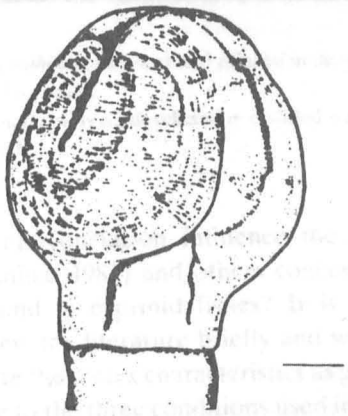


Fig. 8b. *B. gowkongensis*. From Molnár & Murai, 1973: Fig. 2 opposite p. 102. Bar marker = 100 μm.
Fig. 8c. *B. phoxini*. From Molnár & Murai, 1973: Fig. 7 opposite p. 103. Bar marker = 50 μm.
Fig. 9. *B. acheilognathi* scolex from *C. idella* relaxed in distilled water at 10° C for 5 min before fixation in 10% formaldehyde solution. Viewed at 7.2 kv. Markers 76.92 μm each.
Fig. 9a. *B. gowkongensis*. From Musselius, 1973: Fig. 8a p. 28.
Fig. 9b. *B. gowkongensis*. From Molnár & Murai, 1973: Fig. 1 opposite p. 102. Bar marker = 100 μm.
Fig. 9c. *B. phoxini*. From Molnár, 1968: Fig. 1D p. 184.

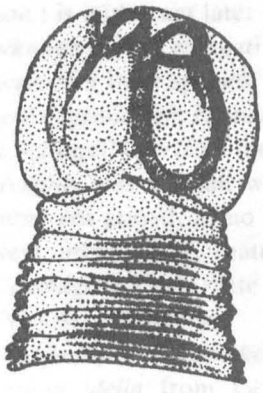






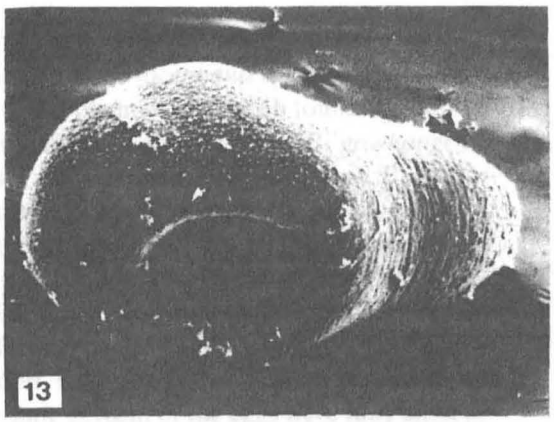
11a

11b



12a

Figs. 10, 11, 12. *B. acheilognathi* scolices from *C. idella* relaxed in distilled water at 10° C for 5 min before fixation in 10% formaldehyde solution. 10. Viewed at 7.2 kv. Markers 71.43 μm each. Same scolex as Fig. 11. Bothrium contracted around intestinal material. 11. Viewed at 7.2 kv. Markers 71.43 μm each. Same scolex as Fig. 10. Other bothrium relaxed. 12. Viewed at 7.2 kv. Markers 71.43 μm each.
Fig. 11a. *B. gowkongensis*. From Protasova, 1977: Fig. 14a p. 88. Bar marker 200 μm.
Fig. 11b. *B. phoxini*. From Molnár & Murai, 1973: Fig. 8 opposite p. 103. Bar marker = 50 μm.
Fig. 12a. *S. fluviatilis*. From Akhmerov, 1960: Fig. 4 p. 20.



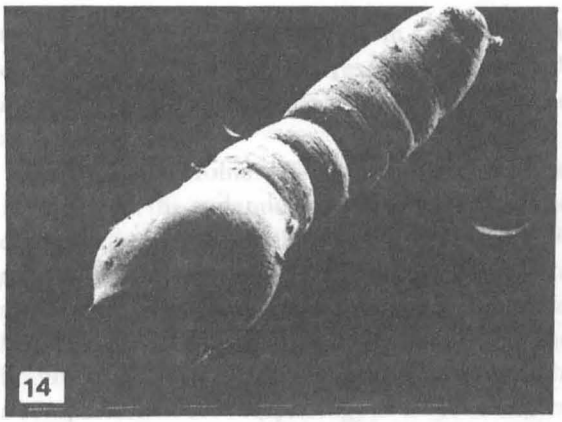


Fig. 13. *B. acheilognathi* late plerocercoid relaxed in distilled water at 10° C for 5 min before fixation in 10% formaldehyde. Viewed at 7.2 kv. Markers 7.7 μm each.

Fig. 14. *B acheilognathi* early adult relaxed in distilled water at 10° C for 5 min before fixation in 10% formaldehyde. Viewed at 7.2 kv. Markers 5.9 μm each.

How does this conclusion influence the proposals of Dubinina (1982) and others concerning the species found in cyprinid fishes? It is convenient to review the literature briefly and where possible to relate the scolex characteristics as given in the literature to the three conditions used in our study.

Yamaguti (1934) described *B. acheilognathi* from a single worm 80 mm long found in the intestine of *Acheilognathus rhombea* (Temminck & Schlegel) (Cyprinidae) from Lake Ogura, Japan. Although he thought the worm had a somewhat atypical scolex, the internal anatomy justified its inclusion in the genus *Bothriocephalus*. His illustration is reproduced as Figure 8a beside Figure 8. We are of the opinion that it represents the scolex typical of a worm fixed after relaxation. In the same publication Yamaguti (1934) described a second species of *Bothriocephalus* from a cyprinid fish *Opsariichthys uncirostris* (Temminck & Schlegel) from Lake Biwa and the River Yodo, Japan. Yamaguti misspelt the generic name of the host, it should have been *Opsariichthys*, hence the cestode was incorrectly named *B. opsalichthydis*, although later Yamaguti (1959) emended the spelling to *opsariichthydis*. *B. opsariichthydis* was a common worm, up to more than 100 mm long with an inverted heart-shaped scolex with a prominent terminal disc and deep bothridial grooves. His illustration of the scolex is reproduced as Figure 6b beside Figure 6. We believe it represents a worm fixed immediately after removal from the host intestine. Yamaguti (1934) noted in *B. opsariichthydis* that the eggs contained embryos which were segmented but not yet fully embryonated (July). This point is important later in connection with *B. gowkongensis*. Yamaguti (1952) subsequently described a third species, *B. fluviatilis*, from a Japanese cyprinid fish *Hymenophysa curta* (Temminck & Schlegel) also from the River Yodo, Japan. *B. fluviatilis* was a small worm 22.5 mm long, presumably not gravid, as no details of egg dimensions were provided. A mature proglottis was figured (Yamaguti, 1952, Plate I, Fig. 1, opposite p. 76), but not the scolex.

Yeh (1955) described *B. gowkongensis* from *Ctenopharyngodon idella* from Gowkong, near Canton, South China. The worms were 35–80 mm long, with a large inverted heart-shaped scolex. Yeh figured three scolices.

Two of Yeh's figures (his Figs. 1 and 2) correspond to two of our worms fixed immediately after removal from the fish intestine (Fig. 5 and 6) and are reproduced as Figures 5a and 6a; his three illustrations show a range of variation characteristic of this situation. In *B. gowkongensis* the eggs

were fully embryonated when laid, according to Yeh the first *Bothriocephalus* species recognized as showing this phenomenon. Yeh found variation in a large number of specimens of *B. gowkongensis* and as a consequence suggested that *B. opsariichthydis* was a synonym of *B. acheilognathi*. It should be noted that Yeh (1955) corrected the spelling of *B. opsalichthydis* to *B. opsariichthydis*.

In 1956 Liao & Shih demonstrated that the degree of embryonation of the eggs of *B. gowkongensis* varied with season and water temperature. Eighty-nine percent of the eggs were fully embryonated on release from April to October (24 to 29° C), whereas only 2% were from November until February (14.8 to 21.3° C).

Baer & Fain (1958) also suggested that *B. opsalichthydis* (= *opsariichthydis*) was a synonym of *B. acheilognathi* owing to the fact that they could not separate the two species on morphological features. Yamaguti (1959) accepted that *B. opsariichthydis* and *B. acheilognathi* were synonyms as well as emending the original spelling of the specific epithet *opsalichthydis* as indicated earlier.

It is interesting to note that Baer & Fain (1958) considered *Clestobothrium crassiceps* (Rudolphi, 1819) to be in the same genus as *B. acheilognathi* and *B. kivuensis* Baer & Fain, 1958 on the basis of their similar scolex morphology. They reduced *Clestobothrium* to a subgenus and renamed the three species *B.(C). crassiceps, B.(C.) acheilognathi* and *B.(C.) kivuensis*. This view was supported by Tadros (1967), who transferred *B. acheilognathi* and *B. kivuensis* to the genus *Clestobothrium* and emended the diagnosis of the genus.

An examination of the descriptions of the genera *Clestobothrium* and *Bothriocephalus* provided by Wardle & McLeod (1952) indicates that they differ most notably in the presence of operculate eggs in *Bothriocephalus* and their absence in *Clestobothrium*. Therefore, despite the similarity in scolex morphology, *C. crassiceps* should not be included in the same genus as *B. acheilognathi*.

Bothriocephalus phoxini Molnár, 1968 was described from *Phoxinus phoxinus* (L.) (Cyprinidae) in a tarn near Lake Balaton, Hungary. In this host the worms had a maximum length of 45 mm and an inverted heart-shaped scolex. A terminal disc and two bothria were readily visible in relaxed specimens. Such a description corresponds to our findings (Figs. 8–12) and Molnár's figure is reproduced as Figure 9c.

According to Molnár (1968) the range of the host *P. phoxinus*, the endemic nature of the infection he discovered and the small size of individuals of *B. phoxini* readily served to differentiate them from all known European bothriocephalids and also from *B. opsariichthydis* from the Far East. The finding of *B. gowkongensis* in Hungary resulted in a comparison of the morphology of *B. gowkongensis* and *B. phoxini* by Molnár & Murai (1973) which substantiated the separation of the two species. Molnár & Murai (1973) fixed their materials using mostly hot 5% formalin. (See Figs. 8b, 8c, 9b, 11b.) Molnár (1977), by means of cross infection experiments using eggs from tapeworms found in *Cyprinus carpio* and *Phoxinus phoxinus*, demonstrated that typical *B. gowkongensis* worms were recovered from *C. carpio* and typical *B. phoxini* specimens were recovered from *P. phoxinus*.

Otte *et al.* (1972) considered the *Bothriocephalus* spp. found in *Cyprinus carpio* imported into Austria from Hungary to be *B. acheilognathi*, with *B. opsariichthydis* as a synonym. Körting (1974) commented about *B. gowkongensis* or *B. opsalichthydis* (see Figs. 3b, 5b, 5c), he stated 'the name of the species is not yet finally established'. By 1975 Körting observed that, in the event of the three species *B. acheilognathi, B. opsariichthydis* and *B. gowkongensis* being accepted as synonymous, the priority should be with *B. acheilognathi*. The *Bothriocephalus* sp. he studied from *Cyprinus carpio* in southern Bavaria appeared to be *B. opsariichthydis* as no fully embryonated eggs were detected when they were expelled from the uterus. However, in the light of Liao & Shih (1956) establishing that the maturity of the eggs of *B. gowkongensis* was dependent upon the season of the year and that the tapeworm could deliver both fully embryonated and half-embryonated eggs, together with the fact that *B. opsariichthydis* and *B. gowkongensis* could not be differentiated by morphological features, Körting (1975) favoured the opinion that the two species were identical. As a consequence of the cross-infection experiments

performed by Molnár (1977) he proposed that the names *B. opsariichthydis* Yamaguti, 1934, *B. gowkongensis* Yeh, 1955 and *B. phoxini* Molnár, 1968 were synonyms of a single species which, by virtue of priority, should be called *B. acheilognathi* Yamaguti, 1934.

In Japan Nakajima & Egusa (1974) examined *Bothriocephalus* in *Cyprinus carpio* from farm ponds in Nagano, Yamagata and Akita prefectures. They decided that the criteria for identification were: (i) shape and structure of the body – their species resembled *B. acheilognathi, B. opsariichthydis, B. fluviatilis* and *B. gowkongensis*, all of which had been found in Japan or China; (ii) their species differed from *B. gowkongensis* in that the egg was never embryonated when laid; (iii) the body length of their worms (average of 10 of the largest mature individuals, 173 mm) was close to *B. opsariichthydis* (more than 100 mm long according to Yamaguti, 1934), but differed from *B. acheilognathi* (about 80 mm) and *B. fluviatilis* (22 mm); (iv) Nakajima & Egusa (1974) proposed that *B. acheilognathi* and *B. fluviatilis* were young stages of *B. opsariichthydis*. Their Figure 2 A–D (not reproduced herein) clearly revealed how the form of the scolex was changed according to condition and angle of view.

To summarize at this point, the opinion held by most authorities was that there was one species in cyprinids (Nakajima & Egusa, 1974; Körting, 1975; Molnár, 1977). This view was adopted by Andrews *et al.* (1981) and Chubb (1981) when *Bothriocephalus* were found in three fish farms in the British Isles during 1979.

However Dubinina (1982) proposed an alternative view, based largely on the hypothesis that for the identification of the species of the genus *Bothriocephalus* special attention should be given to the structure of the scolex. On the basis of scolex morphology she suggested that *B. opsariichthydis* and *B. acheilognathi* were distinct species. She further suggested that *B. gowkongensis* and *B. phoxini* were synonyms of *B. opsariichthydis*, and *Schyzocotyle fluviatilis* Akhmerov, 1960 was a synonym of *B. acheilognathi*.

The figures of the scolex of *Schyzocotyle fluviatilis* provided by Akhmerov (1960) clearly show it to be a bothriocephalid as proposed by Dubinina (1982) (Fig. 1a, 4a and 12a), although the sketch of the scolex (Fig. 4a) is perplexing. It may correspond to our Figure 4 or may be a damaged scolex. The details of the proglottis confirm the worm as a *Bothriocephalus*.

Dubinina (1982) suggested that Yamaguti (1959) confirmed the independence of the two species *B. acheilognathi* Yamaguti, 1934 and *B. opsariichthydis* Yamaguti, 1934. Yamaguti (1959) has two entries: p. 44 '*B. acheilognathi* Yamaguti, 1934, in *Acheilognathus rhombea*; Japan. Also in *Gnathopogon elongatus suwae*, Japan.' and p. 46 '*B. opsariichthydis* Yamaguti, 1934,[1]) syn. of *B. acheilognathi* — Yeh, 1955, in *Opsariichthys uncirostris;* Japan.' The footnote on p. 46 reads: '[1]) Original spelling *opsalichthydis* emended to *opsariichthydis*'. In our opinion the dash between the two sections of the entry for *B. opsariichthydis* on p. 46 leaves no doubt that Yamaguti accepted the proposal of Yeh (1955) that *B. opsariichthydis* was a synonym of *B. acheilognathi*. In this respect Körting (1975) and Protasova (1977) were correct. Nakajima & Egusa (1974) were also in agreement, but they opted for the alternative view, of *B. acheilognathi* Yamaguti, 1934 as a synonym of *B. opsariichthydis* Yamaguti, 1934.

A number of other authors have figured a scolex of *Bothriocephalus* from cyprinid fishes, including Dubinina (1971), Musselius (1973) and Protasova (1977). The Dubinina (1971) scolex (as *B. opsariichthydis*, see Fig. 3a) corresponds broadly to our Figure 3, a specimen fixed *in situ* in 10% formaldehyde; that of Musselius (1973, as *B. gowkongensis*, see Fig. 9a) approaches our Figure 9, an individual relaxed in cold water and fixed in 10% formaldehyde, and that of Protasova (1977, as *B. gowkongensis*, see Fig. 11a) resembles our Figure 11, a specimen also fixed after relaxation.

Some other *Bothriocephalus* spp. need a mention. *B. capillicollis* Mégnin, 1883 was found in a 'carpe de mer' from the coast of Norwegian Lapland. According to Gozmány (1979) the 'carpe de mer' is *Labrus bergylta* Ascanius, 1767 (Labridae) and is therefore not relevant here, although Linstow (1889) listed it from *Idus melanotus* and Zschokke (1903) from *Leuciscus idus*. We agree

with Protasova (1977) that this form should be treated as a *species inquirenda*. Rudolphi (1810) described *B. granularis* in 'Cyprinus' observed by Zeder in 1788. The description given by Rudolphi provides too little information for precise evaluation of the worm, so that again we agree with Protasova (1977) that it should be treated as a *species inquirenda*.

B. kivuensis, from *Barbus altianalis altianalis* Blgr. in Lake Kivu, has a scolex resembling *Bothriocephalus acheilognathi*, but its great length, 700 mm to 1 m, more than three times as long as the largest *B. acheilognathi* specimen we have examined, and the presence of a vaginal sphincter suggest that it is a distinct species. *B. aegyptiacus* Ryšavý & Moravec, 1975 was recorded from *Barbus bynni* in the River Nile. The scolex is similar to that of *Bothriocephalus opsalichthydis*, but is has fewer testes, 60–100 as compared with 100–200 and is somewhat larger (502–611 × 3.8–4.3 mm compared to 100 × 1.25 mm). The above two species require further critical investigation to validate their taxonomic status.

Prigli (1974, 1975) noted the role of aquatic birds in the spread of *B. acheilognathi* (as *B. gowkongensis*) in Hungary. Borgarenko (1981) observed this phenomenon in Tadzhikstan and figured a scolex from the intestine of the little bittern *Ixobrychus minutus* L. Shinde & Jadhav (1977) recorded a single specimen of *B. opsariichthydis* in a marine fish *Histophorus gladius* at Veraval on the west coast of India. Although they described and figured this worm we doubt their identification and suspect it was a marine species of *Bothriocephalus*.

We dispute the conclusion of Dubinina (1982) that *B. opsariichthydis* Yamaguti, 1934 and *B. acheilognathi* Yamaguti, 1934 are separate species. We are of the opinion that the scolex characters utilized by Dubinina in this instance represent no more than variation of form produced by different methods of fixation. Thus, as indicated earlier, the Yamaguti (1934) specimen of *B. acheilognathi* has a scolex form consistent with a worm fixed after relaxation but viewed from a semi-dorsoventral angle (as our Fig. 8). The Yamaguti (1934) specimen of *B. opsariichthydis* (as *B. opsalichthydis*) has a scolex corresponding with a worm fixed immediately on removal from the host intestine (as our Fig. 6). We agree with Dubinina (1982) that *Schyzocotyle fluviatilus* Akhmerov, 1960 (see Fig. 1a) is a species of *Bothriocephalus*, it having a scolex corresponding to our Figure 1, a worm fixed *in situ* in the host intestine.

We propose the continued use of the name *B. acheilognathi* Yamaguti, 1934 in preference to that of *B. opsariichthydis* Yamaguti, 1934, as emended by Yeh (1955) and Yamaguti (1959). Although Yamaguti reserved the specific diagnosis of *B. acheilognathi* until additional specimens came to hand, he did describe a gravid individual, and there is no difficulty with the spelling of the specific epithet as exists for *B. opsariichthydis*. In addition the name *B. acheilognathi* has been accepted into common usage, and no rule of priority is contravened by its continued use. We agree with the other authorities noted earlier in this section that *B. opsariichthydis* Yamaguti, 1934, *B. fluviatilis* Yamaguti, 1952, *B. gowkongensis* Yeh, 1955, *B. phoxini* Molnár, 1968 and *Schyzocotyle fluviatilis* Akhmerov, 1960 are synonyms of *B. acheilognathi* Yamaguti, 1934.

In conclusion, we consider our experiment with cestodes having highly mobile scolices without any hard structures has clearly shown the importance of adopting a standardized fixation procedure. We have also shown how if a standardized procedure is not used considerable taxonomic confusion can result. We agree with Dubinina (1982) that scolex characteristics play an important role in the identification of species of the genus *Bothriocephalus*, and also other cestodes, but only if the exact conditions of fixation are clearly stated.

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Short Communication

The Control of *Bothriocephalus acheilognathi* in Grass Carp, *Ctenopharyngodon idella*, using Praziquantel

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The pathological effects of the tapeworm *Bothriocephalus acheilognathi* Yamaguti, 1934 on its cyprinid definitive hosts have been well documented. Heavy infestations may cause intestinal blockage, inflammation and perforation, generalized emaciation, reduced growth rate, and even death (Liao & Shih, 1956; Bauer, Musselius & Strelkov, 1969; Edwards & Hine, 1974; McDaniel, 1979; Scott & Grizzle, 1979; Hoffman, 1980). The prevalence of the infection may reach 100% in intensive and semi-intensive pond culture systems, where the parasite is a particular threat to underyearling cyprinids.

In order to select an anthelmintic which could be used to treat a batch of 30,000 newly imported grass carp (*Ctenopharyngodon idella* Valenciennes), which were found to be infected with *B. acheilognathi*, trials were carried out in the laboratory using three anthelmintics: praziquantel (*Droncit*, Bayer), niclosamide (*Mansonil*, Bayer) and mebendazole (*Mebenvet*, Crown Chemicals).

Various dose rates of these anthelmintics were given to groups of ten fish from the infected batch in static water, 50-litre aquaria at 18–20°C. The anthelmintics were suspended in 0.9% saline and administered as a single dose via stomach tube (after Andrews & Riley, 1982). The fish were approximately 15 cm long (and weighed 25–30 gm each), and the prevalence of the infestation with *B. acheilognathi* had previously been established as 50–80%.

At a dose rate of 150 mg kg^{-1} body weight, niclosamide did not achieve satisfactory control and there were signs of toxic effects on the fish. Mebendazole was used at dose rates of 35–500 mg kg^{-1} b.w., but was ineffective in eliminating the parasite. In addition, at dose rates of 50 mg kg^{-1} b.w. (and above) there were some signs of toxic effects on the fish.

At dose rates of 35–100 mg kg^{-1} b.w. praziquantel was completely successful at eliminating the tapeworm infestation. There were no signs of toxic effects on the fish and the tapeworms usually disappeared from the gut of the fish within 7 days.

In view of these results (and the large number of fish to be treated), it was decided to use praziquantel and to administer the anthelmintic with pelleted food. The infected grass carp were divided into two batches, and the first batch of approximately 15,000 fish were brought into an indoor recirculating system consisting of eight 1600-litre fibreglass tanks and a large biological filter. The temperature was increased from 6 to 24°C at a rate of 2°C day^{-1}. The fish were then offered trout pellets (*Fingerling 1*, B.P. Nutrition) for several days, during which time any lethargic or moribund (and hence non-feeding) fish were removed and destroyed. As soon as the fish were feeding well, they were starved for 3 days and then offered anthelmintic-medicated feed which had been prepared as follows. Praziquantel powder was obtained

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from Bayer AG. Using a little cooking oil to bind the powder to the pellets, sufficient praziquantel was added to 3 days' ration to achieve a dose rate of (over the 3-day period) 125 mg kg^{-1} b.w. To help ensure that all the fish had access to the medicated feed, automatic feeders were used to provide a 2-min feed every 30 min during daylight hours. Nine days after the end of the treatment period, 300 grass carp (i.e. sufficient to provide a 95% chance of detecting a 1% level of infection) were dissected and none found to harbour intestinal tapeworms.

The recirculating system was allowed to cool to ambient (winter) temperatures and this batch of fish was then released into a previously disinfected pond. The remaining infected fish were brought into the recirculating system and acclimated as described above. These fish were also fed on non-medicated pellets for several days and any obviously non-feeding fish removed and destroyed. The fish were then starved for three days and then fed (as before) on medicated pellets. On this occasion sufficient praziquantel was used to achieve (over the 3-day period) a dose rate of 105 mg kg^{-1} b.w. Six days after the end of the treatment period, 300 grass carp were dissected and none found to contain *B. acheilognathi*. After allowing the recirculating system to cool these fish were released into the same pond as the first batch of treated fish, and the previously infected pond was drained, limed and left exposed to winter temperatures for several weeks (see Mitchell & Hoffman, 1980). During the time the fish were in the recirculating system, water quality was monitored and found to lie within the following limits: pH 7.1–7.8, total ammonia (as N) <0.3 mg l^{-1}, nitrite (as N) 0.05–0.7 mg l^{-1}, dissolved oxygen 70–100% saturation.

The acclimation of the fish to indoor tanks and to pelleted feed was considered very important, as was the removal of any obviously non-feeding fish before the medicated pellets were used. About 500 non-feeding fish were removed, which is less than 1.7% of the 30,000 fish which were imported.

The dose rates of praziquantel employed at this incident were far greater than those normally used to achieve tapeworm control. This was to allow for a certain amount of anthelmintic wastage (bearing in mind the method of administration), and to provide the best possible chance of complete tapeworm elimination. Further work is aimed at establishing the minimum effective-dose rate of praziquantel for treating *B. acheilognathi* and other helminth parasites of fish.

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